Vectors and Gene Delivery to the Retina

Arthur Planul, Deniz Dalkara

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Arthur Planul[1,2,3] and Deniz Dalkara[1,2,3]

[1]Inserm, Institut de la Vision, UMR S968, 75012 Paris, France; email: arthur.planul@inserm.fr, deniz.dalkara@gmail.com

[2]Sorbonne Universités, UPMC Université Paris 6, UMR S968, 75012 Paris, France

[3]CNRS, UMR 7210, 75012 Paris, France

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Abstract

One of the great advantages of the retina as a target tissue for gene delivery is the wide array of genetic tools that have been developed in the past decade. This includes a variety of vectors for therapeutic gene delivery to most types of retinal neurons and glia, as well as cell type–specific promoters for restricted gene expression in distinct neuronal subtypes. Within the scope of neuroscience applications and for gene therapy, it is now routine to express reporter genes, replacement genes, neuronal activity indicators, and microbial opsins in specific neuronal types in the mouse retina. However, there are considerable anatomical, physiological, immunological, and behavioral differences between the mouse and the human that limit the usefulness of these tools in humans and nonhuman primates. Several advances are now being made toward the goal of applying viral targeting tools to understand the primate retina. Here, we describe these advances, consider their potential to advance our understanding of the primate retina, and describe what will be needed to move forward.

1. INTRODUCTION

There has been significant progress in discovering genes that are responsible for human retinal disease, and representative animal models carrying similar gene defects have been identified. In parallel, new methodologies like optogenetics that use light and genetics to manipulate and monitor the activities of defined neural populations have gained ground in asking questions on neuronal functioning and circuitry in an unprecedented way. With these advances, researchers started using retinal gene transfer extensively to develop gene-based therapies for blinding disorders and to investigate retinal circuitry. With the efficacy of gene delivery being central to the success of both gene therapy and understanding of retinal circuits, the development of adequate vectors has become critical. Therefore, there has been an exponential growth in the number of vectors for gene delivery to the mammalian retina in the past ten years. In this review, we discuss these vector technologies in view of their advantages and shortcomings for specific applications. More recently, several successful clinical gene therapy trials have paved the way to consider a number of retinal diseases with an expanded range of cellular targets for future therapies. In parallel, neuroscientists have moved on to understanding how different types of neurons contribute to circuit function in higher primates. There is now a growing demand to extend the vector toolbox for efficient gene delivery to retinas of higher primates. We discuss recent developments and research avenues in the development of vectors suitable for application in humans and higher primates in the Future Issues section.

2. GENE DELIVERY VECTORS

2.1. What Is a Vector?

In molecular biology, a vector is broadly described as a carrier that transfers genetic material into cells. This review focuses on vector systems for gene delivery to the mammalian retina. There are two main categories of vectors for gene delivery: viral and nonviral vectors. Viruses have evolved to efficiently deliver their genetic material into cells. Viral infection involves receptor-mediated intracellular uptake of viral particles and delivery of viral DNA into host cell nuclei, both of which are ensured by the viral capsid (Kay et al. 2001). The viral capsids have coevolved with the constraints of their hosts to overcome and hijack the cell and nuclear entry mechanisms (**Figure 1**). For nonviral gene delivery, however, nuclear entry and persistent transgene expression are significant obstacles (Naik et al. 2009). To improve nonviral gene delivery, chemical and molecular techniques need to be pushed toward a system that can approach the efficiency of natural biological processes of viral infection. In the unique environment of the retina, it might be possible to achieve this in the years to come. Nonviral vectors offer advantages over viral vectors in the size of the gene they can carry and in reduced risk of eliciting immune responses. Below, we discuss each type of vector in view of its potential for retinal gene delivery.

2.2. Synthetic Vectors

Synthetic vectors are often referred as nanoparticles (NPs) because of their small size (usually ranging from 10 to 500 nm). Synthetic vectors can be broadly defined as an assembly of cations that complex DNA into small-sized particles (**Figure 2**). NPs offer a unique set of advantages: They are easy to synthesize and usually have a low production cost compared to viruses (Trapani et al. 2014b). Their molecular structure can be easily manipulated. NPs also have a large transport capacity of up to 20 kb (Fink et al. 2006), which allows them to carry genes like *ABCA4* or *USH2A*.

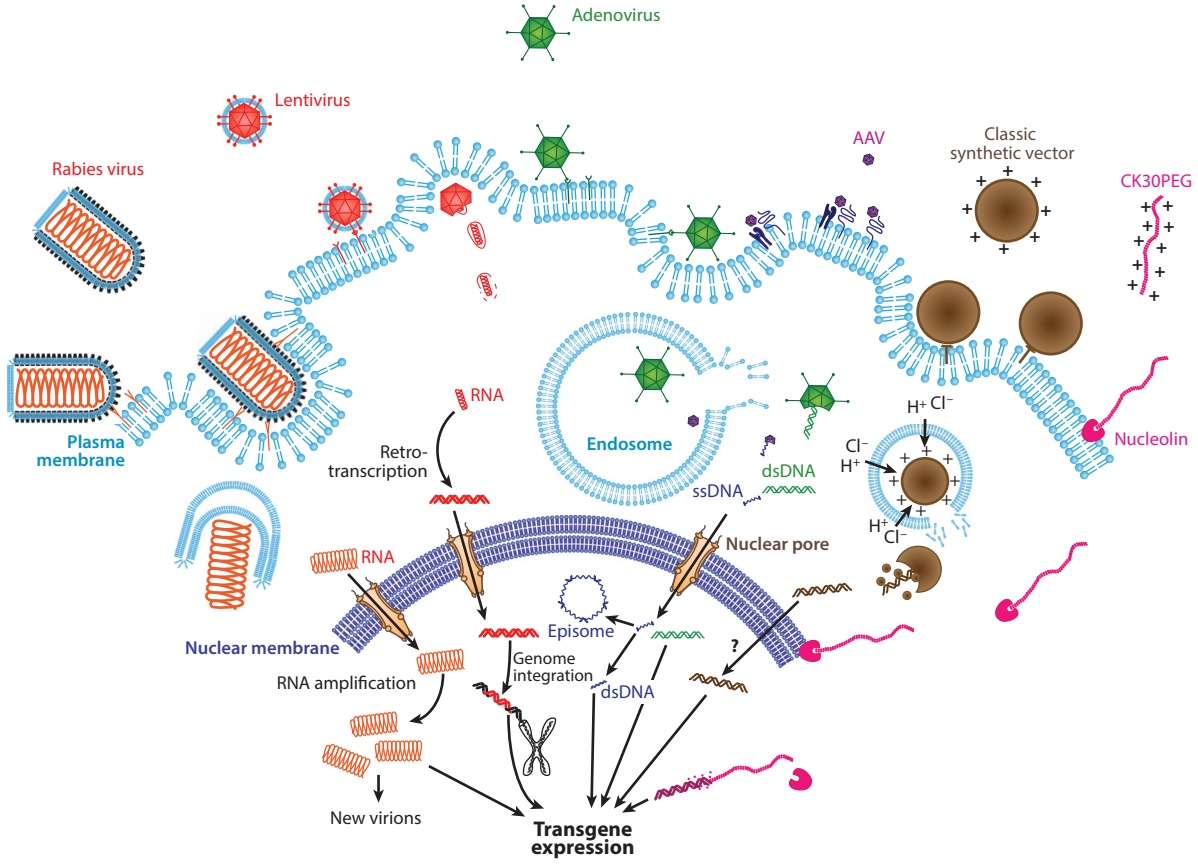

Figure 1

Cellular entry of gene delivery vectors. The main steps in gene delivery are the cell entry, the escape from the endosome, and the entry into the nucleus for all vectors. The enveloped viruses reach the cytoplasm by membrane fusion, whereas the nonenveloped viruses and most of the synthetic vectors destabilize the endosome to escape into the cytoplasm. The DNA carried by synthetic vectors has difficulties entering the nucleus compared to viral DNA, except for CK30PEG, which is directly transported to the nucleus by nucleolin. DNA from lentivirus integrates randomly into the host genome. Rabies virus amplifies its RNA in the host cell, leading to the creation of new virions. Abbreviations: AAV, adeno-associated virus; dsDNA, double-stranded DNA; ssDNA, single-stranded DNA.

Moreover, they possess a favorable safety profile due to their low immunogenicity and the absence of integration into the genome, alleviating insertional mutagenesis.

The key factors governing the efficacy of the NPs are (*a*) their cellular uptake (the NP should be able to protect the DNA in the extracellular medium and then to penetrate the cell), (*b*) their escape from the endosome into the cytosol, and (*c*) their ability to transfer the DNA through the nuclear pores, which block any particle bigger than ~25 nm. There are different categories of synthetic vectors for gene delivery. This review focuses only on synthetic vectors that are able to transduce efficiently the neural retina or the retinal pigment epithelium (RPE) without toxicity (Adijanto & Naash 2015).

CK30PEG is a 30-mer cationic polylysine conjugated with 10-kDa polyethylene glycol (PEG), which produces compacted DNA NPs. By using acetate as the lysine counterion, it is possible to obtain rod-shaped CK30PEG NPs efficient for retinal gene delivery (Farjo et al. 2006, Fink et al. 2006). These vectors can efficiently transfect both the RPE and photoreceptor cells as early as 2 days

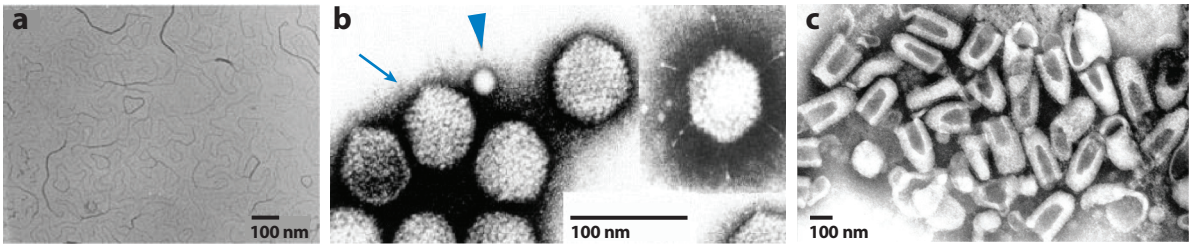

Figure 2

Structural differences between synthetic vectors and viral vectors. (*a*) Rod-shaped CK30PEG nanoparticles with minor diameter of 8 to 11 nm. (*b*) The adeno-associated virus (*arrowhead*) is smaller than the adenovirus (*arrow*), allowing it to cross the inner and outer limiting membrane after intraocular delivery. (*c*) The bullet-like rabies virus. Panel *a* adapted from Farjo et al. (2006). Panel *b* adapted from an image from the International Committee on Taxonomy of Viruses (**http://www.virology.net/Big_Virology/BVDNAadeno.html**). Panel *c* adapted from micrograph from F.A. Murphy, University of Texas Medical Branch, Galveston, TX (**https://www.utmb.edu/virusimages/**).

postinjection into the subretinal space (Han et al. 2012b). Remarkably, their efficacy at 14 days postinjection (PI) is comparable to that of AAV2 and can last for up to 1 year. However, after 14 days PI, the expression levels resulting from the CK30PEG NPs' transduction become lower than for AAV2 and are strongly dependent on the promoter. CK30PEG NPs have the advantage of being internalized by nucleolin, which transfers the plasmid directly into the nucleus. Moreover, their small minor diameter (8–11 nm) seems to help their entry into the nucleus, infiltrating through the nuclear pores. CK30PEG NPs were capable of mediating structural and functional improvements in mouse models carrying mutations in photoreceptor-specific genes like the Abca4$^{-/-}$ model of Stargardt disease (Han et al. 2012a).

Polysaccharide NPs have also been used to deliver genes to the retina. Chitosan is a water-soluble product derived from chitin, the main structural component of crustacean exoskeleton and fungi cell wall. Chitosan NPs were shown to transduce photoreceptors and RPE after a subretinal injection and some retinal ganglion cells (RGCs) after intravitreal injection (Puras et al. 2013). Nevertheless, the efficiency of these NPs seems relatively low. The chitosan NPs formed are cationic, but modifications of functional groups are often added to increase their solubility at neutral pH. Glycol-chitosan NPs have been shown to be able to transport the *GFP* gene under the control of two different promoters and transfect exclusively RPE cells after subretinal injections, without toxicity (Mitra et al. 2014). This limitation to the RPE could be due to the formulation of the NPs, which formed a highly viscous gel that prevents the NPs from achieving a wide distribution into the retina.

Solid lipid nanoparticles (SLNs) are considered to be among the most effective lipid-based vectors. They consist of a solid lipid matrix surrounded by a layer of surfactants in an aqueous dispersion (Battaglia et al. 2016). SLNs coupled with hyaluronic acid (HA-SLNs) transporting plasmids expressing *GFP* and *RS1* were shown to transduce the RPE, the photoreceptors, and some RGCs. Remarkably, HA-SLNs transporting the human *RS1* gene were able to improve the phenotype of a mouse model of X-linked juvenile retinoschisis (Apaolaza et al. 2016).

Despite progress in NP-mediated gene delivery, some challenges remain as there is high variability in transduction efficacy depending on the transported plasmid, and an increase in efficacy is often related to an increase in toxicity. All those problems lead to the conclusion that, for now, the synthetic vectors are not as efficient as viral vectors like adeno-associated viruses (AAVs) to

deliver genes into the retina. However, the high transport capacities of the synthetic vectors leave more space for modification of the transported plasmid, thereby allowing the introduction of full promoter regions, introns, or scaffold/matrix attachment regions that can improve their efficacy (Koirala et al. 2013).

2.3. Viral Vectors

The retina has several characteristics that make it a favorable tissue for gene delivery. First, accessing the retina is easy from a surgical standpoint. Second, injected vectors are trapped into the vitreous or under the retina, without any propagation into peripheral organs. This allows the retina to be immune privileged (Willett & Bennett 2013), thus diminishing risks of adverse effects due to an immune reaction against the viral vectors. Despite those advantages, the retina also has drawbacks limiting the use of certain vectors. The neurosensory retina is shielded by inner and external limiting membranes that restrict access of particles larger than 30 nm into the retina (Inatani & Tanihara 2002). Thus, for gene delivery to the neural retina, AAV is the only vector that can be used via intraocular administration routes. AAVs are members of the Parvoviridae family of single-stranded DNA viruses. It is a dependovirus, which cannot replicate by itself, requiring coinfection with an adenovirus or a herpes simplex virus (HSV). AAV is a nonenveloped virus and has a large number of capsid variants displaying different cell tropisms (Cearley & Wolfe 2006) (**Figure 3**). These are discussed in view of their utility for gene therapy or investigating retinal circuits in the next sections.

2.3.1. Vectors for probing the retina. The visual system is a great model for probing the connectivity and function of neural circuits. With the mouse visual system being genetically traceable, it provides opportunities to understand these processes and link how identified circuit elements contribute to visual perception and behavior. Technologies like optogenetics are playing an important role in this process. Optogenetic actuators such as channelrhodopsin or halorhodopsin allow neuronal control, whereas a large spectrum of sensors like GCaMP synaptopHluorin, GluSnFRs, or ArcLightning allow optical recording of various aspects of neuronal activity. Viral and transgenic technologies enable restriction of both optogenetic control and optical recordings to genetically defined neuronal subpopulations.

The Gene Expression Nervous System Atlas (GENSAT) generated hundreds of mouse lines that express GFP or cre driven by different bacterial artificial chromosomes (Heintz 2001). Both GFP (Tang et al. 2013) and cre (Wang 2009) can be used in combination with viral vectors to confine expression of optogenetic actuators or sensors in the retina in a circuit-specific manner. A recombination event between two DNA recognition sites called loxP sites is catalyzed by cre-recombinase. A DNA sequence in between loxP sites is said to be "floxed." The location and relative orientation of these loxP sites determine the product of cre-based recombination: DNA between oppositely oriented loxP sites will be inverted, whereas the sequence between loxP sites with the same orientation will be eliminated. For example, coinjection of AAVs transporting an inverted optogene sequence flanked by two loxP sites in a mouse line expressing cre in a subset of retinal neurons would allow specific delivery of the optogene into the retinal circuit. Furthermore, transgenic GFP reporter lines can be used for functional studies because of a method that transforms GFP into a molecule that enables genetic manipulation (Tang et al. 2013, 2015). In this system, a cre recombinase dependent on GFP (cre-DOG) is used to directly induce cre/loxP recombination in GFP-expressing cells. Using AAV viral vectors, cre-DOG can be delivered to multiple GFP mouse lines and induce effective recombination selectively in GFP-labeled cells.

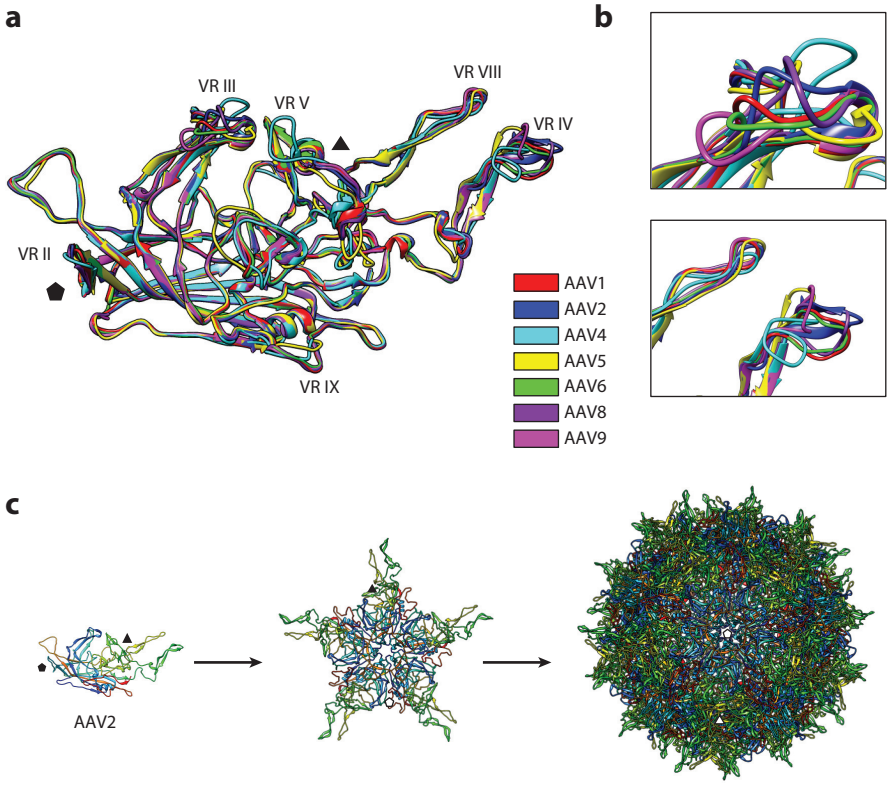

Figure 3

Organization of the adeno-associated virus (AAV) capsids. (*a*) Superposition of the VP1 protein of different AAV serotypes. AAV1 (*red*), AAV2 (*blue*), AAV4 (*cyan*), AAV5 (*yellow*), AAV6 (*green*), AAV8 (*purple*), AAV9 (*magenta*). (*b*) Hypervariable regions III, VIII, and IV. The structural differences are greatest among the nine hypervariable regions of the VP1 protein. This region determines the receptor-binding properties of the capsid and thus its tropism. (*c*) AAV2 capsid formed by 60 subunits assembled in an icosahedral fashion. The pentagon shows the place of the fivefold symmetry in the capsid. The triangle shows the place of the threefold symmetry; the more external regions of the capsid determine cellular tropism. These structures have been obtained using the UCSF Chimera software. The RCSB Protein Data Bank access numbers that have been used are PDB 5EGC (AAV1), PDB 1LP3 (AAV2), PDB 2G8G (AAV4), PDB 3NTT (AAV5), PDB 3OAH (AAV6), PDB 2QA0 (AAV8), and PDB 3UX1 (AAV9).

Nevertheless, the transgenic approaches using cre and GFP are mostly limited to mice. To go beyond the mouse model, viral vectors can be used in combination with cell type–specific promoters to target various subpopulations of retinal neurons. There are not enough characterized promoters to cover the diversity of retinal neurons as of today, but the discovery of new cell-specific promoters and enhancers (Shen et al. 2016) will open new avenues to expand our understanding of the neuronal circuitry of the visual system.

Although AAVs remain the main viral vectors used within the retina, transsynaptic viruses like the rabies virus in combination with the retrograde HSV or AAVs are useful to probe the brain retina connections at a cellular resolution (Yonehara et al. 2013). HSVs or AAVs can be used to transduce RGCs via their axon terminals in the lateral geniculate nucleus. This in turn allows the somas of the RGCs to be infected with a G-protein–deficient rabies virus coated with EnvA protein transporting a reporter protein (EnvA-G-GCaMP; **Figure 4*a***). As the EnvA protein recognizes

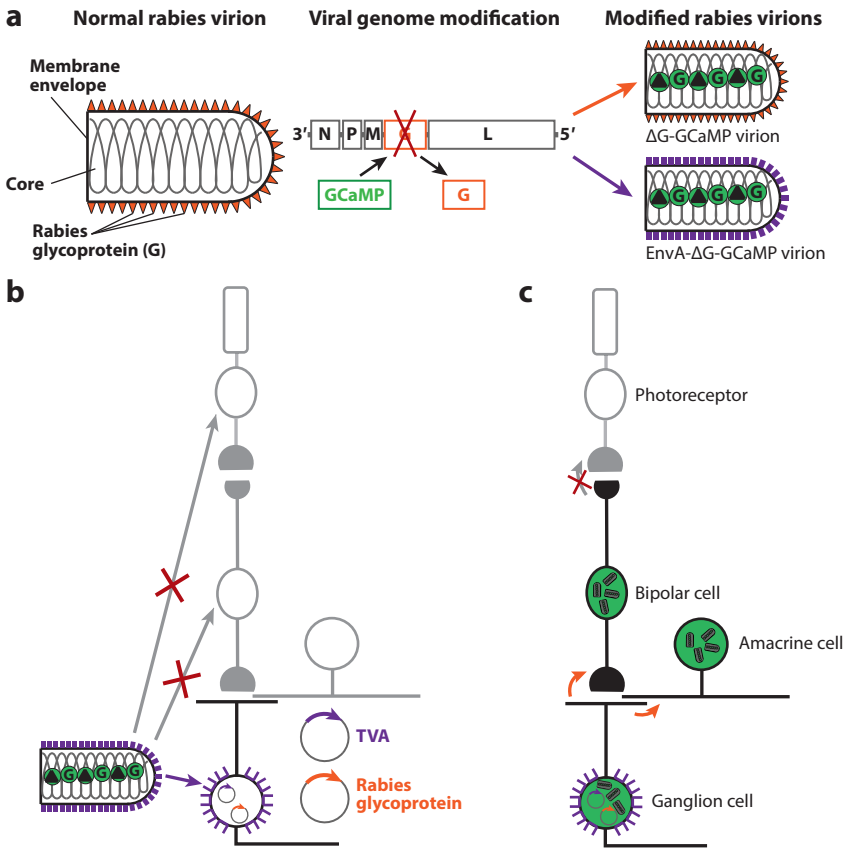

Figure 4

Pseudotyped rabies virus for studying the connections in the retina. (*a*) Normal and modified rabies virions. Replacing the rabies G-protein gene with the GCaMP gene will lead to the production of GCaMP-expressing viruses and will allow the replacement of the G-protein at the surface of the virus by EnvA, which specifically recognizes the TVA receptor (EnvA-ΔG-GCaMP). (*b*) Selective infection. Ganglion cells are transfected with two plasmids, one coding for the TVA receptor and one coding for the rabies G-protein. Thus, only those cells expressing the TVA receptor will be recognized by EnvA and infected by the EnvA-ΔG-GCaMP virions. (*c*) Monosynaptically restricted spread. The rabies G-protein coding plasmid will allow the newly produced rabies virions to be coated with G-protein, creating ΔG-GCaMP virions able to cross one synapse in a retrograde fashion. As the synaptically connected cells do not have the G-protein coding plasmid, the new virions cannot cross one more synapse. The GCaMP will stain the TVA coated cells and the cells synaptically connected to the TVA coated cells (bipolar and amacrine cells). Adapted from Callaway (2008).

the TVA receptor exclusively, only the cells infected with the retrograde virus will express the activity reporter GCaMP (**Figure 4*b***). Then, with the addition of the G-protein coding gene in those RGCs, the rabies virus is able to cross only one synapse and express GCaMP also in the presynaptic cells projecting to the RGCs (Callaway 2008; **Figure 4*c***). This method has been used with success to study the origin of the direction selectivity (DS) in ON DS ganglion cells in mouse retinas (Yonehara et al. 2013). However, it is difficult to use rabies viruses for long-term in vivo studies because of the toxicity of the virus, which modifies cell signaling and eventually leads to cell death (Granstedt el al. 2009).

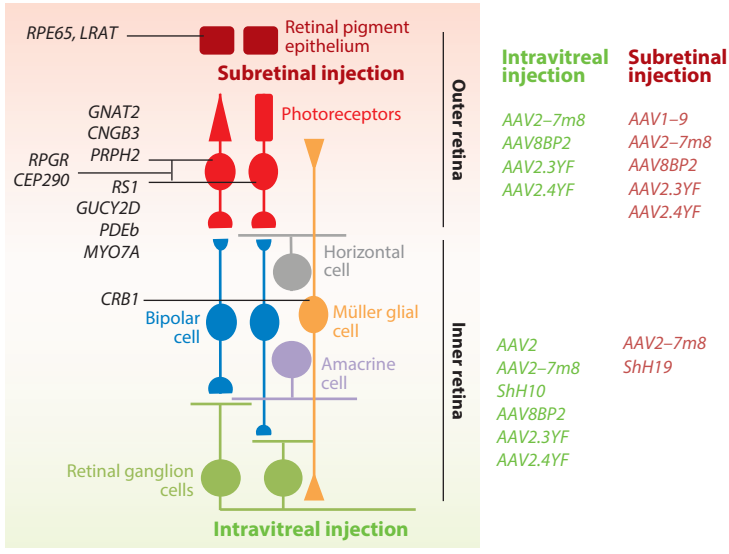

Figure 5

Schematic showing the retinal cell types and injection routes. Cells in the inner and outer retina and the proteins encoded by candidate genes for gene therapy are indicated. Gene defects in the indicated genes lead to recessive phenotypes that are amenable to gene supplementation. Retinal pigment epithelium (*dark red*), photoreceptors (*red*), horizontal cell (*gray*), bipolar cells (*blue*), Müller glial cell (*orange*), amacrine cell (*purple*), retinal ganglion cells (*green*).

2.3.2. Vectors for retinal gene therapy. Blinding retinal diseases that can benefit from gene therapy include common multifactorial conditions like age-related macular degeneration, glaucoma, diabetic retinopathy, and inherited retinal degenerations due to single-gene defects. The complex diseases can benefit from gene therapy where a viral vector delivers a secretable factor alleviating the symptoms of the disease (MacLachlan et al. 2011). In this case, cell type–specific gene delivery is not necessary. Monogenic retinal degenerations, however, are caused by mutations in any one of over 200 genes and can benefit from gene replacement therapy when the causal mutation is recessive. Recessive mutations causing inherited retinal degenerations (IRD) are found mostly in photoreceptor cells and RPE (**Figure 5**). Retinitis pigmentosa, Usher syndrome, Leber congenital amaurosis, Stargardt disease, and achromatopsia are some of the most common and severe IRDs affecting photoreceptors (Lipinski et al. 2013). Other monogenic conditions like congenital stationary night blindness and Leber hereditary optic neuropathy involve the inner retinal cells. The viral vectors used for gene therapy in these diseases need to have a favorable safety profile in addition to having coherent cell transduction patterns for the disease in question. The choice of viral vectors for retinal gene therapy coalesces around adenoviral, lentiviral, and adeno-associated viral vectors. In general, when RPE is the target cell, all three vectors are capable of providing gene delivery (Trapani et al. 2014b), although lentiviral and adenoviral vectors are more inflammatory. Lentiviruses and adenoviruses can deliver genes exceeding the carrying capacity of AAVs. Nevertheless, the superior safety and efficacy of intraocular AAV administration in humans lead to efforts in engineering AAV-based vector systems for delivering large genes. Dual AAV–mediated large gene delivery in mice (Trapani et al. 2014a) and in pigs (Colella et al. 2014) seems to be a viable solution to AAVs' limited cargo capacity.

3. TARGET CELLS IN THE RETINA

The majority of recessive mutations underlying inherited retinal degeneration are found in the outer retinal cells (RPE and photoreceptors) (**Figure 5**). The photoreceptors and RPE are thus the primary targets of gene replacement therapy. However, other recessive genetic mutations are also found in the Müller glial (Pellissier et al. 2015), bipolar (Scalabrino et al. 2015), and retinal ganglion cells (Koilkonda & Guy 2011), making it necessary to find vectors that can target these cells successfully in a gene therapy setting. In addition to gene supplementation, other modes of gene therapy also require targeting inner retinal cells (Busskamp & Roska 2011). Finally, depending on the neuroscience question, it is equally important to be able to selectively and efficiently target all neuronal subtypes of the retina using viral vectors (Packer et al. 2013). The choice of AAV vectors that can be used to target the main retinal cell types are described below.

3.1. RPE Cells

The RPE cells have the particularity of being among the most actively phagocytic cells in nature (Mazzoni et al. 2014). For this reason, they are easily targeted by virtually any viral vector administered into the subretinal space. AAV serotypes 1, 4, and 6 seem to have a preference for RPE cells rather than their underlying photoreceptors and should be used when exclusive RPE transduction is preferred (Castle et al. 2016). RPE cells can also be transduced via an engineered AAV variant, AAV2–7m8, when the vector is administered intravitreally at high doses (Dalkara et al. 2013).

3.2. Photoreceptors

Mature photoreceptors are efficiently targeted using AAV vectors administered into the subretinal space (Allocca et al. 2007, Castle et al. 2016). Several AAV serotypes and engineered variants have been shown to lead to high-level gene delivery to photoreceptors after subretinal injections. AAV serotypes 2, 5, 6, 7, 8, and 9 and engineered variants AAV2–7m8 and AAV8BP2 all allow efficient photoreceptor gene delivery (Allocca et al. 2007; Boye et al. 2012; Vandenberghe et al. 2011, 2013). Among the naturally occurring serotypes, AAV8 leads to the highest overall photoreceptor transduction (Castle et al. 2016). AAV5, 8, and 9 have the advantage of giving rise to faster onset gene expression compared to AAV2 as well as gene expression in cone photoreceptors (Alexander et al. 2007, Komáromy et al. 2010, Mancuso et al. 2009). Photoreceptors can also be targeted via intravitreal injections of engineered AAV capsids (Cronin et al. 2014; Dalkara et al. 2013; Kay et al. 2013; Petrs-Silva et al. 2009, 2011). Directed evolution and rational design yielded AAV capsids capable of efficient outer retinal gene delivery from the vitreous.

3.3. Inner Nuclear Layer Neurons

The inner nuclear layer neurons (bipolar, horizontal, and amacrine cells) are the most difficult targets for gene delivery as they are separated from the vitreous and subretinal space by a layer of densely packed neurons. It has been impossible to target these neurons using naturally occurring serotypes. AAV engineering has provided solutions to reach these cells efficiently via subretinal (Doroudchi et al. 2011) and intravitreal (Gaub et al. 2015, Macé et al. 2014) routes.

3.4. Müller Glial Cell

One cell type that can be easily targeted within the inner nuclear layer is the Müller glial cell. Müller glia span the entire thickness of the retina with their end-feet localized in the inner and outer limiting membranes. Because of their unique morphology, they can be targeted with naturally

occurring (Allocca et al. 2007, Dorrell et al. 2009) and engineered (Klimczak et al. 2009, Koerber et al. 2009) AAVs administered into the subretinal or intravitreal space. An AAV variant capable of targeting exclusive Müller glial targeting via the vitreous has been described and has the advantage of not requiring cell type–specific promoters to obtain restricted gene expression in this cell type (Klimczak et al. 2009).

3.5. Retinal Ganglion Cells

Finally, the RGCs can be targeted with several AAVs via intravitreal injection route (Caporale et al. 2011, Dalkara et al. 2009, Hellström et al. 2009, Ivanova et al. 2010, Yin et al. 2011). Contrary to subretinal injections, a limited number of AAVs lead to successful ganglion cell transduction despite the proximity of these cells to the injection site. One engineered AAV vector, AAV2–7m8, has been shown to these cells via subretinal injection (Ramachandran et al. 2017).

It is important to note that the tropism and capabilities of each AAV variant have been assessed by over a decade of experimental gene delivery studies, as overall capsid sequence homologies are not predictive of the tropism of AAV vectors. Only a few amino acid differences account for significantly different biological activities across the hundreds of naturally occurring and engineered AAV capsids (Kwon & Schaffer 2008).

4. INJECTION ROUTES

The administration route is a major determinant of efficacy and specificity in gene delivery to the retina. As a rule of thumb, the administration route that delivers the vector in closest proximity to the target cell type is preferred. This means that subretinal injections are relevant for gene delivery to the outer retina, whereas an intravitreal route is preferable for targeting the inner retina (**Figure 5**). However, the choice of delivery route can be more complex depending on the purpose of the gene delivery. Each gene delivery paradigm imposes a certain number of constraints. We focus on two main purposes for gene delivery: therapeutic gene delivery and gene delivery to investigate retinal circuitry.

For therapeutic gene delivery, it is necessary to choose the injection route that will afford therapeutic-level gene expression in target cells without causing toxicity or compromising retinal integrity. Indeed, it has been shown that disease state influences retinal transduction patterns using various viral vectors. In some diseases, retinal detachments are not well tolerated owing to the already compromised state of the retina. For investigating neural circuits, one can be more flexible regarding the safety, as long-term expression might not be necessary to adequately address a given question. The onset of gene expression is another parameter to consider when choosing an adequate delivery route. Subretinal injections of most serotypes lead to fast-onset gene expression in outer retinal cells starting at few days postinjection with the exception of AAV2, which has slower kinetics (Tan et al. 2009). Intravitreal injections lead to gene expression in the inner retina starting at 2–4 weeks postinjection depending on the injected dose. Transduction of the outer retina following intravitreal delivery is longer, varying from 6 to 8 weeks after administration (Dalkara et al. 2013). Finally, gene delivery to the developing retina is possible via systemic injections of AAV (Byrne et al. 2015a, Dalkara et al. 2012). When experimental conditions require gene expression before eyelid opening in mice (before day 15), delivering AAV through systemic injections into the bloodstream is less invasive and less challenging. High-titer injections of AAV9 and variants are able to provide bilateral gene delivery to inner and outer retina when the vector is administered within the postnatal period of 1 to 4 days (Byrne et al. 2015b, Dalkara et al. 2012). **Table 1** can be used to guide the choice of AAV for a given application.

Table 1 Retinal cell transduction with various capsids and injection routes

Capsid	Transduced cell types		
	Intravitreal	Subretinal	Systemic
AAV1	–	RPE, PR	–
AAV2	RGC, MGC	RPE, PR	–
AAV2.3YF	RGC, MGC, AC, HC	PR	–
AAV2.4YF	RGC, MGC, AC, HC, PR, RPE	RPE, PR, HC, RGC	–
AAV2–7m8	RGC, BPC, MGC, AC, HC, PR, RPE	RPE, PR, BPC, RGC	–
AAV4	–	RPE	–
AAV5	–	RPE, PR	–
AAV6	RGC	RPE, PR	–
AAV7	–	RPE, PR	–
AAV8	RGC	RPE, PR	–
AAV8BP2	RGC, BPC, AC, HC, PR	RPE, PR	–
AAV9	RGC	RPE, PR, MGC	RPE, RGC
AAV9.2YF	–	–	RPE, PR, MGC, RGC

Abbreviations: AC, amacrine cells; BPC, bipolar cells; HC, horizontal cells; MGC, Müller glial cells; PR, photoreceptors; RGC, retinal ganglion cells; RPE, retinal pigment epithelium.

5. BARRIERS TO RETINAL TRANSDUCTION USING VECTORS

Depending on the injection route, gene delivery vectors need to overcome certain obstacles to reach the retina. Vectors delivered into the subretinal space need to overcome the external limiting membrane of the photoreceptors to infiltrate the extracellular matrix surrounding the photoreceptors and gain access to the neurosensory retina. The major advantage of the subretinal injection route is that it concentrates the vector in close proximity to the retina. Conversely, intravitreal injections dilute the vectors in the vitreous. Vectors delivered into the vitreous cavity have to overcome the inner limiting membrane, which constitutes a formidable barrier to viral particles (Dalkara et al. 2009). Inner limiting membrane is formed of a dense meshwork of glycosaminoglycans secreted by the Müller cell end-feet during development and isolates the retina from the vitreous. The ability of viral vectors to overcome this barrier depends on their size and the availability of their receptor within the inner limiting membrane. It has been shown that enzymatic lysis of this membrane of its physical peeling helps AAV penetrate better into the retina, increasing the efficacy of several serotypes in gaining access to the retina (Cehajic-Kapetanovic et al. 2011, Dalkara et al. 2009). The inner limiting membrane is much thicker in larger animals like dogs and macaques compared to rodents (Matsumoto et al. 1984). Lastly, the blood–retina barrier limits the access of viral particles administered to the retina through the circulation. There seems to be an optimal time window when AAVs can get across the blood–retina barrier, and it is rather early in development (Byrne et al. 2015b).

The viral dose and the state of the retina are also important in determining the efficacy and extent of gene delivery to retinal cells exposed to the vector (Kolstad et al. 2010, Park et al. 2009). In general, retinal degeneration makes the retina more permissive to transduction by viral vectors as barriers limiting access to the retina are breached during the degenerative process. Viral dose acts as a gear on gene expression; the higher the dose, the more transgene expression there is in transduced cells. Some cells away from the injection site will actually be transduced with a limited

number of viral particles, but expression is not visible in these cells because of the low copy number of the transgene in the nucleus. When viral dose is increased, this weak expression becomes visible, leading to an extended expression pattern.

6. RESTRICTING GENE EXPRESSION TO SUBPOPULATIONS OF NEURONS

The mammalian retina consists of millions of neurons, including dozens of neuronal subtypes. Retinal gene therapy aims to target these neurons in an efficient, safe, and specific way so that therapeutic gene expression can bring on benefit. Neuroscience studies, however, aim to understand the principles organizing retinal circuits and thereby decipher how they process information and account for vision. Recent developments suggest that genetic analysis will play an important role in dissecting neural circuits (Shen et al. 2016). Informative analogies can be made between gene interaction networks that regulate complex biological processes and neural circuits. On the basis of this, the transgenes delivered into retinal cells must contain a promoter, which is a regulatory sequence that will determine where and when the transgene is active. Transgene expression after viral mediated gene delivery is a pentapartite equation between the injection route, the viral capsid, the state of the retina, the promoter, and the viral dose. The first four parameters are discussed above. The promoter is the transcriptional-level parameter determining which cells will express the transgene product. It is a critical sequence required for regulating the spatial and temporal expression pattern of a transgene delivered by a viral vector. Promoter sequences are isolated from upstream regions of endogenous mammalian genes. A promoter sequence normally includes a transcriptional start site as well as transcription regulatory sequences. Many promoters have been reported to successfully restrict expression of transgenes in the retina. Here, we focus on promoters that are compatible with delivery using AAV, as this is the only vector that can efficiently target the neurosensory retina. **Table 2** provides a nonexhaustive list of promoter sequences that have been used with AAV vectors in the retina and their cell specificities.

However, one must remember that the specificity of a promoter is part of a larger equation involving the injection route, state of the retina, viral dose, and capsid. This means that ubiquitous promoters can also lead to cell type–specific expression when combined with a particular capsid and administration route, whereas cell type–specific promoters leading to restricted expression under a given set of circumstances may lose their specificity under others. Finally, the promoter activity also shows species-specific attributes.

7. CHALLENGES IN RETINAL GENE DELIVERY

7.1. Challenges for Basic Science Applications (in the Retina and Beyond the Retina Connectivity to Visual Centers)

There are three main challenges for the study of the retina with gene delivery vectors: the capacity to target the desired neuronal subtype, the ability to trace connectivity between the retina and visual centers without toxicity, and the transition to other, more visual species like nonhuman primates (NHPs).

We previously saw the importance of promoters specific to neuronal subtypes. As the AAVs are for now the only efficient viral vectors in the retina, the size of the promoter is limited because AAVs cannot carry more than 4.8 kb, including the promoter and the transgene. In the absence of promoter sequences that can distinctly target each neuronal subpopulation, the GENSAT project we described previously has been substantially useful. It brought an indirect way of obtaining

Table 2 Targeting expression to subsets of retinal neurons

Promoter	Injection route	Animal model	Cell type	Reference(s)
CMV	SR	Mouse, dog, macaque	RPE, PR	Acland et al. 2001; Allocca et al. 2007; Auricchio et al. 2001, 2002; Lau et al. 2000
	IVT	Mouse, dog, macaque, marmoset	RGC, MGC	Dudus et al. 1999, Guy et al. 1999, Ivanova et al. 2010, Tshilenge et al. 2016, Yin et al. 2011
CBA	SR	Mouse	RPE, PR	Allocca et al. 2007
	IVT	Mouse, rat	RGC, MGC	McKinnon et al. 2002
hSyn	IVT	Mouse	RGC	Caporale et al. 2011
CD365	SR	Rat	RPE	Sutanto et al. 2005
RPE65	SR	Mouse, dogs	RPE	Le Meur et al. 2007
VMD2	SR	Mouse, rat, dog	RPE	Alexander & Hauswirth 2008, Conlon et al. 2013, Guziewicz et al. 2013
MOPS	SR	Mouse	Rods	Flannery et al. 1997, Khani et al. 2007, Liu et al. 2005
IRBPe/GNAT2	SR	Mouse, macaque	PR	Dyka et al. 2014
hRK (GRK1)	SR	Mouse, pig	PR	Boye et al. 2010, Khani et al. 2007, Manfredi et al. 2013, Zou et al. 2011
mBP	SR	Mouse	Cones	Michalakis et al. 2010
HB	SR	Rat, dog	Cones (rods and RPE)	Glushakova et al. 2006, Komáromy et al. 2010
RHO	SR	Mouse	Rods	Allocca et al. 2007
mCAR	SR	Mouse	PR	Busskamp et al. 2010, Dyka et al. 2014
pR2.1	SR	Mouse, rat, ferret, guinea pig	Cones	Li et al. 2008
pR1.7	SR	Mouse, macaque	Cones	Ye et al. 2016
GFAP	IVT	Mouse	MGC	Prentice et al. 2011
Grm6	SR	Mouse	BPC	Doroudchi et al. 2011
4xGrm6	SR, IVT	Mouse	BPC	Cronin et al. 2014, Gaub et al. 2015, Macé et al. 2015
200En-mGluR500P	IVT	Mouse, marmoset	BPC	Lu et al. 2016
In4s-In3–200En-mGluR500P	IVT	Mouse, marmoset	BPC	Lu et al. 2016
Ple22	IVT	Mouse	BPC	de Leeuw et al. 2014, Scalabrino et al. 2015

Abbreviations: BPC, bipolar cells; IVT, intravitreal; MGC, Müller glial cells; PR, photoreceptors; RGC, retinal ganglion cells; RPE, retinal pigment epithelium; SR, subretinal.

restricted expression of genes in neuronal subtypes in the retina. It contributed to the study of the connectivity and activity of several neuronal subtypes of the retina like transient OFF-α and ON-OFF direction-selective RGCs (Huberman et al. 2008, 2009). However, the challenge remains the applicability of this approach in nonrodent species. It is of primary importance to be able to do circuit-specific interventions in NHPs to ask questions about our vision, which is remarkably different than the vision of rodents.

The second challenge is the study of the activity and the connectivity of the retina in relation to higher visual centers. For anterograde tracing of the visual system, the only available vectors are herpes simplex virus 1 (HSV1) (Wojaczynski et al. 2015) and AAV1 (Zingg et al. 2017); however, HSV1 is toxic and will quickly modify the neuronal activity, and the genes transported by AAV1 need to be amplified by a system like cre/loxP to have a sufficient expression level. Moreover, these two vectors are not efficient in infecting the retina via intravitreal route. Thus, HSV1 and AAV1 can be used only via stereotaxic injection to study the visual area of the brain and not the connectivity within the retina. For retrograde tracing, rabies can be used. It is important to note that the immunogenicity of the vectors (i.e., rabies) is less of a problem in basic science applications, though toxicity could impair neuronal activity (Granstedt et al. 2009). New viral vectors can help offer solutions in this context. For example, a new AAV called AAVretro has been developed to allow better retrograde transport in the brain (Tervo et al. 2016). This vector might also be relevant in studying the connectivity between the retina and the brain, although the length of the axons might pose a challenge. Because AAVs are much less toxic than typical transsynaptic viruses like rabies or HSV, they allow in vivo studies of neuronal activity in live animals. Once again, studies involving anterograde and retrograde tracing were carried out mostly on mice, and their applications to other species, particularly NHPs, remains problematic. Rabies (Dum & Strick 2013) and lentivirus (El-Shamayleh et al. 2016) have been used in the brain and the spinal cord of macaques, but AAVs have been most frequently used for work in the retina (Dalkara et al. 2013, El-Shamayleh et al. 2016). Finally, transgenic lines of marmoset are being developed (Belmonte et al. 2015, Sasaki et al. 2009), but their number remains largely inferior to rodents.

7.2. Challenges for Clinical Gene Therapy

A major challenge in bringing successful proof-of-concept gene therapies to the clinic is the lack of vectors tailored for gene delivery in human retinas. Preclinical gene therapy work has shown that viral vectors display substantially different transduction patterns in rodents and larger animals like dogs and NHPs (Ivanova et al. 2010, Lin et al. 2009, Mussolino et al. 2011, Ramachandran et al. 2017, Stieger et al. 2009, Yin et al. 2011). It is very likely that retinal transduction in humans will closely resemble those in NHPs. Much effort has been spent on making AAV vectors better in the context of mouse retina. It is now timely to invest AAV engineering efforts to generate vectors compatible with human use. Because transgene expression is dependent on not only the AAV capsid but also the promoter and disease state, efforts are also needed in adapting and testing promoters for their performance in NHP retinas. Lastly, generation of macaque models displaying hallmarks of retinal degeneration would be incredibly valuable for predicting the outcome of viral vector–mediated gene delivery in a clinical setting.

8. CONCLUSIONS

Both for gene therapy and for investigating the retina, huge progress has been made in the discovery and engineering of viral and synthetic vectors. The most adequate vector systems for efficient in vivo gene delivery today are viral vectors. AAV vectors are the only suitable vectors for gene delivery to the neurosensory retina and need to be coupled to cell type–specific promoters to give specific expression in a desired group of cells. Although transgenic lines are useful for obtaining cell type–specific expression in mice, new vector-promoter combinations are needed to target the primate retina. In particular, gene delivery vectors are not efficient in targeting the inner retina in primates because of the presence of a thick inner limiting membrane. A new set of tools needs to be developed to meet the needs of gene delivery in primates in the years to come.

SUMMARY POINTS

1. Synthetic and viral vectors are capable of gene delivery to the retina.
2. Viral vectors are more efficient for long-lasting, high-level transgene expression.
3. Adenovirus, lentivirus, or adeno-associated virus (AAV) can be used to transduce retinal pigment epithelium cells.
4. AAV is the most robust gene delivery vector in the neural retina.
5. Transgene expression patterns are dictated by delivery route, AAV capsid, state of the retina, viral dose, and promoter.
6. AAV-mediated transgene expression has species-specific attributes.
7. New vectors capable of more efficient and widespread gene delivery to the primate retina are needed.

FUTURE ISSUES

1. Future research will focus on the nonhuman primate retina to get closer to the human retina for both basic science and gene therapy applications.
2. New vectors, promoters (Kotterman & Schaffer 2014), and surgical procedures (Boye et al. 2016, Comander et al. 2016) will need to be developed to obtain better gene delivery and cell specificity in the primate retina.
3. Dosage thresholds and immune reactions to adeno-associated viruses need to be better evaluated to alleviate adverse effects in primates that have more sophisticated immune systems (Kotterman et al. 2014, Vandenberghe et al. 2011).

LITERATURE CITED

Acland GM, Aguirre GD, Bennett J, Aleman TS, Cideciyan AV, et al. 2005. Long-term restoration of rod and cone vision by single dose rAAV-mediated gene transfer to the retina in a canine model of childhood blindness. *Mol. Ther.* 12(6):1072–82

Adijanto J, Naash MI. 2015. Nanoparticle-based technologies for retinal gene therapy. *Eur. J. Pharm. Biopharm.* 95(B):353–67

Alexander JJ, Hauswirth WW. 2008. Adeno-associated viral vectors and the retina. In *Recent Advances in Retinal Degeneration*, ed. RE Anderson, MM LaVail, JG Hollyfield, pp. 121–28. Adv. Exp. Med. Biol. Vol. 613. New York: Springer

Alexander JJ, Umino Y, Everhart D, Chang B, Min SH, et al. 2007. Restoration of cone vision in a mouse model of achromatopsia. *Nat. Med.* 13(6):685–87

Allocca M, Mussolino C, Garcia-Hoyos M, Sanges D, Iodice C, et al. 2007. Novel adeno-associated virus serotypes efficiently transduce murine photoreceptors. *J. Virol.* 81(20):11372–80

Apaolaza PS, del Pozo-Rodríguez A, Solinís MA, Rodríguez JM, Friedrich U, et al. 2016. Structural recovery of the retina in a retinoschisin-deficient mouse after gene replacement therapy by solid lipid nanoparticles. *Biomaterials* 90:40–49

Auricchio A, Behling KC, Maguire AM, O'Conner EE, Bennett J, et al. 2002. Inhibition of retinal neovascularization by intraocular viral-mediated delivery of anti-angiogenic agents. *Mol. Ther.* 6(4):490–94

Auricchio A, Kobinger G, Anand V, Hildinger M, O'Conner E, et al. 2001. Exchange of surface proteins impacts on viral vector cellular specificity and transduction characteristics: the retina as a model. *Hum. Mol. Genet.* 10(26):3075–81

Battaglia L, Serpe L, Foglietta F, Muntoni E, Gallarate M, et al. 2016. Application of lipid nanoparticles to ocular drug delivery application of lipid nanoparticles to ocular drug delivery. *Expert Opin. Drug Deliv.* 13(12):1743–57

Belmonte JCI, Callaway EM, Churchland P, Caddick SJ, Feng G, et al. 2015. Brains, genes, and primates. *Neuron* 86(3):617–31

Boye SE, Alexander JJ, Boye SL, Witherspoon CD, Sandefer KJ, et al. 2012. The human rhodopsin kinase promoter in an AAV5 vector confers rod- and cone-specific expression in the primate retina. *Hum. Gene Ther.* 23(10):1101–15

Boye SE, Alexander JJ, Witherspoon CD, Boye SL, Peterson JJ, et al. 2016. Highly efficient delivery of adeno-associated viral vectors to the primate retina. *Hum. Gene Ther.* 27(8):580–97

Boye SE, Boye SL, Pang J, Ryals R, Everhart D, et al. 2010. Functional and behavioral restoration of vision by gene therapy in the guanylate cyclase-1 (GC1) knockout mouse. *PLOS ONE* 5(6):e11306

Busskamp V, Duebel J, Balya D, Fradot M, Viney TJ, et al. 2010. Genetic reactivation of cone photoreceptors restores visual responses in retinitis pigmentosa. *Science* 329(5990):413–17

Busskamp V, Roska B. 2011. Optogenetic approaches to restoring visual function in retinitis pigmentosa. *Curr. Opin. Neurobiol.* 21(6):942–46

Byrne LC, Dalkara D, Luna G, Fisher SK, Clérin E, et al. 2015a. Viral-mediated RdCVF and RdCVFL expression protects cone and rod photoreceptors in retinal degeneration. *J. Clin. Investig.* 125(1):105–16

Byrne LC, Lin YJ, Lee T, Schaffer D V, Flannery JG. 2015b. The expression pattern of systemically injected AAV9 in the developing mouse retina is determined by age. *Mol. Ther.* 23(2):290–96

Callaway EM. 2008. Transneuronal circuit tracing with neurotropic viruses. *Curr. Opin. Neurobiol.* 18(6):617–23

Caporale N, Kolstad KD, Lee T, Tochitsky I, Dalkara D, et al. 2011. LiGluR restores visual responses in rodent models of inherited blindness. *Mol. Ther.* 19(7):1212–19

Castle MJ, Turunen HT, Vandenberghe LH, Wolfe JH. 2016. Controlling AAV tropism in the nervous system with natural and engineered capsids. In *Gene Therapy for Neurological Disorders*, ed. FP Manfredsson, pp. 133–49. Meth. Mol. Biol. 1382. New York: Springer

Cearley CN, Wolfe JH. 2006. Transduction characteristics of adeno-associated virus vectors expressing cap serotypes 7, 8, 9, and Rh10 in the mouse brain. *Mol. Ther.* 13(3):528–37

Cehajic-Kapetanovic J, Le Goff MM, Allen A, Lucas RJ, Bishop PN. 2011. Glycosidic enzymes enhance retinal transduction following intravitreal delivery of AAV2. *Mol. Vis.* 17:1771–83

Colella P, Trapani I, Cesi G, Sommella A, Manfredi A, et al. 2014. Efficient gene delivery to the cone-enriched pig retina by dual AAV vectors. *Gene Ther.* 21(4):450–56

Comander J, Carvalho LS, Xiao R, Langsdorf A, Wassmer S, , et al. 2016. Characterization of the limitations to gene transfer and associated inflammation following intravitreal AAV injection in nonhuman primates. *ARVO* 57:771

Conlon TJ, Deng W-T, Erger K, Cossette T, Pang J-j, et al. 2013. Preclinical potency and safety studies of an AAV2-mediated gene therapy vector for the treatment of *MERTK* associated retinitis pigmentosa. *Hum. Gene Ther. Clin. Dev.* 24(1):23–28

Cronin T, Vandenberghe LH, Hantz P, Juttner J, Reimann A, et al. 2014. Efficient transduction and optogenetic stimulation of retinal bipolar cells by a synthetic adeno-associated virus capsid and promoter. *EMBO Mol. Med.* 6(9):1175–90

Dalkara D, Byrne LC, Klimczak RR, Visel M, Yin L, et al. 2013. In vivo-directed evolution of a new adeno-associated virus for therapeutic outer retinal gene delivery from the vitreous. *Sci. Transl. Med.* 5(189):189ra76

Dalkara D, Byrne LC, Lee T, Hoffmann NV, Schaffer DV, Flannery JG. 2012. Enhanced gene delivery to the neonatal retina through systemic administration of tyrosine-mutated AAV9. *Gene Ther.* 19:176–81

Dalkara D, Kolstad KD, Caporale N, Visel M, Klimczak RR, et al. 2009. Inner limiting membrane barriers to AAV-mediated retinal transduction from the vitreous. *Mol. Ther.* 17(12):2096–102

de Leeuw CN, Dyka FM, Boye SL, Laprise S, Zhou M, et al. 2014. Targeted CNS delivery using human MiniPromoters and demonstrated compatibility with adeno-associated viral vectors. *Mol. Ther. Methods Clin. Dev.* 1:5

Doroudchi MM, Greenberg KP, Liu J, Silka KA, Boyden ES, et al. 2011. Virally delivered channelrhodopsin-2 safely and effectively restores visual function in multiple mouse models of blindness. *Mol. Ther.* 19(7):1220–29

Dorrell MI, Aguilar E, Jacobson R, Yanes O, Gariano R, et al. 2009. Antioxidant or neurotrophic factor treatment preserves function in a mouse model of neovascularization-associated oxidative stress. *J. Clin. Investig.* 119(3):611–23

Dudus L, Anand V, Acland GM, Chen S-J, Wilson JM, et al. 1999. Persistent transgene product in retina, optic nerve and brain after intraocular injection of rAAV. *Vis. Res.* 39(15):2545–53

Dum RP, Strick PL. 2013. Transneuronal tracing with neurotropic viruses reveals network macroarchitecture *Curr. Opin. Neurobiol.* 23(2):245–49

Dyka FM, Boye SL, Ryals RC, Chiodo VA, Boye SE, Hauswirth WW. 2014. Cone specific promoter for use in gene therapy of retinal degenerative diseases. In *Retinal Degenerative Diseases*, ed. J Ash, C Grimm, J Hollyfield, R Anderson, M LaVail, C Bowes Rickman, pp. 695–701. Adv. Exp. Med. Biol. Vol. 801. New York: Springer

El-Shamayleh Y, Ni AM, Horwitz GD. 2016. Strategies for targeting primate neural circuits with viral vectors. *J. Neurophysiol.* 116:122–34

Farjo R, Skaggs J, Quiambao AB, Cooper MJ, Naash MI. 2006. Efficient non-viral ocular gene transfer with compacted DNA nanoparticles. *PLOS ONE* 1(1):e38

Fink TL, Klepcyk PJ, Oette SM, Gedeon CR, Hyatt SL, et al. 2006. Plasmid size up to 20 kbp does not limit effective in vivo lung gene transfer using compacted DNA nanoparticles. *Gene Ther.* 13(13):1048–51

Flannery JG, Zolotukhin S, Vaquero MI, LaVail MM, Muzyczka N, et al. 1997. Efficient photoreceptor-targeted gene expression in vivo by recombinant adeno-associated virus. *PNAS* 94(13):6916–21

Gaub BM, Berry MH, Holt AE, Isacoff EY, Flannery JG. 2015. Optogenetic vision restoration using rhodopsin for enhanced sensitivity. *Mol. Ther.* 23(10):1562–71

Glushakova LG, Timmers AM, Pang J, Teusner JT, Hauswirth WW. 2006. Human blue-opsin promoter preferentially targets reporter gene expression to rat S-cone photoreceptors. *Investig. Opthalmol. Vis. Sci.* 47(8):3505

Granstedt AE, Szpara ML, Kuhn B, Wang SSH, Enquist LW. 2009. Fluorescence-based monitoring of in vivo neural activity using a circuit-tracing pseudorabies virus. *PLOS ONE* 4(9):e6923

Guy J, Qi X, Wang H, Hauswirth WW. 1999. Adenoviral gene therapy with catalase suppresses experimental optic neuritis. *Arch. Ophthalmol.* 117(11):1533–39

Guziewicz KE, Zangeri B, Komáromy A, Iwabe S, Chiodo VA, Boye SL. 2013. Recombinant AAV-mediated *BEST1* transfer to the retinal pigment epithelium: analysis of serotype-dependent retinal effects. *PLOS ONE* 8(10):e75666

Han Z, Conley SM, Makkia RS, Cooper MJ, Naash MI. 2012a. DNA nanoparticle-mediated *ABCA4* delivery rescues Stargardt dystrophy in mice. *J. Clin. Investig.* 122(9):3221–26

Han Z, Conley SM, Makkia R, Guo J, Cooper MJ, Naash MI. 2012b. Comparative analysis of DNA nanoparticles and AAVs for ocular gene delivery. *PLOS ONE* 7(12):e52189

Heintz N. 2001. BAC to the future: the use of bac transgenic mice for neuroscience research. *Nat. Rev. Neurosci.* 2(12):861–70

Hellström M, Ruitenberg MJ, Pollett MA, Ehlert EME, Twisk J, et al. 2009. Cellular tropism and transduction properties of seven adeno-associated viral vector serotypes in adult retina after intravitreal injection. *Gene Ther.* 16:521–32

Huberman AD, Manu M, Koch SM, Susman MW, Lutz AB, et al. 2008. Architecture and activity-mediated refinement of axonal projections from a mosaic of genetically identified retinal ganglion cells. *Neuron* 59(3):425–38

Huberman AD, Wei W, Elstrott J, Stafford BK, Feller MB, Barres BA. 2009. Genetic identification of an On-Off direction- selective retinal ganglion cell subtype reveals a layer-specific subcortical map of posterior motion. *Neuron* 62(3):327–34

Inatani M, Tanihara H. 2002. Proteoglycans in retina. *Prog. Retin. Eye Res.* 21(5):429–47

Ivanova E, Hwang G-S, Pan Z-H, Troilo D. 2010. Evaluation of AAV-mediated expression of Chop2-GFP in the marmoset retina. *Investig. Ophthalmol. Vis. Sci.* 51(10):5288–96

Kay CN, Ryals RC, Aslanidi GV, Min SH, Ruan Q, et al. 2013. Targeting photoreceptors via intravitreal delivery using novel, capsid-mutated AAV vectors. *PLOS ONE* 8(4):e62097

Kay MA, Glorioso JC, Naldini L. 2001. Viral vectors for gene therapy: the art of turning infectious agents into vehicles of therapeutics. *Nat. Med.* 7:33–40

Khani SC, Pawlyk BS, Bulgakov OV, Kasperek E, Young JE, et al. 2007. AAV-mediated expression targeting of rod and cone photoreceptors with a human rhodopsin kinase promoter. *Investig. Opthalmol. Vis. Sci.* 48(9):3954

Klimczak RR, Koerber JT, Dalkara D, Flannery JG, Schaffer DV. 2009. A novel adeno-associated viral variant for efficient and selective intravitreal transduction of rat Müller cells. *PLOS ONE* 4(10):e7467

Koerber JT, Klimczak R, Jang J-H, Dalkara D, Flannery JG, Schaffer DV. 2009. Molecular evolution of adeno-associated virus for enhanced glial gene delivery. *Mol. Ther.* 17(12):2088–95

Koilkonda RD, Guy J. 2011. Leber's hereditary optic neuropathy-gene therapy: from benchtop to bedside. *J. Ophthalmol.* 2011:179412

Koirala A, Makkia RS, Conley SM, Cooper MJ, Naash MI. 2013. S/MAR-containing DNA nanoparticles promote persistent RPE gene expression and improvement in RPE65-associated LCA. *Hum. Mol. Genet.* 22(8):1632–42

Kolstad KD, Dalkara D, Guerin K, Visel M, Hoffmann N, et al. 2010. Changes in adeno-associated virus-mediated gene delivery in retinal degeneration. *Hum. Gene Ther.* 21(5):571–78

Komáromy AM, Alexander JJ, Rowlan JS, Garcia MM, Chiodo VA, et al. 2010. Gene therapy rescues cone function in congenital achromatopsia. *Hum. Mol. Genet.* 19:2581–93

Kotterman MA, Schaffer DV. 2014. Engineering adeno-associated viruses for clinical gene therapy. *Nat. Rev. Genet.* 15(7):445–51

Kotterman MA, Yin L, Strazzeri JM, Flannery JG, Merigan WH, Schaffer DV. 2014. Antibody neutralization poses a barrier to intravitreal adeno-associated viral vector gene delivery to non-human primates. *Gene Ther.* 22(2):116–26

Kwon I, Schaffer DV. 2008. Designer gene delivery vectors: molecular engineering and evolution of adeno-associated viral vectors for enhanced gene transfer. *Pharm. Res.* 25(3):489–99

Lau D, McGee LH, Zhou S, Rendahl KG, Manning WC, et al. 2000. Retinal degeneration is slowed in transgenic rats by AAV-mediated delivery of FGF-2. *Investig. Ophthalmol. Vis. Sci.* 41(11):3622–33

Le Meur G, Stieger K, Smith AJ, Weber M, Deschamps JY, et al. 2007. Restoration of vision in RPE65-deficient Briard dogs using an AAV serotype 4 vector that specifically targets the retinal pigmented epithelium. *Gene Ther.* 14(4):292–303

Li Q, Timmers AM, Guy J, Pang J, Hauswirth WW. 2008. Cone-specific expression using a human red opsin promoter in recombinant AAV. *Vis. Res.* 48(3):332–38

Lin JY, Lin MZ, Steinbach P, Tsien RY. 2009. Characterization of engineered channelrhodopsin variants with improved properties and kinetics. *Biophys. J.* 96(5):1803–14

Lipinski DM, Thake M, MacLaren RE. 2013. Clinical applications of retinal gene therapy. *Prog. Retin. Eye Res.* 32:22–47

Liu J, Timmers AM, Lewin AS, Hauswirth. 2005. Ribozyme knockdown of the γ-subunit of rod cGMP phosphodiesterase alters the ERG and retinal morphology in wild-type mice. *Investig. Ophthalmol. Vis. Sci.* 46(10):3836–44

Lu Q, Ganjawala TH, Ivanova E, Cheng JG, Troilo D, Pan Z-H. 2016. AAV-mediated transduction and targeting of retinal bipolar cells with improved mGluR6 promoters in rodents and primates. *Gene Ther.* 23:680–89

Macé E, Caplette R, Marre O, Sengupta A, Chaffiol A, et al. 2015. Targeting channelrhodopsin-2 to ON-bipolar cells with vitreally administered AAV restores ON and OFF visual responses in blind mice. *Mol. Ther.* 23(1):7–16

MacLachlan TK, Lukason M, Collins M, Munger R, Isenberger E, et al. 2011. Preclinical safety evaluation of AAV2-sFLT01—a gene therapy for age-related macular degeneration. *Mol. Ther.* 19(2):326–34

Mancuso K, Hauswirth WW, Li Q, Connor TB, Kuchenbecker JA, et al. 2009. Gene therapy for red-green colour blindness in adult primates. *Nature* 461(7265):784–87

Manfredi A, Marrocco E, Agostina P, Giulia C, Sinnekka A, et al. 2013. Combined rod and cone transduction by adeno-associated virus 2/8. *Hum. Gene Ther.* 24(12):982–92

Matsumoto B, Blanks JC, Ryan SJ. 1984. Topographic variations in the rabbit and primate internal limiting membrane. *Investig. Ophthalmol. Vis. Sci.* 25:71–82

Mazzoni F, Safa H, Finnemann SC. 2014. Understanding photoreceptor outer segment phagocytosis: use and utility of RPE cells in culture. *Exp. Eye Res.* 126:51–60

McKinnon SJ, Lehman DM, Takzib NG, Ransom NL, Reitsamer HA, et al. 2002. Baculoviral IAP repeat-containing-4 protects optic nerve axons in a rat glaucoma model. *Mol. Ther.* 5(6):780–87

Michalakis S, Mühlfriedel R, Tanimoto N, Krishnamoorthy V, Koch S, et al. 2010. Restoration of cone vision in the CNGA$3^{-/-}$ mouse model of congenital complete lack of cone photoreceptor function. *Mol. Ther.* 18(12):2057–63

Mitra RN, Han Z, Merwin M, Al Taai M, Conley SM, Naash MI. 2014. Synthesis and characterization of glycol chitosan DNA nanoparticles for retinal gene delivery. *Chem. Med. Chem.* 9(1):189–96

Mussolino C, della Corte M, Rossi S, Viola F, Di Vicino U, et al. 2011. AAV-mediated photoreceptor transduction of the pig cone-enriched retina. *Gene Ther.* 18(7):637–45

Naik R, Mukhopadhyay A, Ganguli M. 2009. Gene delivery to the retina: focus on non-viral approaches. *Drug Discov. Today* 14(5–6):306–15

Packer AM, Roska B, Häusser M. 2013. Targeting neurons and photons for optogenetics. *Nat. Neurosci.* 16(7):805–15

Park TK, Wu Z, Kjellstrom S, Zeng Y, Bush RA, et al. 2009. Intravitreal delivery of AAV8 retinoschisin results in cell type-specific gene expression and retinal rescue in the Rs1-KO mouse. *Gene Ther.* 16(7):916–26

Pellissier LP, Quinn PM, Henrique Alves C, Vos RM, Klooster J, et al. 2015. Gene therapy into photoreceptors and Müller glial cells restores retinal structure and function in *CRB1* retinitis pigmentosa mouse models. *Hum. Mol. Genet.* 24(11):3104–18

Petrs-Silva H, Dinculescu A, Li Q, Deng W-T, Pang J-j, et al. 2011. Novel properties of tyrosine-mutant AAV2 vectors in the mouse retina. *Mol. Ther.* 19(2):293–301

Petrs-Silva H, Dinculescu A, Li Q, Min S-H, Chiodo V, et al. 2009. High-efficiency transduction of the mouse retina by tyrosine-mutant AAV serotype vectors. *Mol. Ther.* 17(3):463–71

Prentice HM, Biswal MR, Dorey K, Blanks JC. 2011. Hypoxia-regulated retinal glial cell-specific promoter for potential gene therapy in disease. *Investig. Ophthalmol. Vis. Sci.* 52(12):8562–70

Puras G, Zarate J, Díaz-Tahoces A, Avilés-Trigueros M, Fernández E, Pedraz JL. 2013. Oligochitosan polyplexes as carriers for retinal gene delivery. *Eur. J. Pharm. Sci.* 48(1–2):323–31

Ramachandran P, Lee V, Wei Z, Song JY, Casal G, et al. 2017. Evaluation of dose and safety of AAV7m8 and AAV8BP2 in the non-human primate retina. *Hum. Gene Ther.* 28(2):154–67

Sasaki E, Suemizu H, Shimada A, Hanazawa K, Oiwa R, et al. 2009. Generation of transgenic non-human primates with germline transmission. *Nature* 459:523–27

Scalabrino ML, Boye SL, Fransen KMH, Noel JM, Dyka FM, et al. 2015. Intravitreal delivery of a novel AAV vector targets ON bipolar cells and restores visual function in a mouse model of complete congenital stationary night blindness. *Hum. Mol. Genet.* 9342(352):1–32

Shen SQ, Myers CA, Hughes AEO, Byrne LC, Flannery JG, Corbo JC. 2016. Massively parallel *cis*-regulatory analysis in the mammalian central nervous system. *Genome Res.* 26:238–55

Stieger K, Lhériteau E, Lhéariteau E, Moullier P, Rolling F. 2009. AAV-mediated gene therapy for retinal disorders in large animal models. *ILAR J.* 50(2):206–24

Sutanto EN, Zhang D, Lai YKY, Shen W-Y, Rakoczy EP. 2005. Development and evaluation of the specificity of a cathepsin D proximal promoter in the eye. *Curr. Eye Res.* 30(1):53–61

Tan MH, Smith AJ, Pawlyk B, Xu X, Liu X, et al. 2009. Gene therapy for retinitis pigmentosa and Leber congenital amaurosis caused by defects in *AIPL1*: effective rescue of mouse models of partial and complete Aipl1 deficiency using AAV2/2 and AAV2/8 vectors. *Hum. Mol. Genet.* 18(12):2099–114

Tang JCY, Rudolph S, Dhande OS, Abraira VE, Choi S, et al. 2015. Cell type–specific manipulation with GFP-dependent Cre recombinase. *Nat. Neurosci.* 18(9):1334–41

Tang JCY, Szikra T, Kozorovitskiy Y, Teixiera M, Sabatini BL, et al. 2013. A nanobody-based system using fluorescent proteins as scaffolds for cell-specific gene manipulation. *Cell* 154(4):928–39

Tervo DGR, Hwang B-Y, Viswanathan S, Gaj T, Lavzin M, et al. 2016. A designer AAV variant permits efficient retrograde access to projection neurons. *Neuron* 92(2):372–82

Trapani I, Colella P, Sommella A, Iodice C, Cesi G, et al. 2014a. Effective delivery of large genes to the retina by dual AAV vectors. *EMBO Mol. Med.* 6(2):194–211

Trapani I, Puppo A, Auricchio A. 2014b. Vector platforms for gene therapy of inherited retinopathies. *Prog. Retin. Eye Res.* 43:108–28

Tshilenge K-T, Baptiste A, Weber M, Mendes-Madeira A, Nedellec S, et al. 2016. Vitrectomy before intravitreal injection of AAV2/2 vector promotes efficient transduction of retinal ganglion cells in dogs and nonhuman primates. *Hum. Gene Ther. Methods* 27(3):122–34

Vandenberghe LH, Bell P, Maguire AM, Cearley CN, Xiao R, et al. 2011. Dosage thresholds for AAV2 and AAV8 photoreceptor gene therapy in monkey. *Sci. Transl. Med.* 3(88):88ra54

Vandenberghe LH, Bell P, Maguire AM, Xiao R, Hopkins TB, et al. 2013. AAV9 targets cone photoreceptors in the nonhuman primate retina. *PLOS ONE* 8(1):e53463

Wang X. 2009. Cre transgenic mouse lines. In *Transgenesis Techniques: Principles and Protocols*, ed. EJ Cartwright, pp. 265–73. Meth. Mol. Biol. 561. New York: Springer. 3rd ed.

Willett K, Bennett J. 2013. Immunology of AAV-mediated gene transfer in the eye. *Front. Immunol.* 4:261

Wojaczynski GJ, Engel EA, Steren KE, Enquist LW, Card JP. 2015. The neuroinvasive profiles of H129 (herpes simplex virus type 1) recombinants with putative anterograde-only transneuronal spread properties HHS public access. *Brain Struct. Funct.* 220(3):1395–420

Ye G-J, Budzynski E, Sonnentag P, Nork TM, Sheibani N, et al. 2016. Cone-specific promoters for gene therapy of achromatopsia and other retinal diseases. *Hum. Gene Ther.* 27(1):72–82

Yin L, Greenberg K, Hunter JJ, Dalkara D, Kolstad KD, et al. 2011. Intravitreal injection of AAV2 transduces macaque inner retina. *Investig. Ophthalmol. Vis. Sci.* 52(5):2775–83

Yonehara K, Farrow K, Ghanem A, Hillier D, Balint K, et al. 2013. The first stage of cardinal direction selectivity is localized to the dendrites of retinal ganglion cells. *Neuron* 79(6):1078–85

Zingg B, Chou X-l, Zhang Z-g, Mesik L, Liang F, et al. 2017. AAV-mediated anterograde transsynaptic tagging: mapping corticocollicular input-defined neural pathways for defense behaviors. *Neuron* 93(1):33–47

Zou J, Luo L, Shen Z, Chiodo VA, Ambati BK, et al. 2011. Whirlin replacement restores the formation of the USH2 protein complex in whirlin knockout photoreceptors. *Investig. Opthalmol. Vis. Sci.* 52(5):2343–51