REVIEW ARTICLE

Channelrhodopsins provide a breakthrough insight into strategies for curing blindness

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Abstract

Photoreceptor cells are the only retinal neurons that can absorb photons. Their degeneration due to some diseases or injuries leads to blindness. Retinal prostheses electrically stimulating surviving retinal cells and evoking a pseudo light sensation have been investigated over the past decade for restoring vision. Currently, a gene therapy approach is under development. Channelrhodopsin-2 derived from the green alga *Chlamydomonas reinhardtii*, is a microbial-type rhodopsin. Its specific characteristic is that it functions as a light-driven cation-selective channel. It has been reported that the channelrhodopsin-2 transforms inner light-insensitive retinal neurons to light-sensitive neurons. Herein, we introduce new strategies for restoring vision by using channelrhodopsins and discuss the properties of adeno-associated virus vectors widely used in gene therapy.

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Phototransduction pathway

Retinitis pigmentosa (RP) is the most common type of inherited disease that leads to blindness; it has an expected prevalence of one in 4000 cases and its symptoms include night blindness, loss of peripheral visual field, and loss of central vision (Hartong *et al*. 2006). A number of genes responsible for RP – most of them associated with phototransduction pathways – have been identified and their functions elucidated (http://www.sph.uth.tmc.edu/Retnet/home.htm).

In the vertebrate retina, phototransduction is initiated by activation of rhodopsin. In the dark, rhodopsin contains 11-*cis*-retinal as the chromophore, embedded in its protein moiety. Light-induced isomerization of 11-*cis*-retinal to all-*trans*-retinal initiates a G protein-coupled signalling cascade, involving the hydrolysis of cyclic guanosine monophosphate (cGMP) by upregulated phosphodiesterase, which causes closure of a cGMP-regulated cation channel (figure 1). The photoreceptor cells are hyperpolarized, and they transmit light signals to second-order neurons such as bipolar cells. After the isomerization of 11-*cis*-retinal to all-*trans*-retinal by absorption of photon(s), the latter travels from retinal photoreceptor outer segments to the retinal pigment epithelium (RPE) for regeneration of the chromophore. Thus, visual cycle requires sequential propagation of steps, including enzymatic reactions (Lamb and Pugh 2004); any loss of function causes various retinal disorders.

Gene therapy for retinal disorders

Gene therapies have attempted to compensate the loss of function for the treatment of retinal disorders, mainly hereditary diseases. Various types of viral vectors such as retrovirus (Sakamoto *et al*. 1995), lentivirus (Miyoshi *et al*. 1997; Lotery *et al*. 2002), adenovirus (Reichel *et al*. 2001), and adeno-associated virus (AAV) (Ali *et al*. 1996) have been investigated for an efficient transfer of the relevant gene into target cells, and they have been selected specific to each disorder or purpose, e.g. for transient or long-term gene expression according to the target cell type. Viral vector-based gene transfer may cause undesirable side effects such as systemic dissemination of the vector, immune responses, and overexpression of the gene. To deal with these complications, the eyes specifically have the blood-retinal barrier and

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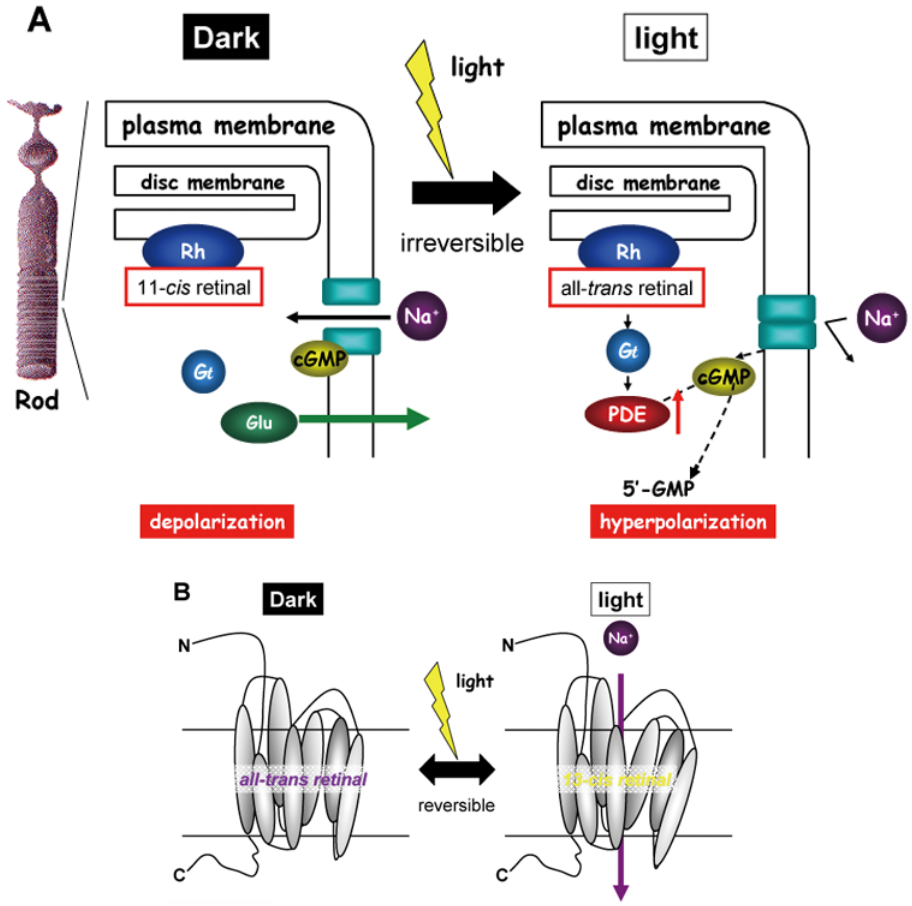

Figure 1. Schematic representation of light-induced phototransduction pathways in (A) rhodopsin, and (B) channelrhodopsin-2. (A) Light induces isomerization of 11-*cis*-retinal to all-*trans*-retinal, which initiates a G protein-coupled signalling cascade. (1) Photons induce isomerization; (2) activated rhodopsin (Rh) activates the guanosine triphosphate (GTP)-binding protein transducin (Gt); (3) activated Gt further activates cyclic guanosine monophosphate (cGMP) phosphodiesterase (PDE); (4) PDE hydrolyses cGMP, coupled to the sodium channel, to 5'-GMP; (5) cGMP hydrolysis leads to closure of the sodium channel; (6) photoreceptor cells are hyperpolarized by the closure of the sodium channel. The isomerization of 11-*cis*-retinal is irreversible, and many enzymatic reactions are needed for the regeneration of 11-*cis*-retinal. (B) Light directly induces conformational change in channelrhodopsin-2 after isomerization, which is a reversible reaction.

the specific immune surveillance system. The blood-retinal barrier is expected to play a role in preventing systemic dissemination of the viral vector, and the eye-specific immune surveillance system may minimize the antibody reaction to the capsid proteins of the viral vector (antigens). Owing to these characteristics of the eye, gene therapy may be better indicated for retinal disorders than systemic disorders.

AAV, which is a small and nonenveloped virus belonging to the parvovirus family, has been well investigated as a vector in studies conducted on gene therapy for retinal disorders (Ali *et al*. 1996; Flannery *et al*. 1997; Jomary *et al*. 1997; Lewin *et al*. 1998). Further, it is used in clinical trials (Bainbridge *et al*. 2008; Hauswirth *et al*. 2008; Maguire *et al*. 2008). Various serotypes of AAV have been identified and modified for use as vectors in gene therapy, each vector having individual characteristics. There are many types of cells in the retina. Each AAV serotype shows different transduction efficiency as a vector, and the time it takes for transgene expression depends on the types of cells in the retina or the species under investigation, such as human, monkeys, or mice. On intravitreal injection of AAV2/2 vector, gene expression is observed mainly in retinal ganglion cells (Ali *et al*. 1998; Guy *et al*. 1998; Martin *et al*. 2002; Qi *et al*. 2007). On subretinal injection of AAV2/2 vector, gene expression is observed in the photoreceptor cells and RPE (Ali *et al*. 1996; Sarra *et al*. 2002); the same is the case with subretinal injection of AAV2/1 vector. However, the time taken for gene expression differs. AAV2/2 and AAV2/1 vectors take 6–8 weeks (Sarra *et al*. 2002) and 3–4 days (Auricchio 2003), respectively, for transgene expression. For each AAV serotype,

the site of and time taken for gene expression are shown in table 1.

Gene therapy for protection of photoreceptor cells

Leber congenital amaurosis (LCA) is a rare retinal dystrophy with a prevalence of one in 30000 (Koenekoop 2004) to one in 81000 (Stone 2007) cases. It is characterized by severe visual loss in the early stages of life, which progresses to blindness. The *RPE65* gene encodes an isomerase enzyme in the RPE, which catalyzes a critical step in the visual cycle, permitting the photoreceptor visual pigments to absorb photons and maintain sight; mutations in this gene have been identified to be responsible for LCA. Recently, two clinical trials of AAV-mediated gene therapy for patients with LCA have been performed (Bainbridge *et al*. 2008; Maguire *et al*. 2008), and successful results have been reported (Bainbridge and Ali 2008; Bainbridge *et al*. 2008; Cideciyan *et al*. 2008; Hauswirth *et al*. 2008; Koenekoop 2008; Maguire *et al*. 2008; Smith *et al*. 2009).

Gene therapy for restoration of vision

Degeneration of photoreceptor cells leads to blindness, even in the case of survival of other retinal neurons. Indeed, inner retinal neurons such as bipolar, horizontal and ganglion cells survive in the retina of patients with RP (Humayun *et al*. 1999; Santos *et al*. 1997); however, some synaptic remodelling occurs (Marc *et al*. 2003; Strettoi *et al*. 2003). Since late 20th century, some approaches such as use of retinal prostheses (Margalit *et al*. 2002; Javaheri *et al*. 2006) and transplantation of retinal cells (Gouras and Lopez 1989; Lopez *et al*. 1989; Sheedlo *et al*. 1991; Lavail *et al*. 1992; Lund *et al*. 1998; Seiler and Aramant 1998; Abe *et al*. 1999; Kaplan *et al*. 1999; Humayun *et al*. 2000; Aramant and Seiler 2002) or stem cells (Schraermeyer *et al*. 2001; Yang *et al*. 2002; Lund *et al*. 2003; Haruta *et al*. 2004) have been employed to restore vision by making use of surviving retinal neurons. Various types of retinal prostheses such as epiretinal (Majji *et al*. 1999; Humayun 2001), subretinal (Chow and Peachey 1998; Peyman *et al*. 1998; Chow and Peachey 1999; Zrenner *et al*. 1999; Zrenner 2002) and suprachoroidal (Sakaguchi *et al*. 2004; Nakauchi *et al*. 2007) implants have been under development worldwide and are being progressively used in clinical trials (Hayes *et al*. 2003; Humayun *et al*. 2003). The discovery of channelrhodopsin-2 (ChR2) (Nagel *et al*. 2003) has provided a new insight into the strategies for restoring vision.

Channelrhodopsin-2

ChR2 is a microbial-type rhodopsin derived from the green alga *Chlamydomonas reinhardtii* (Sineshchekov *et al*. 2002; Nagel *et al*. 2003). Bacteriorhodopsin is a classical example of microbial-type rhodopsin, and functions as a light-driven-proton pump (Subramaniam and Henderson 2000); its structure and functions have been well investigated (Grigorieff *et al*. 1996; Kimura *et al*. 1997). Microbial rhodopsins are 7-transmembrane proteins like retinal rhodopsin, containing all-*trans*-retinal, and not 11-*cis*-retinal, as the chromophore (Tsuda *et al*. 1980). ChR2 functions as a light-driven cation-selective channel (Nagel *et al*. 2003) (figure 2). The reaction occurring with ChR2 after absorption of photons (figure 2) is completely different from that occurring with vertebrate

Table 1. Transduction efficiency of each AAV serotype. All the serotypes, except AAV5/5, contained the genome of AAV2.

Serotype	Intravitreal	Subretinal	Reference
AAV2/1	N. D.	RPE in mice (3–4 days)	Auricchio *et al*. (2001).
AAV2/2	GC in mice, rats	Photoreceptor & RPE in mice (6 weeks)	Ali *et al*. (1998); Martin *et al*. (2002); Sarra *et al*. (2002); Tomita *et al*. (2007)
AAV2/3	N. D.	N. D.	Yang *et al*. (2002)
AAV2/4	N. E.	RPE in dogs and mokeys	Weber *et al*. (2003).
AAV2/5	N. D.	Photoreceptor & RPE in mice, rod in monkeys (3–4 days)	Auricchio *et al*. (2001); Lotery *et al*. (2003)
AAV5/5	N. D.	Photoreceptor & RPE in mice (3–4 days)	Auricchio *et al*. (2001)
AAV2/6	N. E.	RPE in mice	Yang *et al*. (2002a)
AAV2/7	N. D.	Photoreceptor & RPE in mice	Allocca *et al*. (2007)
AAV2/8	N. E.	Photoreceptor & RPE in mice (3–5 days)	
AAV2/9	N. E.	Photoreceptor, RPE and Müller in mice	

N.D., not detected; N.E., not examined. The time taken by each serotype for initial expression of the transgene is given in parentheses.

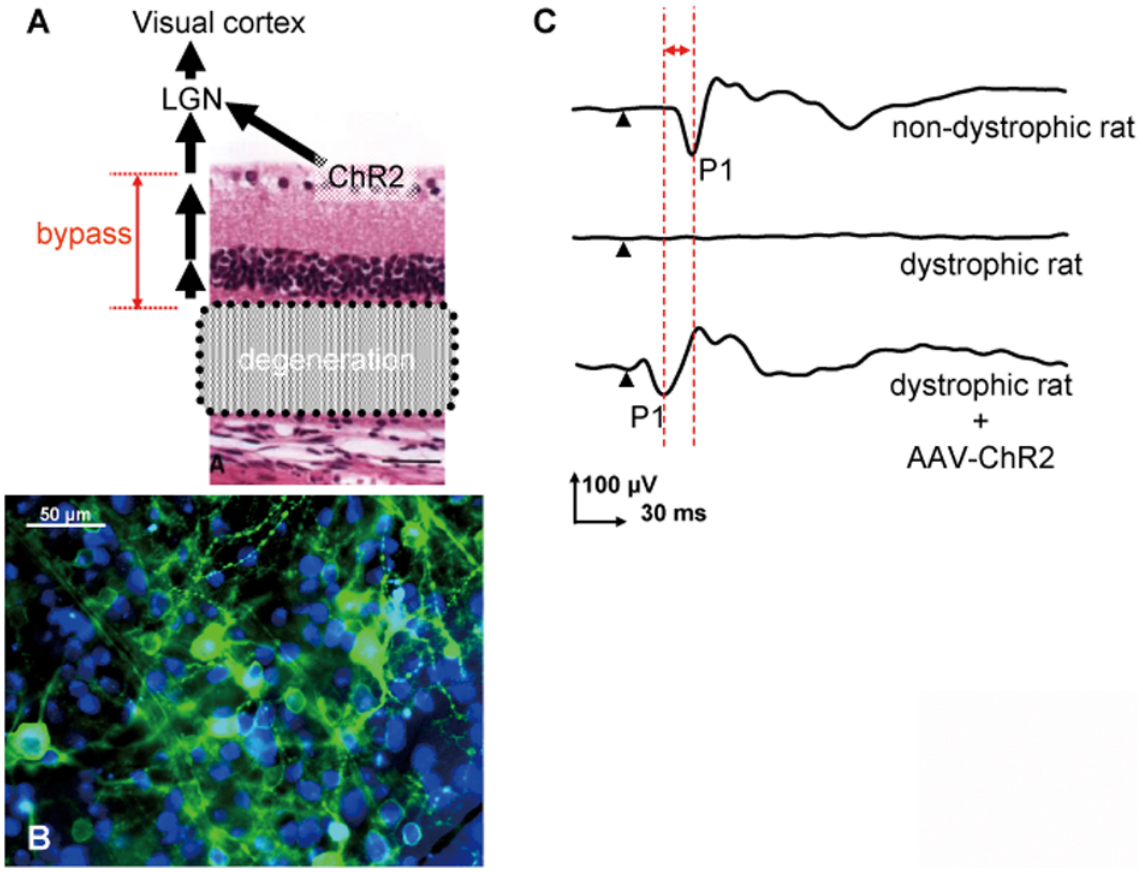

Figure 2. Channelrhodopsin (ChR2)-expressing retinal ganglion cells produce the visually evoked potential. (A) Schematic representation of the visual pathway. In general, a photon is absorbed by photoreceptors, and signals produced by the photoreceptors are transmitted to retinal ganglion cells via second-order neurons such as bipolar cells. However, retinal ganglion cells expressing ChR2 directly absorb the photon. Thus, the visual pathway mediated through the second-order neurons is bypassed by ChR2 expression in retinal ganglion cells (red arrow). (B) About 30% of the total number of retinal ganglion cells expressed ChR2 after a single intravitreal injection of AAV-*ChR2*. (C) The robust amplitude of the visually evoked potential was recorded in the dystrophic rat, which received an intravitreal injection of AAV-*ChR2*. P1 latency of the dystrophic rat is shorter than that of the non-dystrophic rat because of the direct response of retinal ganglion cells to light (red arrow).

rhodopsin (figure 1). The function of ChR2 offers a possibility that ChR2 expression may maintain light sensitivity of neuronal cells (Boyden *et al*. 2005; Ishizuka *et al*. 2006).

ChR2 gene-based strategy for restoring vision

Two *ChR2* gene-based strategies for restoring vision in blind rodents with the photoreceptor degenerative disease RP have been studied. One strategy used ON bipolar cells as the target cells for *ChR2* gene transfer (Lagali *et al*. 2008), whereas the other used retinal ganglion cells (Bi *et al*. 2006; Tomita *et al*. 2007). When the *ChR2* gene is transduced into ON bipolar cells, retinal ON pathway is selectively activated by light. This is a rational way of activating the normal retinal ON pathway, although some methodological difficulties were encountered, such as those pertaining to the mechanism of gene transfer into ON bipolar cells. Lagali *et al*. (2008) successfully expressed the *ChR2* gene in ON bipolar cells by using the mGluR6 promoter, which specifically express in these cells. However, from the perspective of clinical applications, selection of an appropriate vector for transfer of the gene into bipolar cells remains a problem.

On the other hand, it is easy to transfer the gene into retinal ganglion cells. A single intravitreal injection of AAV2/2 vector carrying the *ChR2* gene enables the transfer of the gene into retinal ganglion cells. It is expected that the *ChR2*-expressing retinal ganglion cells directly respond to light and transmit signals to the lateral geniculate nucleus (LGN) without any involvement of the bipolar cell-mediated pathways (figure 2A). We observed that about 30% of the total number of retinal ganglion cells expressed *ChR2* (figure 2B). Royal college of surgeons (RCS) rats are established models of inherited retinal degeneration, becoming blind about three months after birth. Their vision was restored by a single intravitreal injection of AAV-*ChR2*, as determined electrophysiologically (Tomita *et al*. 2007) as well as behaviorally. P1 latency of the *ChR2*-injected rat was

clearly shortened as compared to that of the normal rat (figure 2C). However, it remains unclear what degree of vision is restored by ChR2-expressing retinal ganglion cells. The results of the above mentioned studies give rise to two important questions. There are mainly three types of retinal ganglion cells: ON-ganglion, OFF-ganglion and ON-OFF ganglion cells (Levick 1967; Schiller 1992). Intravitreal injection of AAV vector randomly transfers the gene into all the three types of retinal ganglion cells. The visual signal produced by each type is expected to be different from that produced by nontransduced retinal ganglion cells in the normal visual pathway. Further, primate retinas have the fovea, which lacks ganglion cells. Therefore, only the images obtained from the *ChR2*-expressing retinal ganglion cells may be distorted. Further studies using nonhuman primates, who can undergo the morphological cognition test, are needed to elucidate the images obtained from *ChR2*-expressing retinal ganglion cells.

Prospects of channelrhodopsins

The human eye contains light-sensitive visual pigments—rhodopsin in rods for monochrome dim-light vision and three colour visual pigments in cones for daylight vision; these pigments are sensitive to wavelengths between 350 and 750 nm. The sensitivity of ChR2 is limited to wavelengths of <540 nm, with the peak at 450 nm (Nagel *et al*. 2003). Therefore, even if *ChR2*-expressing ganglion cells can provide useful vision to patients, they can only recognize wavelengths corresponding to blue colour and not green and red colours.

Recently, a few reports have indicated that properties of *ChR2* such as sensitivity to light and its different wavelengths can be improved by modifying ChR2 (Lin *et al*. 2009; Sugiyama *et al*. 2009; Tsunoda and Hegemann 2009; Wang *et al*. 2009). During the efforts to improve different properties of ChR2 by using molecular engineering techniques, a new channelrhodopsin was identified from an unknown microbial rhodopsin—the red-shifted 'ChR2', named as 'VChR1,' since it was identified from the spheroidal alga *Volvox carteri*; it has a ChR2-related sequence and shows a robust wavelength shift towards red. VChR1 can also be useful for restoring vision (Zhang *et al*. 2008).

The central nervous system consists of numerous subtypes of excitatory, inhibitory and modulatory neurons. Signal activities (activation or inhibition) in retinal neurons are bidirectionally controlled by the input information. Channelrhodopsins such as ChR2 and VChR1, transduced into retinal neurons, function as light-gated cation-selective channels and cause depolarization of the neurons by absorbing specific wavelengths of light. Halorhodopsin derived from *Natronomonas pharaonis* (NpHR) is a light-driven chloride pump (Lanyi 1990) and shows peak sensitivity to the wavelength of ~580 nm (yellow). Thus, bidirectional control of neuronal firing can be possible by the transduction of both ChR2 and NpHR in the neurons, because of the difference in the excitation wavelength of the two ion pumps (ChR2, blue; NpHR, yellow) (Evanko 2007; Han and Boyden 2007; Zhang *et al*. 2007a,b).

Channelrhodopsins have generated considerable interest with regard to restoration of vision, and investigation of New World 'channelrhodopsins' is gaining momentum. In the clinical field, expectations are rising with respect to restoration of vision. In the near future, channelrhodopsins might contribute to restoration of lost vision in patients with RP.

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