Optogenetic Approaches to Restoring Vision

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Abstract

Severe loss of photoreceptor cells in inherited or acquired retinal degenerative diseases can result in partial loss of sight or complete blindness. The optogenetic strategy for restoration of vision utilizes optogenetic tools to convert surviving inner retinal neurons into photosensitive cells; thus, light sensitivity is imparted to the retina after the death of photoreceptor cells. Proof-of-concept studies, especially those using microbial rhodopsins, have demonstrated restoration of light responses in surviving retinal neurons and visually guided behaviors in animal models. Significant progress has also been made in improving microbial rhodopsin-based optogenetic tools, developing virus-mediated gene delivery, and targeting specific retinal neurons and subcellular compartments of retinal ganglion cells. In this article, we review the current status of the field and outline further directions and challenges to the advancement of this strategy toward clinical application and improvement in the outcomes of restored vision.

INTRODUCTION

Photoreceptor Degenerative Diseases and Blindness

Vision begins in the retina when light signals that have been converted to electric signals in rod and cone photoreceptor cells are then conveyed through inner retinal neurons to higher visual centers of the brain for formation of vision. Photoreceptor cells are vulnerable to many retinal degenerative diseases. Two leading photoreceptor degenerative diseases are retinitis pigmentosa (RP) and age-related macular degeneration (AMD). RP consists of a group of inherited diseases that are due to genetic defects that damage photoreceptor cells directly or by causing dysfunction of retinal pigment epithelium (RPE) cells (Hartong et al. 2006). PR affects 1 in 4,000 people worldwide. AMD is the leading cause of visual impairment and blindness among older people, and it is the result of the progressive deterioration of the photoreceptor cells in the macula (de Jong 2006). The severe loss of photoreceptor cells in these diseases leads to partial or complete blindness. At present, no effective treatment is available to restore vision once photoreceptor cells have been lost.

Strategies for Vision Restoration

A number of treatment approaches have been introduced to reverse, stop, or slow down the course of retinal degeneration (Berson et al. 2012, Cai et al. 2000, Cideciyan 2010, Cuenca et al. 2014, Wen et al. 2012). Unfortunately, in most cases to date, the death of photoreceptors remains unavoidable. Once photoreceptor cells have been lost, the only retina-based strategies that could restore vision are the reintroduction of photoreceptor cells or the restoration of the retinal light responses. The options for implementing these strategies include transplanting normal photoreceptor cells or progenitor/stem cells (Lamba et al. 2009, Lund et al. 2001, West et al. 2009), implanting retinal devices that provide direct electrical stimulation to the surviving retinal neurons (Weiland et al. 2005, Zrenner 2002), and more recently, using optogenetics and chemical-based photoswitches. The optogenetic strategy is the focus of this article.

OPTOGENETIC STRATEGY

The optogenetic strategy for vision restoration is based on a simple concept—genetic conversion of remaining light-insensitive retinal neurons to photosensitive cells to impart retinal light sensitivity after the death of rod and cone photoreceptors. Two issues are important for the feasibility of this strategy.

Status of Photoreceptor Degenerated Retinas

Fundamental to the feasibility of optogenetics is the anatomical and functional preservation of the inner retinal neurons after the death of photoreceptor cells. This prerequisite has also been the key for other retina-based vision restoration strategies such as device implantation and photoreceptor transplantation. Early studies of postmortem tissues from humans (Milam et al. 1998, Santos et al. 1997, Stone et al. 1992) reported the anatomic preservation of second- and third-order retinal neurons long after the death of photoreceptors. Survival of the inner retinal neurons has been extensively documented in animal models (Chang et al. 2002, Olshevskaya et al. 2004), although progressive retinal remodeling caused by the death of photoreceptors has also been revealed (Fariss et al. 2000, Marc et al. 2003).

Optogenetic Tools

Critical to the feasibility of this strategy is the availability of suitable light sensors that meet two criteria: (*a*) They are genetically encoded so they can be permanently inserted into retinal neurons, and (*b*) activation by light can alter the cell's membrane potential.

Animal rhodopsins. The obvious candidates for such light sensors, or optogenetic tools, would be rhodopsins, which are the natural light sensors of the biological system. Rhodopsins are classified in two groups, animal and microbial rhodopsins (Palczewski 2006, Spudich 2006). Animal rhodopsins belong to a G protein–coupled receptor superfamily, and they use 11-*cis*-retinal as a chromophore. Photoisomerization of 11-*cis* retinal to the all-*trans* configuration activates the G protein–coupled signal cascade to control permeability of membrane channels through pathways that differ between vertebrates and invertebrates: In vertebrates, excitation of rhodopsins hyperpolarizes the membrane by closing cGMP-gated cation channels, whereas in invertebrates, it depolarizes photoreceptors by opening inositol (1,4,5)-trisphosphate- and diacylglyercol-dependent nonspecific cation channels. Also, in vertebrates the all-*trans* isomer detaches from the opsin and needs to be replaced by 11-*cis* retinal, whereas in the invertebrates, the all-*trans* isomer remains bound to opsin and reverses back to the *cis* form, thus making the invertebrate rhodopsin a more permanent pigment. Therefore, the use of animal rhodopsins as light sensors requires reconstructing their respective G protein–coupled signal cascades, and, in the case of vertebrate rhodopsins, exogenously supplying the chromophore. Early studies explored vertebrate and invertebrate rhodopsins to confer light sensitivity in neurons (Lechner et al. 2002, Li et al. 2005, Tan et al. 2006, Zemelman et al. 2002), but the need to reconstruct multiple components for the signaling cascade in neurons creates a major drawback.

Melanopsin, a homolog to vertebrate rhodopsin photopigment, expressed in a small population of intrinsically photosensitive retinal ganglion cells that regulate non-image-forming light reactions such as the circadian rhythm and the pupillary reflex (Berson et al. 2002, Hattar et al. 2002, Lucas et al. 2001), functions differently from photopigments in the rods and cones. Melanopsin harbors 11-*cis* retinal as a chromophore, but its isomerization is reversible and the downstream signaling cascade for melanopsin is more like that found in invertebrate rhodopsins (Hatori & Panda 2010) (**Figure 1*a***). Moreover, the components required for the melanopsin signal pathway were reported to exist in all retinal ganglion cells (Lin et al. 2008). Thus, the expression of melanopsin alone is able to provide photocurrent in non-light-sensitive retinal ganglion cells. However, the use of melanopsin for optogenetics also has a major drawback: its slow kinetics of activation and deactivation.

Microbial rhodopsins. Microbial rhodopsins use all-*trans* retinal as a chromophore group and operate in a much simpler way than animal rhodopsins. The conformational change caused by photoisomerization of the chromophore is directly coupled to ion movement through the membrane, either as light-driven pumps (Oesterhelt 1998) or as light-gated channels (Nagel et al. 2002, 2003) (**Figure 1*b–d***). The photoisomerization of the chromophore from the all-*trans* to the 13-*cis* retinal group is reversible, and both isomers remain stably attached to the protein. In addition, the all-*trans* retinal is efficiently supplied directly by the diet. Although the previously discovered pump-based microbial rhodopsins, bacteriorhodopsin (Oesterhelt & Stoeckenius 1973) and halorhodopsin (NpHR) (Spudich & Spudich 1982), could be used to manipulate the membrane potential (Nagel et al. 1995), it was the discovery of light-gated cation channels, channelrhodopsins (ChRs), by Nagel et al. (2002, 2003) that enabled a rapid expansion of the field of optogenetics in general (Boyden et al. 2005, Fenno et al. 2011) and the exploration

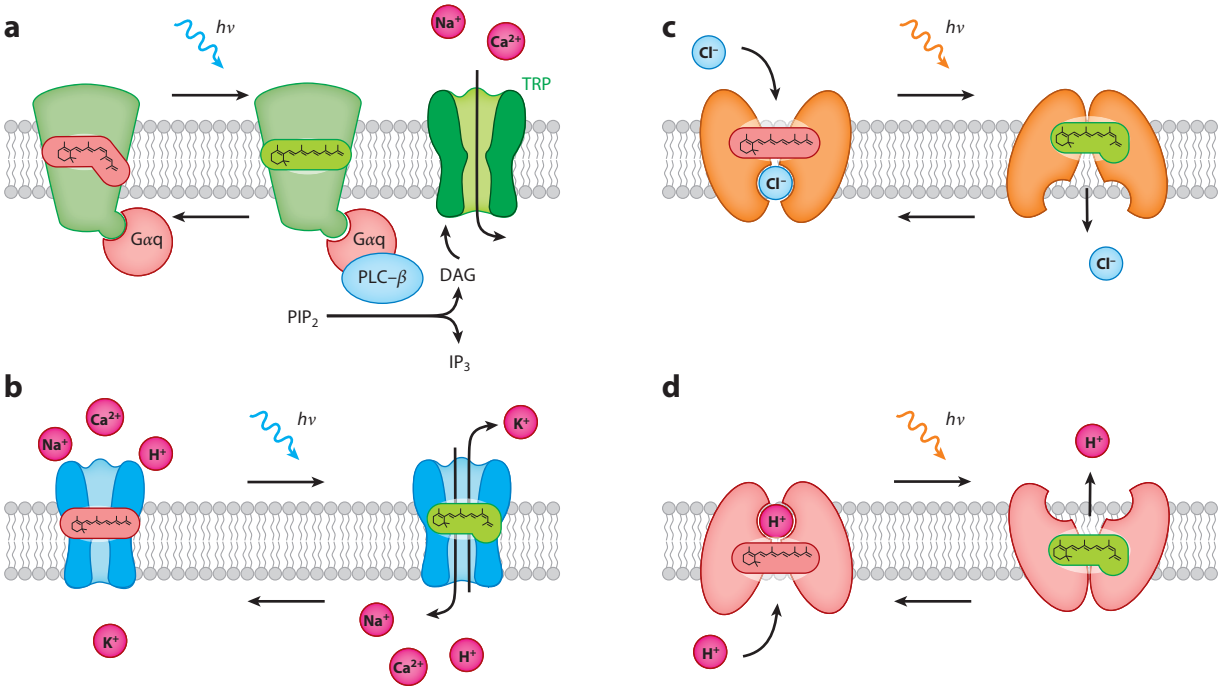

Figure 1

Optogenetic tools: (*a*) melanopsin, (*b*) channelrhodopsins, (*c*) light-driven inward chloride pumps, and (*d*) light-driven outward proton pumps. The diagram of the downstream signal cascade of melanopsin is adapted from Hatori & Panda (2010), with permission. Abbreviations: DAG, diacylglycerol; IP_3, inositol trisphosphate; PLC-β, phospholipase C–β; PIP_2, phosphatidyl inositol 4,5-bisphosphate; TRP, transient receptor potential channels.

of optogenetics for vision restoration in particular (Bi et al. 2006, Flannery & Greenberg 2006). The success of ChRs rekindled exploration of the pump-based microbial rhodopsins as hyperpolarizing optogenetic tools. Currently, microbial rhodopsins present a favored choice for vision restoration because of their simplicity, fast response kinetics, and availability as both depolarizing and hyperpolarizing tools. The main drawback of microbial rhodopsin use in vision restoration is its low light sensitivity. However, as discussed below, this limitation has been partially overcome by the recent development of more light-sensitive variants.

PROOF-OF-CONCEPT STUDIES

Ubiquitous Expression in Retinal Ganglion Cells

Soon after the reports of ChRs (Nagel et al. 2002, Nagel et al. 2003), Bi et al. (2006) launched the first comprehensive study exploring the feasibility of an optogenetic strategy for restoring vision. Using adeno-associated virus type 2 (AAV2) vectors as a delivery vehicle, a stable expression of ChR2 fused to green fluorescent protein (GFP) was achieved in the inner retina in primarily third-order neurons in rats and mice. In an *rd1/rd1* degenerative mouse model lacking photoreceptors, the same study restored robust light responses in ChR2-expressing inner retinal neurons in vitro and visual evoked potentials (VEPs) in vivo. The latter revealed that the functional connection between the retina and the higher visual centers is maintained despite an almost complete

degeneration of photoreceptor cells. Tomita et al. (2007, 2010) subsequently observed similar results in a Royal College of Surgeons (RCS) rat model of RP, and they reported that the optomotor responses were restored by ChR2 expression. Lin and colleagues (2008) found that indiscriminate expression of melanopsin in the inner retina of *rd1/rd1* mice also restored retinal light sensitivity and visually guided behaviors. The light sensitivity of melanopsin-expressing retinal ganglion cells was almost three log units more sensitive than that of native ChR2. The high light sensitivity of melanopsin likely is due to the utilization of a transduction/amplification mechanism. As a trade-off, the melanopsin-mediated light responses were very slow, and the behavioral responses of *rd1/rd1* mice were limited to general discrimination between darkness and light.

Targeting Retinal Bipolar Cells

Subsequent efforts were made to target the optogenetic tools to second-order retinal neurons. Lagali et al. (2008) performed the first targeting of bipolar cells by selectively expressing ChR2 in ON bipolar cells of electroporated *rd1/rd1* mouse retinas using an mGluR6 receptor promoter (Kim et al. 2008). They restored antagonistic surround properties among retinal ganglion cells and optomotor response behavior in treated blind mice. Light sensitivity was increased nearly tenfold when measured at the level of retinal ganglion cells.

Several groups were successful in delivering ChR2 to bipolar cells by AAV vectors. Doroudchi et al. (2011) were the first to achieve its expression in ON bipolar cells under the control of the mGluR6 promoter via a subretinal injection of an AAV2/8 vector combination that harbored a capsid mutation and a self-complementary AAV cassette; this improved viral transduction efficiency in ON bipolar cells and restored visually guided behavior in multiple blinded mouse lines. Two recent studies further improved transduction efficiency in ON bipolar cells by using a new AAV2/2-based capsid variant (Mace et al. 2015) and the combination of a capsid variant of AAV2/8 and an enhanced mGluR6 promoter (Cronin et al. 2014).

Targeting Surviving Cone Photoreceptors

Furthermore, Busskamp et al. (2010) reported their approach to resensitize cone photoreceptors; it was based on the observation that in *rd* animal models and in RP patients, cone photoreceptors could partially survive degeneration even though they were rendered non–light sensitive by the loss of the outer segment (Milam et al. 1998). AAV-delivered targeted expression of NpHR using cone photoreceptor-specific promoters in degenerative mouse models allowed them to observe restoration of retinal signal processing features such as ON, OFF, and ON/OFF light responses; lateral inhibition; and directional selectivity. The light sensitivity reportedly increased by two log units compared with the ChR2 expression in retinal ganglion cells.

Taken together, the proof-of-concept studies that use various optogenetic tools and different approaches to partially restore vision in blind animals have convincingly demonstrated the validity of this strategy.

REFINING THE OPTOGENETIC APPROACHES

Intrinsic Visual Processing Features in the Retina

Early proof-of-concept studies were limited by the availability of cell-specific promoters; the expression of depolarizing optogenetic tools, ChR2 and melanopsin, was mainly on the ganglion cells (Bi et al. 2006, Lin et al. 2008, Tomita et al. 2007), which indiscriminately converted all transduced cells to ON-type depolarization in response to light. This prompted the question of

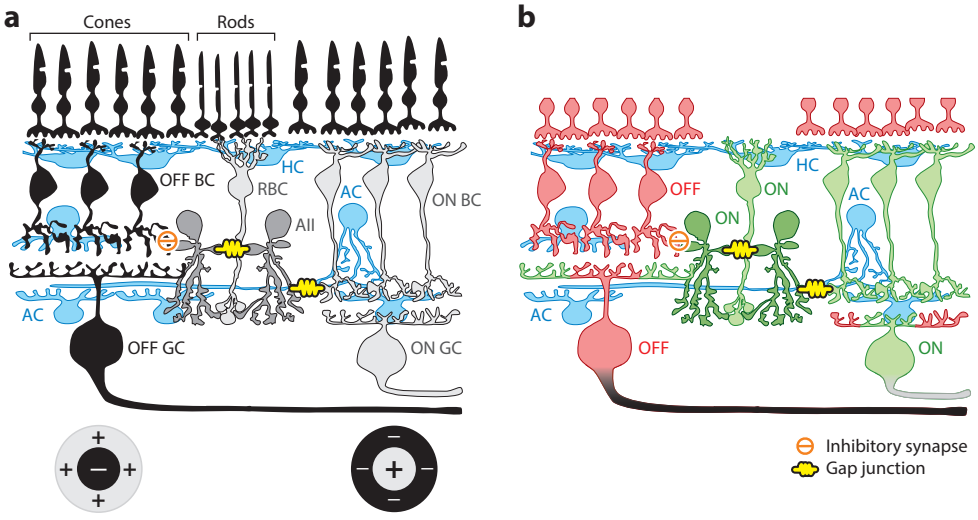

Figure 2

(*a*) Parallel visual processing pathways in the retina, including illustration of the rod/cone, of ON/OFF pathways, and of the center–surround antagonistic receptive fields of retinal ganglion cells. ON cells, including rod bipolar cells and AII amacrine cells, are shown in gray tones, and OFF cells are shown in black. Horizontal and other amacrine cells are shown in blue. The ON and OFF regions of the receptive field for ganglion cells are indicated by + and −, respectively. (*b*) Multiple possible optogenetic targeting approaches for vision restoration in the photoreceptor degenerated retina. ON cells could be targeted with depolarizing optogenetic tools (*light* and *dark green*), whereas OFF cells could be targeted with hyperpolarizing optogenetic tools (*red*). The center–surround receptive fields of retinal ganglion cells could be created by differentially targeting depolarizing (*green*) and hyperpolarizing (*red*) optogenetic tools with subcellular motifs. The motif targeting could also reduce the expression in the axons. Abbreviations: AC, amacrine cells; AII, AII amacrine cells; BC, bipolar cells; GC, ganglion cells; HC, horizontal cells; RBC, rod bipolar cells.

whether the conversion of all inner retinal neurons to ON cells could restore useful vision. Clinical trials for testing implanted devices that indiscriminately deliver a depolarizing current stimulation to the inner retinal neurons suggest that some useful vision can be restored (Humayun et al. 2012, Stingl et al. 2013), which in turn suggests that the brain may have sufficient plasticity to interpret the altered visual signals (Huxlin 2008).

However, a restoration of visual response that mimics the intrinsic visual processing features would likely result in a better outcome. It has been well established that visual signals are processed in the retina through multiple parallel pathways (Wassle 2004) (**Figure 2*a***). In particular, the bipolar cells and the ganglion cells are divided into ON and OFF types that depolarize and hyperpolarize, respectively, in response to light. The segregation of visual signals into the ON and OFF pathways facilitates high contrast sensitivity (Schiller et al. 1986). Furthermore, the receptive fields of both bipolar cells and ganglion cells are organized in a center–surround antagonistic manner, which is a fundamental feature of visual information processing in the retina (Boycott & Wassle 1999, Jacobs & Werblin 1998).

Developing Multiple Targeted Approaches

The optogenetic strategy has the capability to restore the intrinsic visual processing pathways and features in the retina (**Figure 2*b***). One approach is to target more distal retinal neurons

such as expressing hyperpolarizing optogenetic tools in photoreceptors (Busskamp et al. 2010), depolarizing optogenetic tools in ON bipolar cells (Doroudchi et al. 2011, Lagali et al. 2008), or simultaneous expression of depolarizing and hyperpolarizing optogenetic tools in ON and OFF bipolar cells, respectively. Other options are to target depolarizing optogenetic tools to rod bipolar cells or to AII amacrine cells. The targeting of either cell type takes advantage of the unique rod pathway in the mammalian retina because the signals of rod bipolar cells are conveyed to ON and OFF cone bipolar cells and then ganglion cells via AII amacrine cells (see **Figure 2*a***). The advantage of targeting these distal retinal neurons is in the utilization of the remaining retinal circuitry, which may result in partial retention of the intrinsic retinal processing pathways. This may also increase the light sensitivity and spatial resolution of the restored vision on the basis of the general signal convergence from the distal to the proximal retinal neurons.

A potential disadvantage of targeting distal retinal neurons is that during retinal degeneration, the inner retina undergoes progressive and time-dependent remodeling (Jones et al. 2012, Marc et al. 2003, Strettoi & Pignatelli 2000). Although the extent of the remodeling varies among different degenerative diseases, the distal retinal neurons appear to be the first or the most affected. In addition, there is evidence to suggest alternations in receptor expression and neuronal circuits at the level of bipolar cells, including the downregulation of mGluR6 receptor expression (Puthussery & Taylor 2010). Therefore, optogenetic treatment that targets bipolar cells or photoreceptors (the latter even more so) may be questionable in patients with advanced stages of retinal degeneration. In contrast, retinal ganglion cells were reported to be the most resistant to remodeling (Damiani et al. 2012, Mazzoni et al. 2008). In the case of advanced severe retinal degenerations, retinal ganglion cells could be the only plausible target for optogenetic therapies.

Considering the heterogeneity of retinal degenerative diseases, multiple targeting approaches are needed to treat the blindness that results from different conditions.

Reconstruction of Intrinsic Visual Processing Features in Retinal Ganglion Cells

To directly photosensitize retinal ganglion cells, the intrinsic visual processing features, such as ON, OFF, and ON/OFF light responses and center–surround receptive fields, need to be reconstructed within the retinal ganglion cells. Expression of depolarizing optogenetic tools such as ChR2 and melanopsin can create ON-type cells (Bi et al. 2006, Lin et al. 2008). Creating an OFF light response can be achieved by using hyperpolarizing optogenetic tools such as NpHR (Han & Boyden 2007, Zhang et al. 2007). Indeed, Zhang et al. (2009) showed that retinal ganglion cells that express NpHR produce robust spiking after termination of the light. However, in order to restore the intrinsic ON and OFF light responses, the depolarizing and hyperpolarizing optogenetic tools need to be targeted to the intrinsic ON and OFF ganglion cells, presumably by using the respective cell type–specific promoters (**Figure 2*b***), and this has not yet been achieved.

There is a strategy that could be used to circumvent the need for cell-specific targeting. The peak of sensitivity for NpHR is near 580 nm, whereas that for ChR2 is approximately 460 nm. On that basis, Zhang et al. (2009) coexpressed ChR2 and NpHR in retinal ganglion cells under the control of a ubiquitous promoter to generate their ON, OFF, and ON/OFF light responses using different wavelengths of the stimulus light. This coexpression strategy could provide a way not only to create ON/OFF cells, but also to control the light response polarities by light wavelength. However, the success of this strategy relies on the technical feasibility of identification and selective stimulation of different ganglion cell classes. Because the dendritic processes of retinal ganglion cells with ON, OFF, and ON/OFF light responses are generally organized in different inner plexiform layers, focusing the light stimuli of different wavelengths on different inner plexiform layers could be a solution (Reutsky-Gefen et al. 2013).

The recreation of center–surround receptive fields in retinal ganglion cells is possible by differentially expressing the depolarizing and hyperpolarizing optogenetic tools at the center versus the peripheral regions of the dendritic trees of retinal ganglion cells using subcellular targeting motifs (**Figure 2*b***). Greenberg et al. (2011) reported the creation of center–surround receptive fields in ganglion cells by expression of ChR2 and NpHR, with the center and the peripheral targeting motifs derived from ankyrin-G and postsynaptic density (PSD) protein, respectively. Furthermore, Wu et al. (2013) identified two motifs that are suitable for AAV-mediated targeting in mice. The first was derived from voltage-gated K^+ channel 2.1 (Kv2.1) and was shown to target ChR2 or NpHR to the soma and proximal dendrites of retinal ganglion cells, which renders it able to establish a center. The second was derived from neuroligin-1 and targeted the somatodendritic region of retinal ganglion cells to establish a surround. For both motifs, the expression in distal axons was significantly reduced, which is important for two reasons: First, the responses generated by retinal ganglion cell axons could interfere with the retinotopic projection in higher visual centers. Second, there is a concern of immunogenicity with the spread of microbial proteins beyond the retina to the brain. Again, restoration of the intrinsic center–surround receptive fields for retinal ganglion cells would require targeted expression in ON and OFF retinal ganglion cells. Nevertheless, before such a restoration is achieved, the use of a center-targeting motif via expression of optogenetic tools in the soma and proximal dendrites of retinal ganglion cells could convert them to midget-like ganglion cells, thus improving spatial acuity.

Encoding Retinal Ganglion Cell Spike Firing

Another optogenetic strategy for restoring vision at the level of retinal ganglion cells is to directly replicate the code of retinal ganglion cells (i.e., patterns of action potentials) for outside world images (Nirenberg & Pandarinath 2012); this requires the use of an encoder to mimic retinal visual processing by converting visual signals into the spike code. The expression of depolarizing optogenetic tools, such as ChR2 in retinal ganglion cells, serves as a transducer to precisely drive the required spike fire patterns. Although in principle this strategy is elegant, it faces technical challenges. Because there are more than 20 different retinal ganglion cell types, each of which has distinct visual coding properties, the major challenge is the ability to drive each of these ganglion cells to fire its distinct code. In addition, although ChRs with fast kinetics could be used to drive temporally precise spikes at high frequency, a drawback of using fast ChRs is their low light sensitivity. As discussed below, for all known ChRs, kinetics are inversely correlated with light sensitivity.

Table 1 summarizes representative studies using different optogenetic approaches for vision restoration in animal models and their electrophysiological recording and/or animal behavioral outcomes.

COMPARISON WITH OTHER APPROACHES

Traditional Gene Therapy

Gene therapy in vision restoration usually relies on compensation for the lack of a normal gene. This approach is applied to recessive mutations that cause loss of normal gene function in RPE and photoreceptors, such as in Leber's congenital amaurosis (LCA), which is caused by an *RPE65* gene deficiency that disables production of the 11-*cis* retinal required for rods and cones. Gene function replacement in RPE, delivered by an AAV vector, has been shown to improve vision in LCA (Cideciyan et al. 2011, Jacobson et al. 2012, Testa et al. 2013). However, the full restoration of photoreceptor function by gene delivery still presents a substantial challenge when it comes to the

specificity of the gene delivery and proper control of the targeted gene expression (Jacobson et al. 2012). In all cases, the most important prerequisite for this type of gene therapy is the presence of viable photoreceptor cells. LCA blindness, caused primarily by the loss of photoreceptor function rather than massive loss of the photoreceptors per se, should be more suitable for the delivery of the required functional gene. Recessive mutations in the human *GUCY2D* gene together with the *RPE65* gene constitute one of the most frequent causes of LCA (Stone 2007). LCA is diagnosed as an early-onset loss of vision that can be caused by severe degeneration of photoreceptors (Koenekoop & Traboulsi 1998). However, patients with mutations in *GUCY2D* typically retain near-normal retinal structure but lack proper *GUCY2* function (Jacobson et al. 2012), resembling a mouse model in which the ortholog gene is deleted (Yang et al. 1999). Because the feasibility of long-term restoration in that animal model has been successfully demonstrated using AAV-mediated delivery (Boye et al. 2013, Mihelec et al. 2011), the results of the latest clinical studies (Jacobson et al. 2013) make *GUCY2D*-linked LCA a prospective target for gene replacement therapy in human patients.

Much more conceptually challenging for gene therapy are dominant forms of blindness caused by photoreceptor dystrophies secondary to either uncontrolled (gain-of-function) enzymatic activities or accumulation of unfolded proteins. Gene therapy in such cases would require not the delivery of normal genes, but inactivation of mutated genes. Gene suppression utilizing short interference RNA (siRNA) may be a viable option for rescuing photoreceptors from death, but it faces serious obstacles. Proper selectivity between the normal and the mutated alleles is highly problematic in the case of a single-base substitution because the siRNA suppresses the expression of both the mutant and the normal alleles. This problem can be partly mitigated if the targeted function is partially redundant. For example, mutated constitutively active guanylyl cyclase activating protein (GCAP) 1 triggers rod degeneration in a mouse model mimicking photoreceptor degeneration in humans (Jiang et al. 2011, 2014; Woodruff et al. 2007). Interference RNA therapy rescues photoreceptors from death by suppressing mutated GCAP1 expression, unless the mutant protein is expressed at high levels (Jiang et al. 2011, 2014). Although siRNA suppresses expression of both the mutated and the wild-type GCAP1, mouse rods express a homologous protein, GCAP2, that can partially compensate for the absence of GCAP1 function in rods; therefore, light sensitivity is maintained in the rescued retina (Makino et al. 2012). However, the latest emerging technologies such as the CRISPR-Cas9 genome editing system (Hsu et al. 2014) provide a promising option for the treatment of retinal degenerative diseases, especially for the dominant forms of blindness.

To summarize, the main advantage of the more traditional gene therapy approach that targets rods and cones is the enabling of the retinal neuronal network, where the native synaptic connections between the first-order and second-order neurons have been already established, to route the light signal by restoring the missing first step in retinal processing. However, there is a critical limitation to this approach: It cannot be applied to patients with a massive loss of photoreceptors caused by advanced stages of retinal degeneration. This limitation does not apply to the optogenetic approach, which targets the remaining secondary and tertiary neurons of the retina.

Device Implant

The goal of using retinal prosthetic devices is to impart light sensitivity to retinas that have severe photoreceptor degeneration but have retained secondary and/or tertiary neuronal networks capable of transmitting electrical signals to the visual cortex. The use of a retina-stimulating prosthesis requires surgical implantation of the device. Electrical stimulation of the retina using electronic photosensitive implants is known to elicit spatial sensations of light known as

Table 1 A summary of representative studies using different optogenetic approaches

Cell types	Optogenetic tools	Animal model	Transduction method	Retinal recording	Light sensitivity (photons cm^{-2} s^{-1})[a]	Cortical recording	Behavioral	Major results	Reference(s)
GCs	ChR2	*rd1* mice	AAV2/2	WC, MEA	At low 10^{15}	VEP	NT	Restore ON light response in the retina and VEP in the cortex	Bi et al. 2006
	ChR2	RCS rats	AAV2/2	ERG	ND	VEP	Optomotor	Restore VEP in the cortex; observe optomotor response	Tomita et al. 2007, 2010
	Melanopsin	*rd1* mice	AAV2/2	WC	$\sim 10^{14}$[b]	VEP	Pupillary light reflex, light-dark box test, and water maze	Restore ON light response in the retina; observe pupillary light reflex and avoidance behavior	Lin et al. 2008
	NpHR or ChR2/NpHR	*rd1* mice	AAV2/2	WC, MEA	At low 10^{16}	NT	NT	Restore ON, OFF, ON/OFF responses	Zhang et al. 2009
	ChR2/eNpHR	Rabbit	Electroporation	WC, extra-cellular recording	ND	NT	NT	Create ON and OFF antagonistic center–surround receptive field with subcellular targeting motifs	Greenberg et al. 2011
	ChR2/NpHR	C57BL/6J mice	AAV2/2	MEA	ND	NT	NT	AAV-mediated targeting with subcellular motifs for creating ON and OFF antagonistic center–surround receptive field	Wu et al. 2013
	ChR2	*rd1* mice	ChR2 transgenic mice	MEA	ND	NT	Optomotor	Restore vision by driving RGCs with their spiking codes; observe optomotor response	Nirenberg & Pandarinath 2012
	ChR2 mutants[c]	*rd1* mice	AAV2/2	MEA	10^{13}	NT	NT	Improve light sensitivity	Pan et al. 2014a

ON BCs	ChR2	*rd1* mice	Electroporation	WC, MEA	10^{14}	VEP	Optomotor and locomotor activity	Target ChR2 to ON BCs with mGluR6 promoter; restore ON light response and lateral inhibition at the RGC level; observe optomotor response and light-dependent locomotor activity.	Lagali et al. 2008
	ChR2	*rd1*, *rd10*, and *rd16* mice	Sc-AAV2/8 subretinal	MEA	At low 10^{16}	NT	Water maze	AAV-mediated targeting to ON BCs; observe visually guided behavior	Doroudchi et al. 2011
	ChR2(*L132C*)	*rd1* mice	AAV2/8BP2 subretinal	MEA	At low 10^{15}	NT	NT	Improved transduction efficiency in ON BCs; observe ON, OFF, and ON/OFF light responses at the RGC level	Cronin et al. 2014
	ChR2(*H134R*)	*rd1* mice	AAV2-7m8 intravitreal	MEA	$\sim 10^{14}$	VEP	Light avoidance	Improved transduction efficiency in ON BCs; observe both ON and OFF light responses at the RGC level and light avoidance behavior	Mace et al. 2015
Cones	eNpHR	*rd1* mice; $Cnga3^{-/-}$ $Rho^{-/-}$ mice (slow rd)	AAV2/8 subretinal	WC, MEA	At low 10^{13}	VEP	Optomotor and light/dark box test	Restore ON, OFF, and ON/OFF light responses, lateral inhibition, and directional selectivity at the RGC level; restore VEP in cortex; observe optomotor responses and light avoidance behavior	Busskamp et al. 2010

[a] Light sensitivity is shown as the estimated light intensity that is required to evoke the threshold spike activity of retinal ganglion cells.
[b] Estimated based on the light intensity of 100 lux.
[c] ChR2 mutants: *L123C/T159C* and *L123C/T159S*.
Abbreviations: AAV, adeno-associated virus; MEA, multielectrode array recordings; ND, not determined; NT, not tested; ON BCs, ON-type bipolar cells; RCS, Royal College of Surgeons; RGCs, retinal ganglion cells; VEP, visual evoked potential; WC, whole-cell patch-clamp recordings.

phosphenes. Subretinal or epiretinal multiarray electrodes, designed to excite secondary and/or tertiary neurons, have been successfully implanted to stimulate the retina (Weiland et al. 2005). The phosphenes produced by this method have the potential to be deciphered by the brain as low-resolution pixilated images that enable the tested blind patients to recognize light sources and shapes of objects (Chader et al. 2009, Zrenner et al. 2011). A critical challenge remains: to achieve sufficient spatial resolution. To obtain high resolution will require adequate development of analog microelectronics, wireless transfer and data packaging, as well as stimulating electrodes that do not cause adverse effects on stimulated retinal neurons in the long term (Weiland et al. 2005).

Although retinal prosthetic devices have been approved for use in patients, they are still in an early stage of development and will require major progress in bioengineering, material science, and microelectronics before good vision resolution can be produced. In contrast to the retinal chip implants, optogenetics relies on the use of the intrinsic neural network and offers the potential for high spatial resolution but does not require surgical implantation and does not constitute a threat to the long-term viability of neurons.

Chemical-Based Photoswitches

Because the alteration of a cell's membrane potential can also be achieved by manipulating a cell's intrinsic membrane channels or receptors, chemically synthesized photosensitive molecules have been employed for manipulating neuronal activity (Kramer et al. 2009). The photosensitive molecules, termed optopharmacological photoswitches, are compounds that contain two linked components. One is a ligand that is either a channel blocker or a receptor agonist or antagonist. The second is a photoisomerizable group. The reversible change of the photoisomerizable group from all-*trans* to *cis* configuration controlled by light alters the ability of the ligand to block/unblock channels or to activate/inactivate receptors. Currently, azobenzenes have been the choice for the photoisomerizable group (Broichhagen & Trauner 2014). Optopharmacological photoswitches have been developed to regulate membrane channels (Polosukhina et al. 2012, Tochitsky et al. 2014) and ligand-gated receptors such as GABA receptors (Yue et al. 2012) and glutamate receptors (Adesnik et al. 2005, Volgraf et al. 2007). The optopharmacological photoswitches can also be covalently attached to genetically modified proteins, channels, or receptors. The latter approach is thus termed optogenetic pharmacology. As an advantage, optogenetic pharmacology allows the engineered channels or receptors to be targeted to specific neurons. Therefore, selective regulation of the light response of specific neurons is possible. Banghart et al. (2004) demonstrated the first optogenetic pharmacological photoswitches, SPARK, which is attached to an engineered K^+ channel. Since then, the same strategy has been extended to target the photoswitches to other K^+ channels (Fortin et al. 2011, Sandoz et al. 2012), ionotropic and metabotropic glutamate receptors (Levitt et al. 2013, Volgraf et al. 2007), and nicotinic acetylcholine receptors (Tochitsky et al. 2012).

The chemical-based photoswitches have been used in proof-of-concept studies for vision restoration. Azobenzene-quaternary amines such as acrylamide-azobenzene-quaternary ammonium (AAQ) can block voltage-gated K^+ channels when azobenzene is in the *trans* configuration in the dark or under 500-nm light while opening the channels in the *cis* configuration when exposed to 380-nm light. Intraocular injection of AAQ (Polosukhina et al. 2012) in *rd1/rd1* mice enables retinal ganglion cells to increase their firing rate at 380 nm and to decrease it at 500-nm light stimuli as well as restore visually guided behavior. More recently, the same group (Tochitsky et al. 2014) successfully used an improved photoswitch compound, diethylamine-azobenzene-quaternary ammonium (DENAQ), which regulates hyperpolarization-activated cyclic nucleotide-gated (HCN) channels and has a red-shifted (450–500 nm) absorption spectrum.

In a study in which a genetically modified ionotropic glutamate receptor (LiGluR) carried a mutation that enabled the receptor to covalently attach a photoisomerizable agonist, maleimide-azobenzene-glutamate (MAG), the optogenetic pharmacological approach restored vision in blind mice (Caporale et al. 2011). The expression of LiGluR in retinal ganglion cells was first achieved via AAV-mediated delivery, and the light sensitivity of the LiGluR-expressing neurons was then conferred by intraocular injections of MAG, leading to restoration of light responses in the retina and primary visual cortex as well as visually guided behaviors in *rd1/rd1* mice. More recently, targeting LiGluR to ON bipolar cells by using an improved photoisomerizable agonist, $MAG0_{460}$ (MAG0 with peak efficiency at 460 nm), has also been reported (Gaub et al. 2014).

The chemical-based approaches, optopharmacology and optogenetic pharmacology, represent good alternatives to the optogenetic approach alone. A major issue for these approaches is the short life span of the photoisomerizable molecules. Therefore, a continuous intraocular supply of the molecules is required. However, the temporal nature of the chemical-based photoswitches may present an advantage over optogenetics in the initial development of clinical applications because it can be interrupted more easily if unwanted adverse effects occur.

Table 2 summarizes the pros and cons of the vision-restoration approaches that have been explored to date.

FURTHER DEVELOPMENTS AND CHALLENGES

Development of Optogenetic Tools

Expansion of depolarizing optogenetic tools. Since the discovery of ChR1 and ChR2, the microbial-based optogenetic toolbox has been rapidly expanding through molecular engineering of the existing molecules and discovery of newer variants in nature. Today, a wide variety of ChR-based depolarizing optogenetic tools with altered kinetics, light sensitivity, and spectral selectivity are available.

ChRs with fast kinetics. ChRs possess a superior quality as optogenetic tools: their fast-response kinetics are such that spike firing by neurons can be controlled by light with high temporal precision (Boyden et al. 2005). The highest frequency driven by a ChR is limited by the off-rate (τ) of its photocurrent. Because ChR2 has an off-rate of ~10 ms, it could drive fire frequencies of only up to 40 Hz, but subsequently developed ChR variants have even faster off-rates. Two engineered variants, ChR2 (*E123A*) ($\tau = 4.5$ ms) and ChIEF ($\tau = 5.2$ ms) (Gunaydin et al. 2010, Lin et al. 2009), are able to drive the spikes at frequencies of up to 200 Hz, and an even faster variant, Chronos ($\tau = 3.6$ ms), was recently discovered in nature (Klapoetke et al. 2014). Thus, ChRs that can be used to drive precise spike firing at the high frequencies typical of retinal ganglion cells are now available. However, considering that the spike firing of retinal ganglion cells is driven by bipolar cells with graded membrane potentials, the modulation of spike frequency of retinal ganglion cells can be accomplished by using ChRs with slow kinetics as well.

ChRs with increased light sensitivity. The low light sensitivity of wild-type ChR2 was initially considered to be a major limitation of its use for vision restoration. The threshold light intensity required for activating ChR2-expressing retinal ganglion cells is at least 4–5 log units above the cone threshold (**Figure 3**). However, since the discovery of ChR2, a number of ChR2-based mutants or chimeras have been reported to have improved light sensitivity: *H134R* (Nagel et al. 2005), ChRGR (Wen et al. 2010), ChIEF (Lin et al. 2009), *T159C* (Berndt et al. 2011), *L132C* (also called CatCh) (Kleinlogel et al. 2011), *L132C/T159C*, and *L132C/T159S* (Pan et al. 2014a,

Table 2 A comparison of the pros and cons of the currently explored approaches for restoring vision

Approach	Pros	Cons
Traditional gene therapy	■ Prevents photoreceptor degeneration ■ Has the potential to restore normal vision	■ Requires the presence of viable photoreceptor cells ■ Requires the delivery of specific genes and the proper control of the targeted gene expression
Cell transplantation	■ Replaces or rescues photoreceptor degeneration ■ Has the potential to restore good vision	■ Requires the presence of viable photoreceptor cells or the ability to form synapses with downstream retinal neurons ■ Not suitable to treat advanced retinal degeneration
Device implantation	■ Suitable to treat severe retinal degeneration ■ Low associated risks	■ Requires surgical procedures ■ High cost ■ Limited spatial resolution and visual field size ■ Challenge to maintain long-term stability
Optogenetics	■ Broadly applicable to treat blindness caused by retinal degeneration ■ Simple treatment procedures and low cost ■ Has the potential to restore intrinsic visual processing features ■ Has the potential to restore high-acuity vision	■ Potential immunogenicity and toxicity associated with the expression of the exogenous gene ■ Requires long-term stable expression of the exogenous gene
Optopharmacology	■ Broadly applicable to treat blindness caused by retinal degeneration ■ Can be interrupted if unwanted adverse effects occur ■ Has the potential to restore good vision	■ Needs a continuous intraocular supply of the photoisomerizable molecules ■ Potential toxicity associated with the long-term use of photoisomerizable molecules
Optogenetic pharmacology	■ Broadly applicable to treat blindness caused by retinal degeneration ■ Can be interrupted if unwanted adverse effects occur ■ Has the potential to restore intrinsic visual processing features ■ Has the potential to restore high-acuity vision	■ Needs a continuous intraocular supply of the photoisomerizable molecules ■ Potential toxicity associated with the long-term use of photoisomerizable molecules ■ Requires long-term stable expression of the modified gene

Prigge et al. 2012). Basically, a ChR with high light sensitivity requires production of a large photocurrent at low light stimulation, which is also defined as operational light sensitivity (referred to as light sensitivity for simplicity hereafter) (Mattis et al. 2012). On the basis of this criterion, the *L132C/T159S* ChR2 mutant exhibits highest light sensitivity (Pan et al. 2014a). A side-by-side comparison of almost all the reported ChR variants revealed that the increase in the light sensitivity closely correlated with the slower kinetics or the increase in off-rate (Mattis et al. 2012, Pan et al. 2014a); this suggests that the increase in light sensitivity was mainly due to the prolonged channel opening, which decreases the temporal resolution. When expressed in retinal ganglion cells, the respective light sensitivities of the *L132C/T159C* and *L132C/T159S* ChR2 mutants were 1.5 and nearly 2 log units higher than in wild-type ChR2 (**Figure 3**), whereas the modulation of their spike activities in response to low light followed light flicker frequencies up to 20 and 10 Hz, respectively (Pan et al. 2014a). Considering that the visual system could tolerate relatively slow temporal resolution, these mutants, albeit with slower kinetics, may be better suited to restore useful vision. Because of the development of these more light-sensitive ChRs and the possibilities

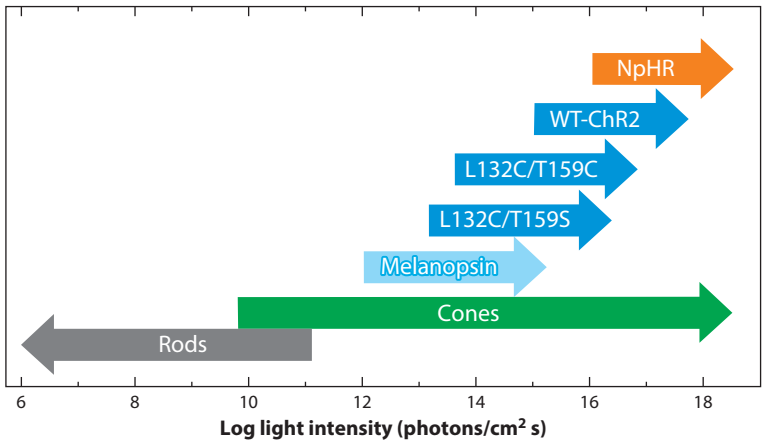

Figure 3

Comparison of the light sensitivities of photoreceptors and melanopsin-expressing retinal ganglion cells with those of halorhodopsin (NpHR), wild-type channelrhodopsin 2 (ChR2), and ChR2 mutants. The estimated light sensitivities for photoreceptors are taken from Dacey et al. (2005); those for melanopsin are from Lin et al. (2008); those for ChR2 and its mutants are from Pan et al. (2014a), and those for NpHR are from Zhang et al. (2009). The light sensitivities are based on in vitro retinal whole-mount recordings from retinal ganglion cells.

for further improvement, the low light sensitivity of ChRs may no longer be a critical hurdle for vision restoration (see **Figure 3**).

ChRs with altered spectral sensitivity. The original ChR2 is sensitive mainly to blue light, with the peak spectrum at ~460 nm (**Figure 4**). Several ChR2 mutants, such as *E123T*, and a ChR1/ChR2 chimera (ChRGR) show moderately red-shifted spectra (Gunaydin et al. 2010, Wen et al. 2010). Even more red-shifted ChRs were developed following the discovery of VChR1, a red-shifted ChR variant from *Volvox carteri*. VChR1 has a peak sensitivity of ~535 nm (Zhang et al. 2007) but exhibits poor trafficking to the membrane. Several VChR1-based chimeras were generated with improved membrane expression, including the C1V1s (Prigge et al. 2012), ReaChR (Lin et al. 2013), and mVChR1 (Tomita et al. 2014). The mVChR1 and ChRGR both have a broader action spectrum. All these VChR1 variants exhibit peak spectral sensitivity similar to that of VChR1 at relatively low light intensities. ReaChR is able to produce a larger photocurrent, likely due to its better membrane expression. A recently reported ChR, Chrimson, has its peak spectral sensitivity red-shifted even further to 590 nm (Klapoetke et al. 2014).

The availabile red-shifted and/or broader spectrum–tuned ChR variants could benefit optogenetic vision restoration in a number of ways. First, they can more efficiently catch the photons of natural daylight and thus improve light sensitivity. Indeed, the expression of red-shifted broader-tuned mVChR1 in retinal ganglion cells enhanced VEP responses and improved the optomotor behavioral outcomes (Tomita et al. 2014). Second, in extraocular image display used for stimulation, the longer wavelength should have better tissue penetration and cause less phototoxicity. Furthermore, the use of multiple opsins to mimic color signal coding in the retina may restore color vision. The human retina contains rhodopsin in rods and three different cone pigments with peak spectral sensitivities at 498, 437, 533, and 564 nm, respectively. ChRs with spectra closely mimicking those of human rods and cones are already available (see **Figure 4**). Major challenges for restoring color vision, however, remain with the retinal cell-type and circuit-specific targeting.

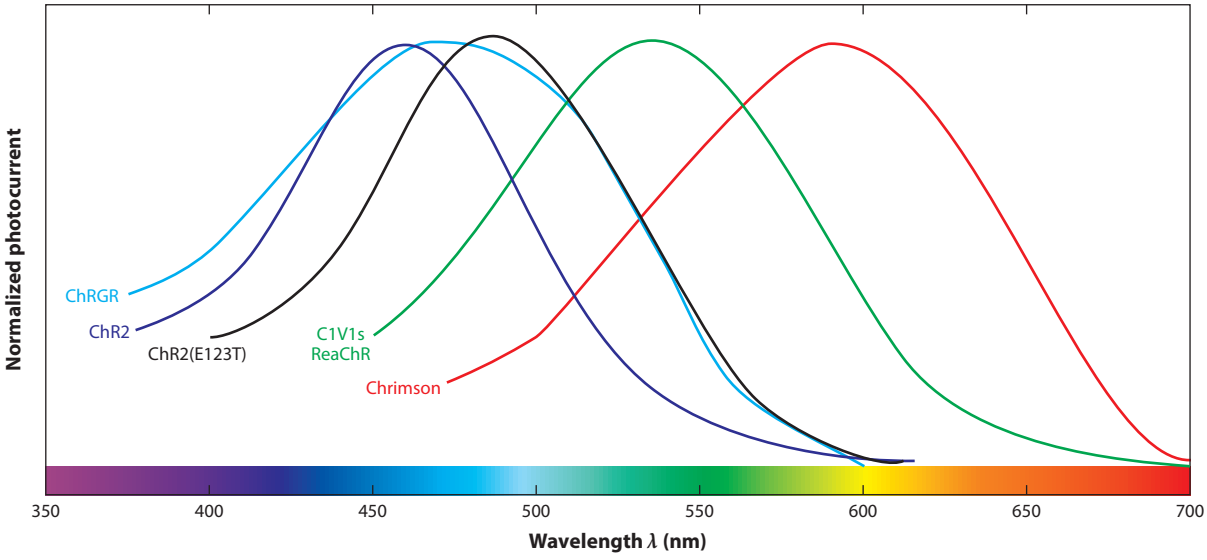

Figure 4

Currently available channelrhodopsin (ChR) variants with distinct spectral sensitivity. The action spectra of ChR2, ChRGR, ChR2(*E123T*), and C1V1s are drawn based on data from Mattis et al. (2012); that of Chrimson is drawn from Klapoetke et al. (2014). The action spectrum of ReaChR is similar to that of C1V1s at relatively low light intensities (T.H. Ganjawala, Q. Lu, Z.-H. Pan, unpublished data).

Expansion of hyperpolarizing optogenetic tools. There is also a substantial expansion of available microbial opsin-based hyperpolarizing optogenetic tools. First, new generations of NpHR (eNpHR 1.0–3.0) have been produced by adding signal motifs that improve their trafficking to the cell membrane (Gradinaru et al. 2008, Gradinaru et al. 2010). Additional outward proton pump microbial rhodopsins that exhibit blue-shifted and/or wider spectra compared with NpHR (~580 nm) have been discovered: (*a*) Mac (~550 nm) and (*b*) Arch and ArchT (~566 nm) (Chow et al. 2010, Han et al. 2011). The improved version of ArchT, eArchT3.0, has the largest photocurrent (Mattis et al. 2012). Another recently discovered inward chloride pump, cruxhalorhodopsin (Jaws), was reported to show a slightly red-shifted spectrum compared with NpHR, but it has improved photocurrents (Chuong et al. 2014). However, the magnitude of the absolute photocurrents to low light for all these pump-based microbial rhodopsins is much smaller than that of ChRs, which renders them less efficient optogenetic tools.

A potential breakthrough in the development of hyperpolarizing optogenetic tools is the recent reports of the conversion of ChRs to light-gated chloride channels. A single amino acid mutation at E90 in ChR2 or a combination of 9 amino acid substitutions in C1V1 made them into the chloride channels ChloC and iC1V1, respectively (Berndt et al. 2014, Wietek et al. 2014); however, the magnitude of photocurrents for both appeared rather small. Introducing additional mutations increased their light sensitivities, but at the expense of decelerating their off-rates by many seconds. Although promising as a concept, the current versions of the chloride ChRs require major improvements to become useful in vision restoration.

Further development required for the optogenetic tools. Rapid advances in the development of optogenetics continue to provide better tools, but further development will be required for these tools to meet the requirements specific for vision restoration. In particular, light sensitivity will

need to be further improved for depolarizing and even more so for hyperpolarizing microbial rhodopsins.

Several strategies can be employed to further improve the light sensitivity of depolarizing optogenetic tools. The light sensitivity of the previously characterized ChRs and their newly reported red-shifted variants may be further improved by molecular engineering to optimize light sensitivity versus kinetics. As discussed above, development of microbial rhodopsins with a broad-turned spectrum can be another way to boost their apparent light sensitivity. To increase light sensitivity without sacrificing the kinetics, an increase in channel conductance would be an ideal solution, although no molecular engineering studies to date have produced such an increase. However, with progress in understanding the structure and function of ChRs (Kato et al. 2012), this may be achieved in the future.

The low light sensitivity for hyperpolarizing optogenetic tools remains a major obstacle to using them in vision restoration. The light sensitivity of NpHR expressed in retinal ganglion cells remains tenfold lower when compared with ChR2 (see **Figure 3**). When targeted to cone photoreceptors in mice, NpHR-induced sensitivity can become higher. This has been determined from the recordings of retinal ganglion cells (Busskamp et al. 2010) and is likely due to the signal convergence from photoreceptors to ganglion cells. In the human fovea, however, signal convergence from cones is low. Therefore, in general, using these pump-based microbial rhodopsins for vision restoration is rather challenging. It remains unclear whether they can be substantially improved through molecular engineering. The low light sensitivity of the pump as a sensor could be a fundamental limitation because only one ion is transported per absorbed photon. In this regard, the recent successful creation of chloride-selective ChRs introduces a better possibility for optimizing their biophysical properties, including light sensitivity. The development of K^+-selective ChR variants would also be very attractive.

Finally, it remains a good strategy to search for better microbial rhodopsins in nature. A new ChR variant from the alga *Platymonas subcordiformis*, PsChR, was reported to have a higher unitary conductance compared with ChR2 (Govorunova et al. 2013). Also, a large number of microbial rhodopsin variants have been reported by de novo transcriptome sequencing of algae (Chow et al. 2010, Klapoetke et al. 2014), including several improved channel-based and pump-based variants (Chuong et al. 2014).

Development of Virus-Mediated Transduction Efficiency and Targeting

AAV vectors for efficient retinal gene delivery. The success of optogenetics also critically depends on the development of gene delivery methods. AAV vectors are considered the most promising vehicles for therapeutic gene delivery because of their nonpathogenic and nonimmunogenic properties toward the host, their efficient transduction rate, and their broad cell and tissue tropisms (Surace & Auricchio 2008). The AAV vectors have already been used in several ocular clinical trials, including the treatment of retinal degenerative diseases (Bainbridge et al. 2008, Hauswirth et al. 2008, Jacobson et al. 2012, Maguire et al. 2008). Consequently, AAV vectors, the current vehicle of choice in delivering optogenetic transgenes to retinal neurons for proof-of-concept studies in animal models, will likely remain the choice for therapeutic optogenetic applications in humans.

One of the major efforts in the development of virus-based retinal gene therapy is to improve the vector accessibility to retinal neurons and the efficiency and specificity of the transgene expression. To facilitate access to the targeted cells, a subretinal viral injection is commonly used to deliver transgenes to photoreceptor cells, whereas an intravitreal viral injection targets inner retinal neurons, especially third-order neurons. Virus delivery through the intravitreal route has

several advantages: It is less invasive, so it would cause less surgical damage to the retina (Jacobson et al. 2012); it is easier to perform; and more importantly, it could achieve a broad homogeneous expression across the entire retina. Therefore, if accessibility and efficiency are not compromised, intravitreal injection would be a preferable route for gene delivery to all retinal neurons.

As discussed above, delivery of transgenes to the bipolar cells is one of the approaches for vision restoration. However, even in rodents, the AAV-mediated transgene expression in bipolar cells via intravitreal injection is usually poor; this is apparently due to the combination of a physical barrier, virus tropism and trafficking, and the promoter strength. With regard to the physical barriers, the inner limiting membrane (ILM) evidently presents a major barrier for the diffusion of viral vectors to the distal retinal neurons (Dalkara et al. 2009). Systematic studies from the Hauswirth group (Petrs-Silva et al. 2009, 2011; Kay et al. 2013) demonstrated that for different AAV serotypes, mutations in the capsid protein could markedly improve transduction efficiency for more distal retinal neurons following intravitreal injection. New AAV variants recently developed through self-directed evolution have also shown markedly improved virus penetration through the retina (Cronin et al. 2014, Dalkara et al. 2013) and enhanced tropism toward retinal bipolar cells (Cronin et al. 2014, Mace et al. 2015).

Virus-mediated targeting to specific retinal cell types. For retinal cell-specific targeting, the viral delivery of optogenetic tools needs to be driven by cell-specific promoters. A major disadvantage of the AAV vectors is their relatively small packaging capacity (<4.8 kb), which limits the use of the large promoters and regulatory elements that can be required for retinal cell type–specific targeting.

To date, AAV-mediated targeting of optogenetic tools has been successfully achieved for several retinal cell types. NpHR was expressed in mouse photoreceptors using photoreceptor-specific gene promoters such as rod opsin, cone red opsin, and mouse cone arrestin-3 promoters (Busskamp et al. 2010). In the case of retinal bipolar cells, the AAV-mediated targeting of ChR to ON bipolar cells has been achieved by using a 200-bp mGluR6 enhancer (Doroudchi et al. 2011) combined with the newly developed capsid variants (Cronin et al. 2014, Mace et al. 2015). The expression efficacy and specificity can be further improved by enhancing and optimizing the mGluR6 promoter and by adding regulatory elements (Cronin et al. 2014, Pan et al. 2014b).

However, the cell type–specific promoters for other inner retinal neurons remain undeveloped. As discussed above, to restore the intrinsic visual processing features by directly photosensitizing retinal ganglion cells, one would need to target the depolarizing and hyperpolarizing optogenetic tools to the respective ON and OFF ganglion cells, but it remains unclear whether such purely ON- and OFF-selective promoters even exist. Promoters that are specific for certain ganglion cell type(s) are likely to exist, but identification of such cell type–specific promoters for virus-mediated delivery could be a challenging task.

Improving virus-mediated transduction efficiency and validating cell-type selectivity in nonhuman primates. For clinical application in a human retina, AAV-mediated delivery needs to be highly effective. However, although AAV-mediated robust transgene expression can be achieved in all types of retinal cells in rodents after intravitreal injection (Dalkara et al. 2009, Petrs-Silva et al. 2011), it remains very poor even in the third-order retinal neurons in nonhuman primates, despite the use of strong promoters (Ivanova et al. 2010, Yin et al. 2011). In marmosets and macaques, the transgene expression was observed mainly surrounding the fovea and in the peripheral retina. The ILM was suggested to be a major barrier for intravitreal gene delivery in primate retinas because the transduction-efficient pattern appeared to inversely follow the thickness of the ILM. The ILM will likely present a similar problem in the human retina, and

methods to improve penetration of the virus should be considered for therapy. Possible methods include an enzymatic treatment to break down the protein structures joining the vitreous to the ILM (Yin et al. 2011) and the removal of the ILM by mechanical peeling at the time of vector injection. Furthermore, AAV-mediated retinal cell-specific targeting developed in rodent models for optogenetic therapy needs to be validated in nonhuman primates.

ISSUES RELATED TO CLINICAL APPLICATIONS

Evaluating Neurotoxicity, Immunogenicity, and Long-Term Safety

Because AAV vectors have already been used in human clinical trials (Bainbridge et al. 2008, Jacobson et al. 2012, Testa et al. 2013), the key concerns for advancing optogenetic approaches toward clinical trials are the neurotoxicity, immunogenicity, and long-term stability of optogenetic transgenes in the retina. From that perspective, the use of melanopsin has an advantage because it is a native photopigment of the retina; therefore, it minimizes biocompatibility concerns.

Although ChR2 is a microbial rhodopsin, all the results to date indicate that it is compatible with mammalian retinal neurons. With a single administration with viral vectors, stable expression of ChR2 has been observed in the inner retina of both normal and *rd1/rd1* mice for close to the entire life span of the animals (Ivanova & Pan 2009). Expression of ChR2 was found to produce neither detectable toxic nor immunologically harmful reactions in vivo in RCS rat retinas for up to 64 weeks of observation (Sugano et al. 2011), and similar results were reported in a mouse *rd10* model (Doroudchi et al. 2011). The expression of ChR2 in retinal ganglion cells and bipolar cells did not compromise these neurons when the animals were exposed to bright light for up to 2 and 10 weeks, respectively (Doroudchi et al. 2011, Ivanova & Pan 2009). In nonhuman primates, the virus-mediated expression and physiological responses driven by the ChR2 remained stable when evaluated for up to 3 months (Ivanova et al. 2010). Thus, the potential for using ChR2 in clinical applications appears quite strong.

Nonetheless, use of the more effective and light-sensitive ChR2 variants in optogenetic therapy requires that similar safety and stability studies be conducted. It should be noted that other microbial rhodopsins, including NpHR, ArchT, and VChR1, encountered various complications. Most of them appeared to exhibit trafficking and expression problems, and they required the use of trafficking signal motifs to improve their expression in mammalian neurons (Mattis et al. 2012). Therefore, long-term stability and neurotoxicity for these other microbial rhodopsins will need to be carefully evaluated before they can be considered for clinical application.

Extraocular Imaging Device and Goggles

To achieve better outcomes, the optogenetic approaches need to be combined with an extraocular imaging device or goggles (for a detailed review, see Barrett et al. 2014). The extraocular device would include a camera and an image processing and/or display system. There are two main reasons for the need for such a device. First, human vision operates over 10 log units of light intensity, whereas all microbial rhodopsins operate within a dynamic range that extends only 2–3 log units. The use of the extraocular imaging device could allow the light intensities of the outside world to be converted to fit the dynamic range of the microbial rhodopsins in the treated retina and thus to expand the operation range of the restored vision. Second, the image processing system could allow compensation for the loss of visual processing for the treated retina because the approaches bypass part of the visual processing pathways. This would be especially important for the optogenetic targeting of more proximal retinal cells such as retinal ganglion cells.

CONCLUSIONS AND OUTLOOK

Restoration of vision in the blind is one of the most exciting and promising applications for optogenetics. In less than ten years since the first publication of optogenetic-based studies (Bi et al. 2006), impressive progress has been made in all major aspects required to advance this strategy toward clinical applications. First, the proof-of-concept studies for this strategy convincingly demonstrated vision restoration from in vitro physiological recordings to a variety of in vivo animal behavioral tests in various animal models of retinal degeneration. Second, significant progress has been made in refining targeted approaches, offering better outcomes in vision restoration. Third, there has been rapid expansion and improvement of optogenetic tools, such that some major hurdles, including low light sensitivity of ChRs, have been partially overcome. Fourth, substantial improvement has been made in virus-mediated gene delivery, targeting, and transduction efficiency in the retina. Last, but not least, the long-term stability and safety of expressing ChR2 has been validated in multiple animal models.

Following the progress achieved in animal studies, multiple groups are working toward clinical trials (e.g., RetroSense Therapeutics and GenSight Biologics). Although the main aim of the initial clinical trials is to determine safety, the functional efficacy of the treatment may also be revealed. In particular, considering the technical feasibility and the extensive safety data for ChR2, the ubiquitous expression of ChR2 in retinal ganglion cells will likely be among the first-generation approaches in clinical trials. In the near future, we hope to learn the functional efficacy of some of these treatment approaches. Because the treatment will also likely require an extraocular imaging system and substantial vision rehabilitation/training following treatment, the results from clinical studies will answer many questions that can't be answered with animal studies and will provide valuable guidance for further refining and developing optogenetic approaches. Clinical trials may also address fundamental questions regarding the plasticity and capacity of our visual system.

Similar to other approaches, such as device implants and stem cell therapies, optogenetic therapy is intended to be a last resort to regain some degree of sight by those patients who have completely lost vision due to the death of photoreceptors. An expectation to regain normal or close to normal sight is not realistic. However, the clinical results from device implants are encouraging. Best visual acuities of 20/1260 and 20/546 were reported with multiarrays of 60 epiretinal-based and 1,500 subretinal-based electrodes, respectively (Humayun et al. 2012, Stingl et al. 2013). Considering the fact that optogenetic approaches could confer photosensitivity on a substantially higher number and density of retinal neurons, the outcome for restored visual function may be more optimistic.

DISCLOSURE STATEMENT

Zhuo-Hua Pan and Alexander Dizhoor are inventors and patent holders for the use of microbial rhodopsins for vision restoration. Zhuo-Hua Pan also serves as a scientific advisor to RetroSense Therapeutics.

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