Looking within for Vision

Channelrhodopsin-2 (ChR2), a directly light-gated cation channel from the green alga *Chlamydomonas reinhardtii* has been shown to be a directly light-switched cation-selective ion channel, which employs 11-*cis* retinal as its chromophore. This is the same chromophore as the mammalian photoreceptor's visual pigment—rhodopsin. Previously, investigators demonstrated that ChR2 can be used to optically control neuronal firing by depolarizing the cell. In this issue of *Neuron*, Bi et al. apply viral-mediated gene transfer to deliver ChR2 to retinal ganglion cells (RGC) in a rodent model of inherited blindness. In this way, the authors have genetically engineered surviving retinal neurons to take on the lost photoreceptive function. The conversion of light-insensitive retinal interneurons into photosensitive cells introduces an entirely new direction for treatments of blinding retinal degeneration.

Inherited retinal degenerations that cause partial or total blindness affect one in 3000 people worldwide. Patients afflicted with Usher syndrome develop progressive deafness in addition to vision loss from retinal degeneration. There are currently no effective treatments or cures for these conditions.

Basic research on treatment approaches for retinal degeneration has long been separated into two groups: treatments to preserve the remaining photoreceptors in patients with retinal degenerative disease and methods to replace photoreceptors lost to retinal degeneration. If one attends a conference of patients afflicted with retinal disease (http://www.ffb.ca/vq_conference2005.php?hc=2&sub=5), attendees often group themselves into patients seeking ways to slow the loss of their diminishing vision and patients who are legally blind or NLP (no light perception), having lost their photoreceptors because of an inherited eye disease or trauma. Currently there are no therapies for inherited blinding disease that restore vision. In the last decade, the development of therapies designed to prevent blindness in patients with inherited retinal disease who retain some functional photoreceptors has come a long way. In particular, neuroprotection with neurotrophic factors (LaVail et al., 1992) and virus-vector-based delivery of wild-type genes for recessive null mutations (Acland et al., 2001) have come the furthest—to the point of a Phase I/II clinical trial (Hauswirth, 2005) (Jacobson, S., Protocol #0410-677, http://www.webconferences.com/nihoba/16_jun_2005.html) gaining approval in the US for AAV-mediated gene replacement therapy for Leber's congenital amaurosis, a form of retinal degeneration.

Up until now, there has been no evidence for the capability of retina to regenerate photoreceptors lost to apoptosis. The genetics of retinal degeneration are complex, with over 50 genes and 150 mutations identified to date (http://www.sph.uth.tmc.edu/retnet/). The great majority of these defects involve photoreceptor specific genes, and it is the rod and cone photoreceptors that are lost in most retinal degenerations. The extremely heterogeneous nature of inherited retinal diseases makes the design of gene replacement therapies challenging. Gene replacement vectors will have to be very mutation specific, further stratifying the patients in what is already an orphan disease population. Nonetheless, there has been remarkable progress in designing effective, rational treatments for animal models of both recessive and dominant retinal degenerations. In addition to gene replacement, neuroprotection by the overexpression of neurotrophins has shown efficacy in slowing the loss of photoreceptors regardless of the mutation (McGee Sanftner et al., 2001). For dominant diseases involving photoreceptor-specific genes, there are studies with siRNA or ribozymes to knockdown the mutant gene product (Lewin et al., 1998). Obviously, all of the aforementioned strategies must be applied before the photoreceptors are lost to apoptosis because blindness resulting from loss of the rods and cones cannot be ameliorated by gene augmentation or pharmaceutical means. It is estimated that there are 130 million photoreceptors in a young adult, converging though a network of interneurons onto ~1 million retinal ganglion cells (RGCs). Fortunately, the great number of photoreceptors translates into a long therapeutic window for these diseases—clinical measures suggest that patients can lose substantial numbers of photoreceptors without significant loss of vision, providing there is not catastrophic loss in the central retina (macula) (as occurs in ARMD—age-related macular degeneration). Histopathology studies in donor retina suggest that the inner retinal cells are preserved for many years after the photoreceptors are lost (Flannery et al., 1989); however, recent studies in rodents demonstrate that these interneurons are not static—there is significant retinal remodeling of the neurons after synaptic input from photoreceptors is lost (Jones et al., 2003) along with reactive gliosis of Müller cells, the primary retinal glial cell (Fisher et al., 2005).

For patients in advanced stages of retinal degeneration, it has been assumed that these therapies are not applicable, and the photoreceptor cells must be replaced. For these individuals, the therapeutic options are more limited and cell based, including transplantation of isolated photoreceptors or intact sheets of retina (Seiler et al., 2005) or methods to differentiate embryonic stem cells into photoreceptors. These therapies are still several years off because the methods to control stem cell fate determination or synaptic connection from transplanted fetal retinal sheets or isolated photoreceptors to the host retina are yet to be developed. A parallel path to therapy for the blind or partially sighted has been the creation of retinal prosthetic "chip" devices (Lakhanpal et al., 2003), which use a photosensitive video camera and electrode array to replace the photoreceptor's light-signaling function. These devices have two primary designs: a subretinal implant designed to have a photoreceptive function and directly electrically stimulate the second order retinal interneurons—the bipolar

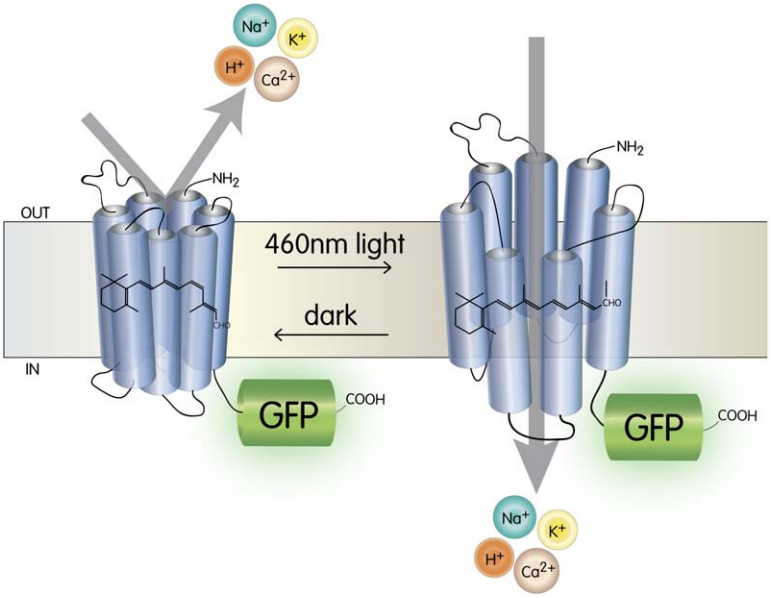

Figure 1. Photoisomerization of Retinal Gates Ionic Currents through Channelrhodopsin-2

Channelrhodopsin-2 (Chop2) is a seven transmembrane domain cation selective channel that becomes photoswitchable when bound to the chromophore 11-*cis* or all-*trans* retinal. The chromophore bound channel (designated ChR2) opens in response to 460 nm light, allowing cation flow in and depolarizing the cell. In response to darkness, the channel closes allowing the cell to once again reach its resting membrane potential. Bi et al. express a truncated Chop2 fragment (Met^{1}-Lys^{315}) fused to green fluorescent protein (GFP) exogenously in retinal ganglion cells of blind mice.

cells—or an alternative design that utilizes an electrode array to stimulate RGCs from the inner face of the retina.

In this issue, the authors (Bi et al., 2006) break new ground in the application of a genetically encoded light-sensitive channel, which they introduced into RGCs of an animal model of blinding photoreceptor degeneration. The channelopsin-2 (Chop2) protein, originally discovered in green algae, utilizes the same light-sensitive chromophore—11-*cis* retinal as the rod photoreceptor photopigment rhodopsin. As a result, its action spectrum is similar to the cells whose function it is designed to replace. Unlike rhodopsin, which only binds the 11-*cis* conformation, Chop2 binds either 11-*cis* or all-*trans* retinal isomers, obviating the need for the all-*trans* to 11-*cis* isomerization reaction supplied by the vertebrate visual cycle. When the retinal chromophore is bound to Chop2, a functional light-sensitive channel is formed, designated ChR2 (Chop2 retinalidene). The authors have capitalized upon advancements in the field by using viral vectors to transfer genes to retinal photoreceptor cells (Flannery et al., 1997). With this strategy, the investigators have made a paradigm shift in the field and opened the possibility of genetically modifying the surviving retinal interneurons to function as a replacement light-sensing receptor.

Experimentally, the authors begin by demonstrating the chosen adeno-associated viral (AAV) vector construct efficiently targets RGCs, effectively delivering the Chop2-GFP cDNA and expressing protein at high levels after intravitreal injection in both normal and diseased retinas. The authors systematically demonstrate that when endogenous retinal is bound to ChR2, it can be photoswitched (Figure 1), that neural activity can be evoked in retinas and at cortical levels. This is shown by several techniques—initially by in vitro patch-clamp recordings of individual dissociated RGCs, followed by multielectrode array recordings of whole-mount retina preparations representative of a large population of RGCs. Finally, they perform in vivo cortical recordings from live blind mice to demonstrate critical connections are functionally maintained to higher visual centers. Their progressive in vitro and in vivo data convincingly suggests ectopic expression of ChR2 could provide a future therapeutic strategy for restoring light sensitivity to a "blind" retina.

Future behavior testing will undoubtedly determine if converting RGCs into light sensors is a viable strategy for restoring functional vision. Although many believe that a highly plastic brain will have the capacity to "learn" to decode this new information presented by light-sensitive RGCs, substantial processing (Roska and Werblin, 2001), which normally occurs in the retina itself, will be bypassed by this strategy. Exciting research of this nature inevitably spawns many more unanswered questions: will the neural activity evoked at V1 be understood as "vision" on a cortical level? How will receptive fields be influenced by having ChR2 expression in the long RGC axons, and therefore potentially local depolarizations, as well as the soma?

The field is admittedly in its infancy, and there are substantial technical challenges to overcome. RGCs are coarsely divided into two ON and OFF subpopulations, based on their polarization response to light. Bi et al. have effectively created an exclusively ON retina by expression of depolarizing ChR2 in all classes of RGCs. What will the visual consequences be of an all ON retina? Future refinements in viral targeting at the cellular transduction and transcription level may aid in delivering Chop2 and other light-sensitive channels to the appropriate class of RGCs in an attempt to restore receptive field properties to the retina. In addition, there is the need to develop channels that serve the opposite function, that is hyperpolarize in response to illumination and target these channels to the OFF RGC subpopulation. There has been progress along these lines by engineering nonlight-sensitive hyperpolarizing ion channels to become light triggered (Banghart et al., 2004). Finally, the "phototransduction cascade" in photoreceptors generates a tremendous signal amplification that is clearly not present in this light-activated channel. As

a result, the RGC's are substantially less light sensitive than the intact retina. These are solvable problems. This publication is clearly a significant first step into this new field of re-engineering retinal interneurons as genetically modified "prosthetic" cells.

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Kissing and Pinching: Synaptotagmin and Calcium Do More between Bilayers

Building on recent findings that synaptotagmin (Syt) participates in synaptic vesicle endocytosis, Poskanzer et al., in this issue of *Neuron*, show distinct mechanisms by which Syt functions in this process. Most significantly, they show (1) that calcium binding to Syt determines the rate but not fidelity of vesicle recycling and (2) that mutations in a different Syt domain affect the shape but not rate of formation of recycled synaptic vesicles.

Calcium triggering of synaptic vesicle exocytosis is mediated by calcium binding to the synaptic vesicle membrane protein, synaptotagmin (Koh and Bellen, 2003). Following exocytosis, synaptic vesicles are retrieved by an endocytic mechanism. Two interesting features of the latter process are that (1) in order to maintain vesicle pools and plasma membrane morphology, the rate of synaptic vesicle endocytosis must be regulated to match the rate of exocytosis; and that (2) endocytic proteins must associate with cytoplasmic domains of synaptic vesicle proteins in the context of plasma membrane, but not in the context of synaptic vesicles. Thus, there are at least two conceptually different regulatory steps: one determines the *rate* of endocytosis and the other the *need* for endocytosis of vesicle proteins. Experiments by Poskanzer et al. (2006) indicate that synaptotagmin participates in both of these regulatory steps, by distinct molecular mechanisms.

Syt's involvement in endocytosis has long been suspected, based not only on its biochemical interactions with several endocytic proteins, but also on phenotypes of *C. elegans* mutants lacking synaptotagmin (Jorgensen et al., 1995). However, strong, direct evidence for Syt function in synaptic vesicle reformation comes from recent, parallel studies of Syt 1 mutant synapses, both at the *Drosophila* neuromuscular junction and in cultured mouse cortical neurons (Nicholson-Tomishima and Ryan, 2004; Poskanzer et al., 2003). These analyses measured kinetics of synaptic vesicle reformation using a pH-sensitive green fluorescent protein (pHluorin) targeted to the luminal domain of synaptic vesicles (Miesenbock et al., 1998). Thus, the differential fluorescence of pHluorin exposed to pH 7 after exocytosis compared to pH ~6 within synaptic vesicles reports on the fraction of vesicles in mature synaptic vesicles. Both papers used clever methods to separate and measure rates of exocytosis and endocytosis that occur simultaneously at nerve terminals. For example, Nicholson-Tomishima and Ryan (2004) used bafilomycin to block reacidification of synaptic vesicles after exocytosis and thus measure the rate of exocytosis under given experimental conditions. Poskanzer et al. (2003) used conditional temperature shifts of *shi*^{ts} mutants or temporally controlled photoinactivation of FlAsH-tagged synaptotagmin to isolate and analyze the role for Syt I in synaptic vesicle endocytosis. Strikingly, both *Drosophila* and mouse studies concluded that Syt I was necessary for normal endocytosis of synaptic vesicles.

Here, exploiting previous structure-function analyses of Syt (Chapman, 2002), Poskanzer et al. analyze how Syt variants, with defects in specific molecular interactions, function in synaptic vesicle recycling. First, they make the very interesting observation that mutations in the Ca^{2+}-coordinating aspartate residues of the C_2B domain (Syt-D3,4N), but not C_2A, inhibit synaptic vesicle recycling. This observation implicates for the first time the calcium-binding function of Syt in vesicle recycling. Direct evidence that the effect of these C_2B mutations is through altered calcium binding is provided by an additional experiment. Increased extracellular calcium substantially enhances recycling rates of synapses