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When green algae give light to blind mice

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The genetic heterogeneity associated with retinal degeneration arises from the large number of genes that have been implicated and the variety of mutations which affect them. This diversity has driven researchers to develop alternatives to gene replacement strategies in order to treat a spectrum of retinal diseases. Complementary strategies also need to be devised to adapt to the different stages of the disease, from early treatments to late-stage therapies. Thus in parallel to gene replacement techniques, advances in neurotrophic factor support and transplantation of photoreceptors into the retina have thus far shown promise. The development of a retinal prosthesis has progressed strongly, allowing retinas which are entirely devoid of photoreceptors to recover some basic visual input to a brain which has already learned to treat that information. In this context, considerable emphasis shoule be placed upon the work of Lagali *et al* and their predecessors who have adapted the function of surviving bipolar and retinal ganglion cells to serve as photoreceptors. The transfer of photosensitive protein complexes to these cells offers a new vista for the restoration of visual function as it evokes brain stimulation and the improvement of visual perception (optomotor response). Although much additional work is required to increase the sensitivity of this genetically adapted retina and to mimic its normal excitatory mode of operation, these early breakthroughs promise exciting potential human applications.

Retinal degeneration affects millions of patients throughout the world for whom almost no therapy exists. The origin is often genetic and results in two main categories of diseases: the hereditary retinal dystrophies (or *retinitis pigmentosa*) and aged-related macular degeneration. During the last years and months important breakthroughs in the ophthalmology field revealed that the treatment of certain forms of retinal degeneration can be really efficient to stop the disease or even restore visual function. Indeed, three recent studies show that gene replacement in young patients, affected by a recessive disease disrupting the *RPE65* gene function in the pigmented epithelium, can restore some retinal and visual functions (Bainbridge *et al.*, 2008; Maguire *et al.*, 2008) and (Jacobson *et al.*, *in press*). Such results are the proof of principle that gene replacement strategy in recessive disease could also be successful for other types of gene

mutations. However, for the condition retinitis pigmentosa, around one hundred different mutated genes may be responsible for this disease. Until now, 45 different genes have been identified as being at the origin of blindness when a mutation alters the function of the encoded protein. Fifty other loci might explain other forms of retinal dystrophies (Hartong *et al*., 2006). In total, 60% of the known diseases may be related to these genetic differences. The complexity increases upon knowing that a same gene, depending on the mutation that it bears, can provoke either a recessive or a dominant form of the disease. These facts reveal the complexity of developing a specific therapy for each disease and different strategies are being developed to prolong vision in affected patients.

Different strategies to maintain and restore vision

As an alternative to gene replacement, a neuroprotective approach has been proposed to enhance the survival of the affected photoreceptors. Neurotrophic factors and chemical compounds with anti-oxidative properties are the main candidates to have been tested until now. Two neurotrophic factors have become of prospective interest and either are, or have actually been tested in clinical trials: the ciliary neurotrophic factor (CNTF; Sieving *et al*., 2006; www.neurotechusa.com/news_press) and the pigmented epithelial derived factor (PEDF; Nguyen *et al*., 2006). To deliver CNTF, human cells were bioengineered to express CNTF cDNA, and those clones that were able to secrete reliably CNTF and to survive *in vivo* within dog eyes, were encapsulated in a semi-permeable membrane to prevent immune rejection and to allow the free diffusion of the factor and nutrients. Such a device, implanted under the sclera in the dorsal part of the eye in patients suffering from various forms of retinitis pigmentosa actually delayed the loss of visual acuity in certain patients, leading to the launch of a Phase II/III clinical trial (www.neurotechusa.com/news_press). PEDF, which has anti-angiogenic as well as neurotrophic actions, was tested in a clinical trial using a non-replicative recombinant adenovirus injected under the retina to transfer the PEDF coding sequence into the retinal pigmented epithelium. In patients suffering from neovascular AMD, PEDF induced the regression of the neo-vessels in certain patients and also prevented the loss of visual acuity performances during the first year after the treatment (Campochiaro *et al*., 2006). These experiments reveal that neurotrophic factors show promise in slowing down retinal degeneration, but not so far actually preventing it.

Another clinical strategy seeks to replace lost photoreceptors by generating photoreceptors *in vitro*. The proof of principle of restoring retinal function through cell transplantation was brought by MacLaren *et al*. (2006) who showed after transplantation, in mice at least, that newly generated post-mitotic photoreceptors (isolated from postnatal day 3 to 7) have a great ability to integrate morphologically and functionally within a healthy or a diseased retina. The grafted cells make adequate connections and enhance retinal sensitivity, as well as the pupil light reflex. In parallel to this study, several groups attempted to generate adequate cell numbers through using different types of stem cells, including those derived from the retina, embryo, bone marrow and iris (for review see Pellegrini *et al*., 2007). Some successes were gained in generating cells that became ‘committed’ to a photoreceptor fate (Merhi-Soussi *et al*., 2006; Osakada *et al*., 2008; Haruta *et al*., 2001). However, no efficient cell integration into the retina was reported with such stem cells (see Pelligrini *et al*. 2007). Moreover, no successful primary cell transplantation has been reported to date for any retina that has lost all its photoreceptors.

Can a retina devoid of photoreceptors be reactivated to restore visual functions?

Depending on the nature of the condition, retinal degeneration may be too fast to be stopped with actual experimental tools, leading to the total loss of photoreceptors. However, both the interneurons and retinal ganglion cells, the latter forming the optic nerve and being the final common pathway for retinal signals, may survive quite some time after the loss of photoreceptors. Even if a rapid remodelling of the circuitry occurs between the remaining neurons (Jones *et al*., 2003; Strettoi *et al*., 2002), it is hypothesized that the enduring retinal ganglion cells may maintain their capacity to transfer coherent information into the brain if they receive the appropriate stimuli. Several groups and biotechnology companies have developed a retina prosthesis that is composed of chips containing multiple micro-electrodes. The device is directly apposed to the retina at the level of the ganglion cell layer, and is stimulated by the electrodes thanks to a camera and an amplifier situated outside the eye (Weiland *et al*., 2004). Over the past decade, it has been shown that acute stimulation of a retina from a blind patient can elicit the sensation and perception of light dots termed ‘phosphenes’, and in some better cases, the reading of large letters or the observation of a large object in a well contrasted environment is possible. Perception of motion has also been reported (Weiland *et al*., 2004). The main problems encountered are: 1) putting sufficient electrodes onto the retina to give an adequate resolution of an image; 2) transferring energy to the electrodes; 3) generating a device that remains attached to the retina; and 4) developing an intelligent device that can be modulated to generate the best picture depending on the structure of the remaining retina. Numerous progressions have been made in this field, but these early devices still require further improvements to enable chronic implantation. Nonetheless, such results unequivocally provide evidence that the retina of a blind person who was previously able to see, may be reactivated to provide some useful information to the brain.

In this context, the paper of Lagali *et al*. (2008) brings a breakthrough by restoring vision in a retina completely devoid of photoreceptors. In 2006, Bi *et al*. (2006) investigated the possibility of rendering the remaining retinal ganglion cells (RGCs) active under light stimulations. The goal was to find a molecule which was able to detect photons and to induce the direct depolarization of RGCs. Within the photoreceptor, such a mechanism necessitates a cascade of numerous proteins from rhodopsin, which traps light energy through its pigment 11-cis-retinal, to the sodium and calcium channels which close to hyperpolarize the cell. These graded changes in membrane potential lead to neurotransmitter release to activate ON bipolar cells, which in turn stimulate RGCs. Bi *et al*. propose to use an evolutionary ancestor of rhodopsin, ChR2, which is found in green algae such as *Chlamydomonas*. ChR2 is a light-gated cation channel which confers two principle properties upon a single protein, namely photosensitivity and a cationic conductance through which to depolarize the cell. Using adeno-associated viral vectors, Bi *et al*., genetically engineered the RGCs, horizontal and amacrine cells of *Rd1/Rd1* blind mice, which characteristically lose all of their rod photoreceptors at around the age of 21 days. Some bipolar cells were also infected. The genetically engineered cells respond to light stimuli, and recordings taken from the cortical regions suggest signal transmission from the retina to the brain. These experiments were remarkable by the fact that they demonstrate that a neuron may be converted into a photosensitive cell that stimulates a neuronal network. However, the RGC population

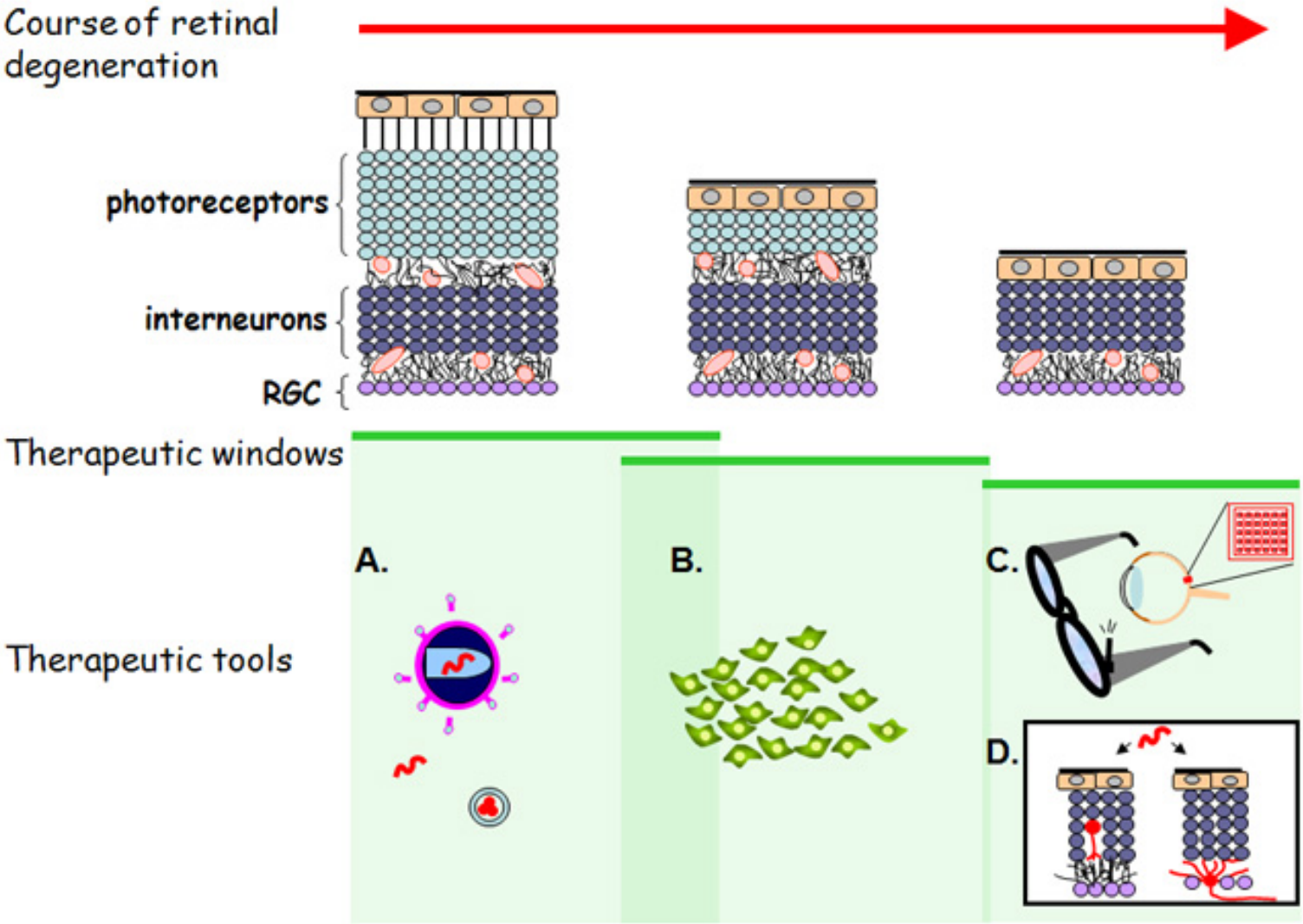
usually receives both excitatory and inhibitory inputs that are necessary to pre-construct an image which is then sent to the brain for further processing and construction. The approach of Bi *et al*., does not allow the cortex or RGCs to discriminate these fine inputs which convey important information concerning such properties as contrast, movement and wavelength. To mimic some of the classical photoreceptor outputs onto the inner retina, Lagali *et al*. (2008) selectively targeted the expression of ChR2 within ON bipolar cells, which normally receive direct photoreceptor input when activated. To achieve this cell specific expression, the Grm6 promoter, which is only active in ON bipolar cells, was included in the transgene construction comprising the ChR2 sequence fused with enhanced yellow fluorescent protein cDNA. The resulting plasmid construct was electroporated into the retina of newborn Rd1 mice (post-natal day 0-1). In total 7% ± 3% of all ON bipolar cells expressed this construct. Their identity could readily be demonstrated by examining the terminals of the ON bipolar cells which only innervate certain synapse boundaries. Using bright light stimuli, they observed different types of RGC spiking activity which correlated with the stimulation period. It is however known that synaptic remodelling occurs during and after photoreceptor loss (Jones *et al*., 2003; Strettoi *et al*., 2002). Interestingly, by using different synaptic blockers, the investigators demonstrated that synaptic connections between ON bipolar cells and RGCs, or ON bipolar cells and amacrine cells, appear to be normal, even 67 days after the treatment. This suggests that the maintenance of ON bipolar cell activity may help to prevent synaptic retraction. It is not known if such a reactivation of bipolar cells at a later stage (and older age) of retinal degeneration may allow the restoration of normal circuitry, although such an approach may be of great potential interest for blind patients of a certain period. Nonetheless, it is not clear whether the treated mice conserve a normal circuitry between the different classes of interneurons and the RGCs, and further investigations are needed to clarify this point. Nevertheless, these experiments show that such genetically engineered ON bipolar cells may react to light to elicit different RGC responses. Moreover, electrophysiological recordings obtained from the visual cortex reveals that the RGCs successfully transmit these light signals to higher brain centres. Even if the activated regions shows some differences from wild-type animals, the responsivity of the treated Rd1 mice is certainly significantly greater than those seen in the absence of cortical stimulation in Rd1 control mice. This cortical responsivity in mice that received the ChR2 transgene suggests that these animals may even have had a visual perception. Indeed, these mice demonstrated light-induced changes in motor behaviour and responded to visual stimuli when exposed to a rotating drum containing black and white strips (an ‘optomotor response’). It is remarkable that such visual restoration is effective when only some 7% of ON bipolar cells are successfully transduced to express a detectable level of gene expression. These experiments have paved the way to restore ‘picture perception’ by the retina in absence of photoreceptors. Such technology appears especially pertinent for patients detected at late stages of retinal degeneration, or for those where the gene or gene function related to their condition is unknown. Nonetheless, several refinements in the technology have to be attained before such applications may be gainfully translated to the treatment of blind persons.

Perspectives

The sensitivity of the bipolar cells containing ChR2 is very low in comparison to that of rods or cone photoreceptors. The engineered cells are in fact 100,000 times less sensitive to light than are

cones. The threshold of perception corresponds to bright light, and only highly contrasted objects may be perceived. It is difficult to imagine that such sensitivity could confer independent mobility upon patients. As a consequence, an increase in the sensitivity of the system has to be developed. In parallel, the remaining retinal circuitry has to be 'requisitioned' to improve visual acuity and contrast. The ideal scenario would be to generate both ON and OFF bipolar cell inputs by appropriately engineering these cells which directly target different functional populations of RGCs. Synthetic 'optical neuromodulators' are already being tested within different neuronal systems and appear promising for the retina.

Such neuromodulators are derived from existing ion channels which have been genetically engineered to accept a photosensitive molecule that can be fixed to the external region of the receptor. Following light stimulation, reversible photoisomerization modulates the conformation of the engineered channels, resulting in changes in their open probability or conductance. This approach has led to the creation of photosensitive channels which may depolarize or hyperpolarize neurons within neuronal circuits which are not normally light sensitive. For example, a glutamate receptor-channel (LiGluR) was engineered to covalently bind a maleimide-azobenzene-glutamate agonist that activates the receptor under 380 nm stimulation, and inhibits it in response to 500 nm light (Gorostiza *et al*., 2007; Szobota *et al*., 2007). This synthetic receptor, when used to genetically engineer zebra fish sensory neurons, allowed the activity of these neurons to be controlled by light stimuli (Szobota *et al*., 2007). For example, the escape response to tactile stimuli could be inhibited via the photoactivation of this receptor. Such experiments demonstrate that neuronal activity may be artificially controlled by unnatural stimuli thanks to the creation of genetically engineered ion channels. This strategy opens new vistas for regaining control of the activity of neurons which have lost their normal sensory or modulatory inputs. The work of Lagali *et al*. (2008) clearly shows that ON bipolar cells which have become disconnected from their normal photoreceptor input may still behave appropriately when excited. Neuron reactivation was already known to some degree through electrode stimulations of brain or retinal tissue through varying stages of neurodegenerative diseases (*e.g.* Parkinson's disease, retinal degeneration). However, the molecular engineering of specific neurons augurs a level of fine neuromodulation which would have been inconceivable at the end of the last Millennium. Such a strategy would enable a precise, unlimited and rapid remote control of any neuronal population which would not have been possible to achieve with diffusible modulators (Gorostiza *et al*., 2007; Kocer *et al*., 2005). Much work has still to be done to fine tune the sensitivity of these synthetic receptors and to target other cell types, but the recent data generated from the genetically engineered retinas of blind mice clearly provide the proof of principle needed to promise the restoration of function within a diseased nervous tissue. It remains a widespread hope that such nascent technologies may be applied to humans in order to circumvent the functional deficits associated with neurodegenerative diseases and nerve injury.



Scheme illustrating the various approaches currently in development to treat retinal degeneration. During the time course of retinal degeneration (red arrow), the photoreceptor layer thins and eventually disappears. This progression defines therapeutic windows (green areas) for which different strategies are explored in order to adjust the treatment to the stage of degeneration. Early therapeutic interventions might comprise gene replacement or neurotrophic support represented by a viral vector, naked DNA and nanoparticles (**A**). With the disappearance of the photoreceptors, transplantation of new cells becomes crucial to replace this missing population (**B**). At a later stage of degeneration, the last approaches which remain to help a retina devoid of phototreceptors to transmit some visual inputs to the brain are implanting a retinal prosthesis (**C**) or gene transfer into secondary neurons (*e.g.* work of Lagali *et al.*) or RGCs to render them photosensitive (**D**).

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