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Retina Repair, Stem Cells and Beyond

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FINAL

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Abstract: In this review, we will explore several studies where stem cells from neural, non-neural and even embryonic cells have been used as potential sources to repair the damage retina. In addition, we will also discuss the possibility of inducing retina regeneration by transdifferentiation of cells present in existing eye tissues, such as, the Retinal Pigmented Epithelium (RPE), the Pigmented Ciliary Margin (PCM) and Müller glia cells.

Key Words: Ciliary marginal zone, Circumferential germinal zone, Müller glia cells, Pigmented Ciliary Margin, Retinal Pigmented Epithelium, Transdifferentiation.

INTRODUCTION

The retina is a complex neural structure and when functioning properly detects, absorbs, and processes visual signals from the environment. However, in humans, there are many retinal degenerative diseases, such as, retinitis pigmentosa, macular degeneration and cone-rod dystrophy, which inevitably lead to blindness impairing the every day activities many of us take for granted. Replacement of the damaged or lost retina becomes the only hope for restoring vision to these patients. Generation of new retina tissue would be the ultimate cure. Studies described in this review including the use of stem cells from ocular and non-ocular tissues to repopulate the diseased retina suggest this may become a possibility in the near future.

Retina Regeneration by Transdifferentiation

RPE (Amphibians, Embryonic Chicks and Mammals)

Many embryonic and larval vertebrates have the ability to regenerate their retinas upon damage through the process of transdifferentiation. While this capacity is lost in most animals during maturation, certain urodele amphibians retain this potential [Lopashov and Stroeveva, 1964; Mitashov, 1996, 1997; Raymond and Hitchcock, 2000; Fischer and Reh, 2001a; Del Rio-Tsonis and Tsonis, 2003]. The process of transdifferentiation involves a cell conversion, where a “terminally differentiated” cell loses its characteristics of origin and reverts back to an undifferentiated state. It is at this stage that the cell can be re-directed to differentiate into a completely different cell. In the adult newt, the cells of the RPE Fig. (1) are the cells that lose their mature characteristics and re-enter the cell cycle to form a neuroepithelial cell layer. This new neuroepithelial layer eventually differentiates into all the different cell types of the retina recapitulating the appearance of retina during development [reviewed in Reyer 1977; Hitchcock and Raymond, 1992; Mitashov, 1996, 1997; Raymond and Hitchcock, 2000; Fischer and Reh, 2001a; Del Rio-Tsonis and Tsonis, 2003; Tsonis and Del Rio-Tsonis, 2004] Fig. (2 A-C). The RPE not only gives

rise to the neuroepithelial cell layer, but it also renews itself by forming a new pigmented monolayer of cells that lie under the new neuroepithelium. Therefore, these RPE cells have a latent capacity to become proliferating precursor cells that can differentiate into the missing cell types as well as renewing themselves.

Embryonic chicks, which can also regenerate their retinas via transdifferentiation of the RPE, lose this layer as it becomes the neuroepithelium. This neuroepithelium seems to develop similar in sequence to that of normal development, but with reverse polarity Fig. (2 D-F). As a result, the rods and cones of the photoreceptor layer are in contact with the vitreous chamber and the ganglion cells face the choroid [Coulombre and Coulombre, 1965; Park and Hollenberg, 1989, 1991, 1993].

RPE cells in mammals can transdifferentiate *in vitro* to form stratified layers of cells that express retinal cell specific markers only if taken at early stages of their development [Neill and Barnstable, 1990; Neill *et al.*, 1993; Zhao *et al.*, 1995]. It is interesting that the *in vitro* transdifferentiation potential of the RPE in rodents is limited to a small window of development, whereas the pigmented cells from the ciliary margin (PCM) retain this potential even during adulthood.

PCM (Mammals In Vitro)

PCM cells in adult rats have been shown to have proliferative potential *in vivo* [Ahmad *et al.*, 2000] and therefore, it is possible that this region in mammals could be induced to give rise to precursors that could replace damaged retinal cells. As described above, adult PCM cells of rodents have the capability to transdifferentiate *in vitro* into retinal specific cells [Ahmad *et al.*, 2000; Tropepe *et al.*, 2000]. Transdifferentiation in this context includes the formation of progenitors/stem cells via a dedifferentiation process that can self renew and/or produce progeny that could differentiate into different cell types. It has been shown that PCM cells cultured *in vitro* de-differentiate, form colonies with progenitor cells that self-renew and express cell markers expressed by retinal progenitor cells such as Chx10. These progenitor cells can give rise to a variety of retinal cells including rod photoreceptors, bipolar neurons and even Müller glia cells [Ahmad *et al.*, 2000; Tropepe *et al.*, 2000]. Populations of PCM cells have also been isolated from

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Received: 11/26/03; Revised: 02/05/04; Accepted: 02/09/04

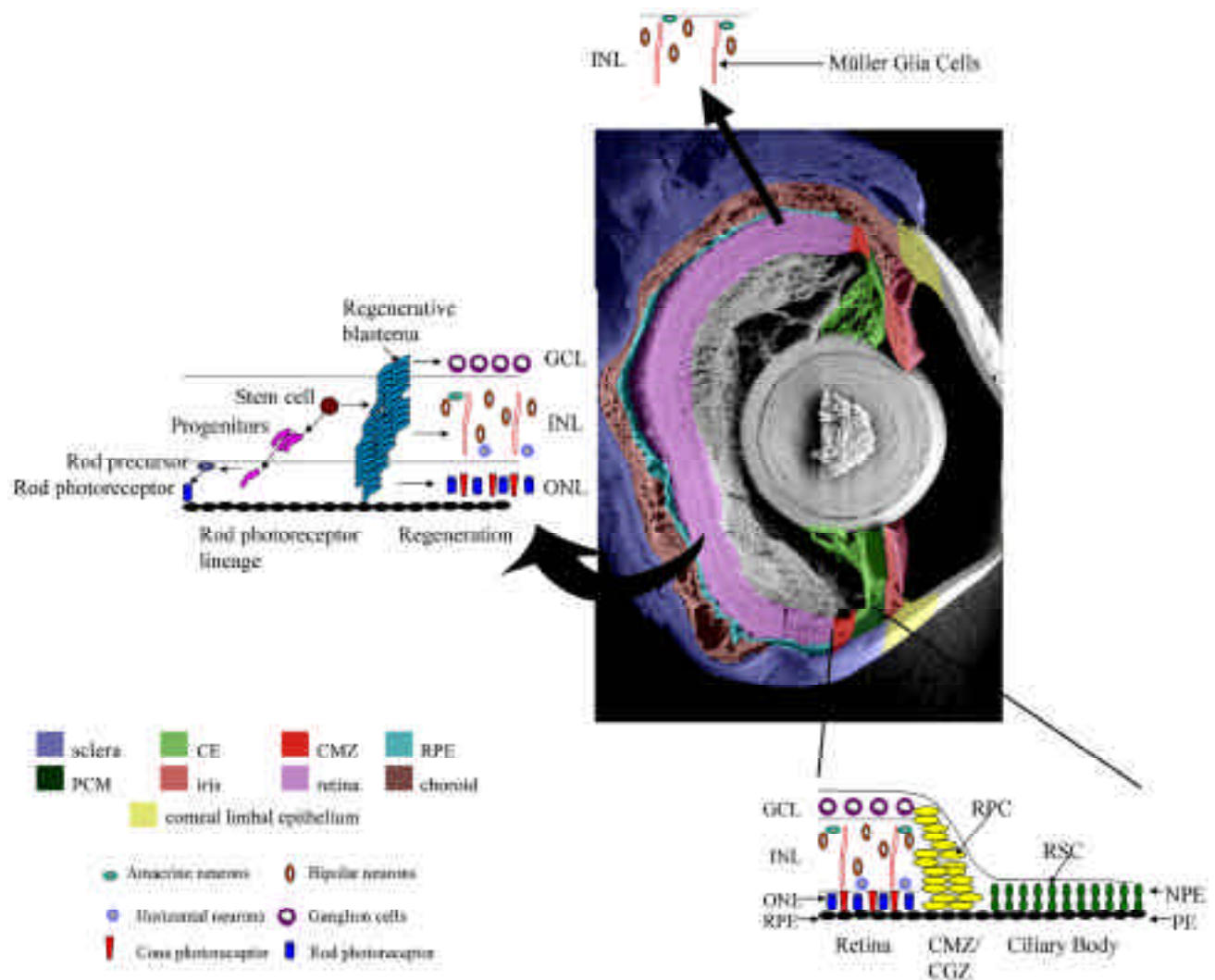


Fig. (1). Regions of the eye containing retinal progenitor or stem cells. A scanning electron micrograph of a vertebrate eye used as a model to represent the different areas (by color) of the eye that have been shown to contain or give rise to retinal progenitors or stem cells in various species. Three different areas in the eye are enlarged: **1)** the anterior region of the eye to depict the location of the CMZ/CGZ and the ciliary body. Retinal Progenitor cells (RPC) have been identified in the CMZ/CGZ of the fish, amphibian, and chick. Retinal stem cells have been identified in the non-pigmented ciliary epithelium (NPE) of the chick. Cells from the pigmented epithelium (PE) in mammals have been shown to transdifferentiate into retinal neurons *in vitro*. (ONL= outer nuclear layer, INL = inner nuclear layer, GCL= ganglion cell layer, RPE = retinal pigmented epithelium). **2)** A region of the inner nuclear layer identifying Müller Glia cells that have been shown to dedifferentiate and form retinal progenitors that give rise to neurons in the adult chick. **3)** A region of the neural retina enlarged to show the location of the intrinsic stem cells of the inner nuclear layer found in teleost fish. The intrinsic stem cells lead to the formation of rod photoreceptors during normal development and can differentiate into all types of retinal neurons in response to damage via the formation of a blastema. The progenitors that make up the blastema are Pax-6 positive cells.

human pars plana and plicata and seem to behave similarly to the rodent PCM [Tsonis and Del Rio-Tsonis, 2004; Personal communication Arsenijevic, 2003] Fig. (1, 3). When the optimal conditions to activate this population of cells *in vivo* are determined and functional assays performed to prove that the new cells are functionally and physiologically active in rodents, then the potential of regenerating lost retinal tissue from the PCM could be a reality in humans.

Müller Glia Cells (Adult Birds)

Recent studies have pinpointed Müller glia cells as potential sources of retinal neural progenitors in the adult

chick eye Fig. (1). These cells respond to local retinal damage by re-entering the cell cycle and de-differentiating into Pax-6, Chx10 and CASH 1 positive cells. Cells in this pool of progenitors eventually differentiate into neurons and glia [Fischer and Reh, 2001b; 2003a; Reh and Fischer, 2001; Fischer *et al.*, 2002b]. The cells that are replaced depend on the type of cells that were originally damaged as well as the availability of growth factors, such as FGF-2 and insulin [Fischer *et al.*, 2002b; Fischer and Reh, 2002; 2003a]. In a sense, these Müller glia cells are analogous to the cells of the RPE in that, when stimulated, are capable of giving rise to a pool of progenitors that will eventually differentiate into the

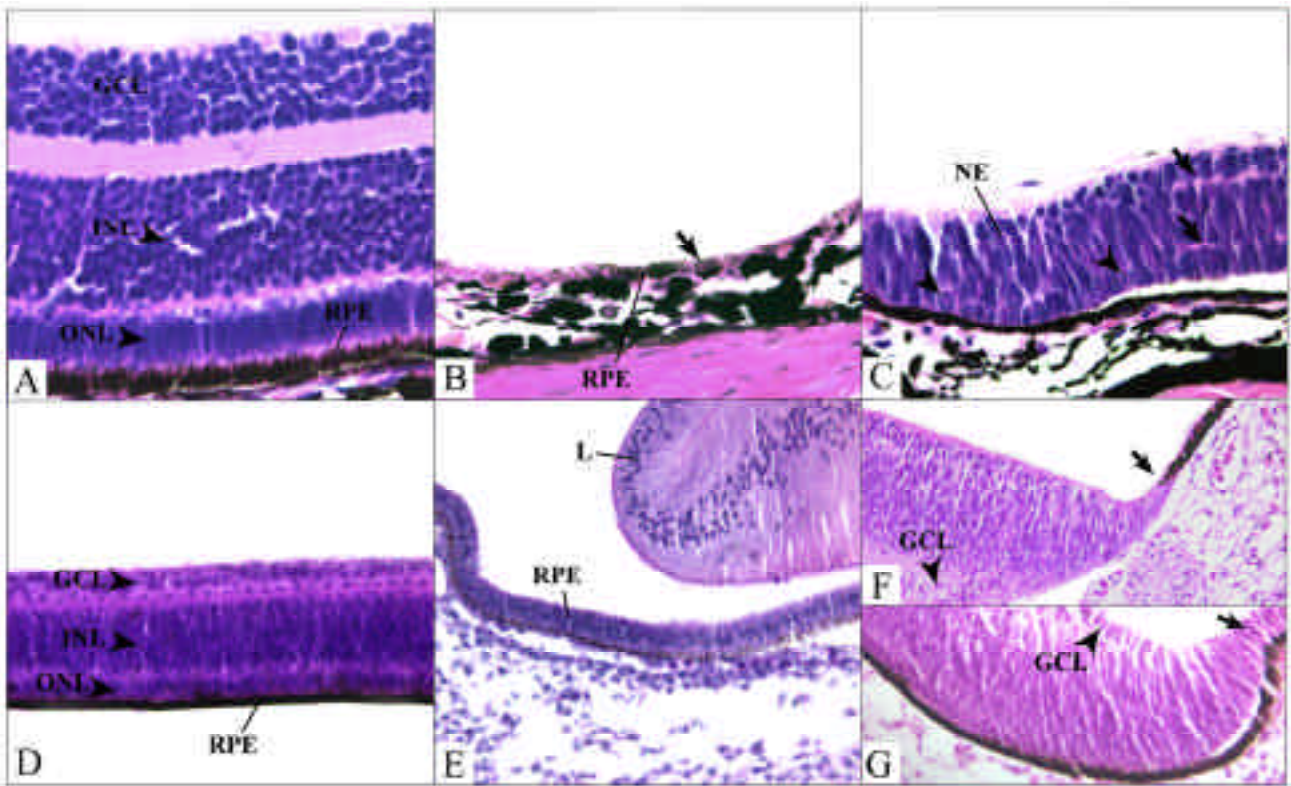


Fig. (2). Regenerating retinas in adult newts and embryonic chicks. A: Intact retina of the adult newt. B: Retinectomized newt eye (10 days post-retinectomy). Here dedifferentiation and proliferation of the RPE cells takes place. Note that the cell shown by the arrow is in the process of dedifferentiation. C: About 20 days post-retinectomy, the regenerated retina starts to stratify into the different retinal layers evident by the formation of extracellular material (arrows). The RPE has also been renewed and the orientation of the newly formed retina is the same as the intact. Also note that some cells in the basal layer are still undergoing cell division (arrowhead). D: Intact developing retina of an E11 chick retina. E: Retinectomized eye at E4 where all neural retina has been removed and only the RPE remains. At this time the RPE has grossly expanded and is mostly non-pigmented. F: Regenerated retina 7 days post-retinectomy that arose via transdifferentiation of the RPE. Note that the RPE did not renew itself in this case. The arrow shows the region where transdifferentiation starts. G: Regenerated retina that arose from the differentiation of stem cell/ retinal progenitors from the ciliary region (CMZ/CB). Arrow points to the anterior region of the eye close to the ciliary region where this retina originated. Note that the retina regenerated via transdifferentiation (F) is in the opposite orientation than the one regenerated via cells of the ciliary region (G) since the GCL faces opposite directions. (ONL= outer nuclear layer, INL = inner nuclear layer, GCL= ganglion cell layer, RPE = retinal pigmented epithelium, NE= neuroepithelium).

different cell types needed. Interestingly though, not all Müller glia cells in a given region respond to damage or growth signals, evident by their lack of BrdU incorporation but positive for glial fibrillary acidic protein (GFAP) staining, a marker for glia cells [Fischer and Reh, 2003a]. In addition, Müller glia cells in the center of the eye lose their ability to respond to stimulus soon after hatching while peripheral Müller glia cells retain this ability longer. This might indicate a readiness to transdifferentiate depending either on their time of genesis or in their function in the different regions of the retina [Fischer and Reh, 2003a].

Retina Regeneration by Differentiation from Ocular Progenitors/stem Cells

Progenitor/Stem Cells Derived from CMZ/CGZ and Ciliary Body (Amphibians, Birds, Fish and Mammals)

The CMZ/CGZ [ciliary marginal zone/ circumferential germinal zone] and the ciliary body, located in the anterior

margin of the eye, have both been shown to contain retinal precursor cells in certain species. Fish, amphibians, and chick contain both a CMZ or CGZ and a ciliary body while higher vertebrates contain only a ciliary body [Müller 1952; Lyall, 1957, Hollyfield, 1968; Morris *et al.*, 1976; Perron *et al.*, 1998, Beebe, 1986; Fischer and Reh, 2003b] Fig. (1). Cells from the CMZ in amphibians and the CGZ in fish proliferate throughout the life of the organism in order to add new neurons to the retina as the eye continues to grow. Although these cells can also be induced to proliferate in response to injury, other mechanisms are primarily used by these species during regeneration of a damaged retina [Reh *et al.*, 1987, Raymond *et al.*, 1988, Hitchcock *et al.*, 1992, Mitashov, 1996; 1997; Del Rio-Tsonis and Tsonis, 2003; Otteson and Hitchcock 2003; Tsonis and Del Rio-Tsonis, 2004].

The retinal progenitor cells of the CMZ in amphibians are capable of differentiating and forming all types of retinal neurons while cells from the CGZ in fish differentiate and

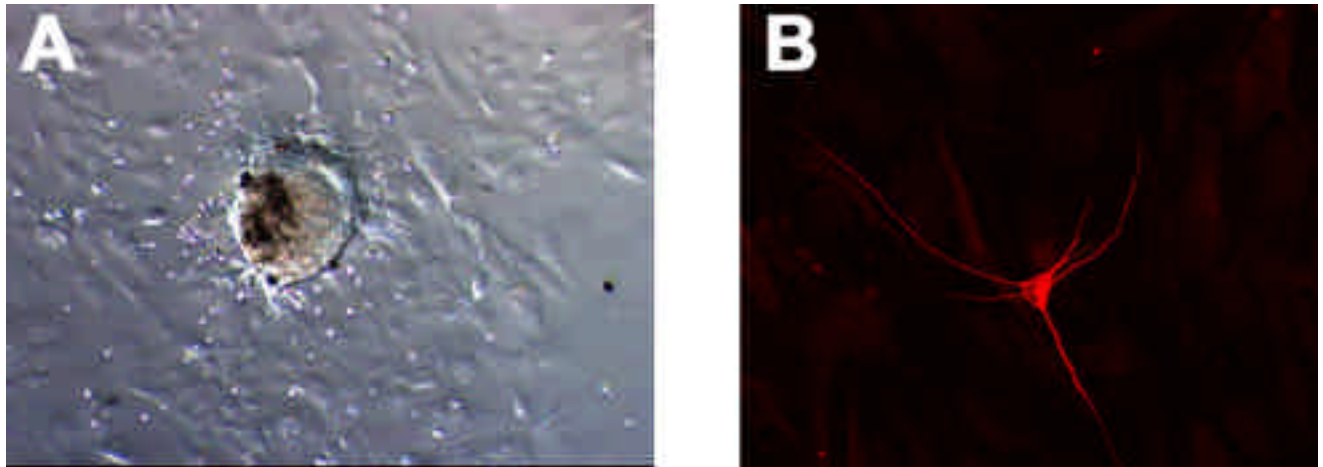


Fig. (3). Cultured cells from the pigmented region of the pars plana and pars plicata of humans. **A:** A cell colony derived from pigmented cells of the pars plana and plicata of the adult human eye grown in the presence of EGF and then plated onto a coverslip coated with poly-ornithin and laminin. **B:** An example of neuron-like cells derived from such colonies (immunolabeling with an antibody directed against the α -tubulin-III antigen). (Courtesy: Dr. Yvan Arsenijevic).

form all cells except rod photoreceptors [Johns and Easter 1977; Johns and Fernald, 1981 and Perron *et al.*, 1998]. These cells arise from rod precursors located in the outer nuclear layer of the retina (see section below). The CMZ of the adult chick is somewhat different in that cells from this region only proliferate for the first three weeks of life and are able to differentiate into amacrine and bipolar neurons but not ganglion cells [Fischer and Reh, 2000] unless exogenous growth factors are present [Fischer *et al.*, 2002a]. These cells, however, do not proliferate in response to injury.

The non-pigmented epithelium (NPE) of the ciliary body in the adult chick contains a different population of cells capable of differentiating primarily into ganglion cells but not bipolar neurons in the presence of certain growth factors. Injury may also induce cells from the NPE since puncture of the eye alone stimulated a few cells to proliferate and differentiate into neurons [Fischer and Reh, 2003b]. Photoreceptors are not formed by either population of cells in the adult chick. Since cells from the ciliary body differentiate into cells normally formed earlier in retinal development, while the cells from the CMZ differentiate into cells normally formed later in retinal development, these populations of cells are referred to as retinal stem cells and retinal progenitor cells respectively [Fischer and Reh, 2000; 2003b]. This gradient of less mature progenitor cells located in the more anterior ciliary region and more mature progenitor cells located in the more posterior ciliary region (CMZ) has also been reported in amphibians [Perron *et al.*, 1998]. In addition, if the CGZ is removed from fish eyes, it will be regenerated from both stem cells in the peripheral neural retina and stem cells from the anterior ciliary body [Jimeno *et al.*, 2003].

Retinal stem and progenitor cells in the adult chick are not capable of producing all retina neural cells, however, cells from this CMZ/ciliary body region in the embryonic chick are capable of regenerating a complete retina in response to injury as long as a supply of FGF is present

[Coulombre and Coulombre, 1965; Park and Hollenberg, 1989, 1991, 1993]. This regenerated retina, in contrast to the retina regenerated during chick embryogenesis from trans-differentiation, forms in the normal orientation and is believed to occur via the use of neural precursors [Coulombre and Coulombre, 1965; Park and Hollenberg, 1991; 1993; Willbold and Layer, 1992]. This multipotency and response to injury displayed by the CMZ/ciliary body region has been observed at least through stage 28 [according to Hamburger and Hamilton, 1951] of chick development (our observations).

The ciliary body of mammals is also being studied for the presence of retinal progenitors or stem cells. As previously mentioned, a population of cells from the adult rodent pigmented ciliary margin (see Fig. (1) for structure) has the capability of forming retinal specific cells *in vitro* but not *in vivo* [Ahmad *et al.*, 2000, Tropepe *et al.*, 2000]. Recently, mice with only one functional allele for *ptc*, the receptor of sonic hedgehog (Shh), show an increased number of proliferating cells in the non-pigmented region of the ciliary body up to 3 months of age [Moshiri and Reh, 2004]. When these *ptc*^{+/-} mice are crossed with mice having a retinal degeneration background (pro23his rhodopsin transgenic), non-pigmented cells of the ciliary body proliferate in response to injury and begin to express retina neuronal markers indicating a potential for retinal regeneration. These proliferating cells are similar in gene expression patterns to the retinal progenitors found in the CMZ of lower vertebrates [Moshiri and Reh, 2004].

In another report, neuron-like cells have also been observed in the non-pigmented epithelium of the ciliary body in monkeys greater than seven years of age [Fischer *et al.*, 2001]. This anterior region of the eye is currently being studied and compared in the above mentioned organisms in order to further decipher the molecular mechanisms necessary for complete retina regeneration in response to injury in mammals.

Progenitor/Stem Cells Derived from the Inner Nuclear Layer (Fish)

In teleost fish, a population of cells in the inner nuclear layer (INL) of the retina has been shown to be responsible for producing new rod photoreceptors during normal development and may also differentiate and produce all retinal neurons during regeneration [Otterson and Hitchcock, 2003] Fig. (1). In contrast to most vertebrate organisms that produce all retina neurons during embryogenesis, rod type photoreceptors are not formed until the larval stage of fish development [Müller, 1952; Lyall, 1957, Johns, 1982]. During larval development, a group of proliferating cells was discovered in the INL of the retina containing two distinct cell populations which can be distinguished by morphology and gene expression patterns. Populations of fusiform shaped cells that are Pax-6 negative are present along with a population of spherical shaped Pax-6 positive cells. These spherical, Pax-6 expressing cells are believed to be undifferentiated embryonic retinal stem cells that are maintained in this layer to form the precursors for rod photoreceptors and may also be capable of producing all retinal neurons if certain damage occurs [Otterson and Hitchcock, 2003]. These cells are therefore, referred to as INL stem cells. These INL stem cells, during normal development, will differentiate into the fusiform, Pax-6 negative expressing cells, referred to as INL progenitor cells, and migrate to the outer nuclear layer (ONL) of the retina where they become committed to form rod photoreceptors [Johns and Fernald, 1981; Johns, 1982, Hagedorn et al., 1998; Julian et al., 1998; Vihtelic and Hyde, 2000; Otterson et al., 2001; Otterson and Hitchcock, 2003] Fig. (1). In the peripheral region of the eye where new neuronal layers are continually being added, INL stem cells are thought to be produced by the cells in the CGZ to allow an increased production of rod photoreceptors needed in this region [Otterson and Hitchcock, 2003]. If damage to the retina occurs, there is a significant increase in INL stem cells, INL progenitors and rod precursors. The current model for regeneration of the damaged retina favors the differentiation of the INL stem cells as the major source of new retinal neurons [Otterson and Hitchcock, 2003] Fig. (1). It is postulated that the Pax-6 positive INL stem cells, upon receiving proper signals, will not follow the rod photoreceptor lineage pathway, but instead, form a regenerative blastema that is ultimately the source for all retinal neurons. The cells of the blastema are Pax-6 positive suggesting it forms from the INL stem cells. It is possible that INL retinal progenitors and/or rod precursors of the ONL also play a role in retina regeneration since they also increase in response to injury [Otterson et al., 2001, Otterson and Hitchcock, 2003]. However, these cells may have to de-differentiate and re-express Pax-6 in order to form the Pax-6 positive cells found in the regeneration blastema or they may contribute to regeneration via a different pathway. For instance, the INL progenitor cells, which form rod precursors during normal development, have been shown to differentiate into cone photoreceptors in response to injury [Wu et al., 2001]. Regardless of the mechanism used for regeneration, it does not occur if the ONL is not damaged. The reason for this is not clear, but damage to the photoreceptors may be necessary for regeneration inducing factors to be secreted.

Progenitor/Stem Cells Derived from Embryonic Neural Retina (Mammals)

Chacko et al. (2000) have isolated retinal progenitors from E 17 rat eyes [Ahmad et al., 1999] and used them for transplantation experiments into host eyes. These cells survived and differentiated into photoreceptor-like cells expressing opsin but did not integrate into the existing retina. In similar experiments, post-natal PN1 retinal progenitors were transplanted into host retinas where mechanical damage was induced showing that retinal damage was essential for retinal integration [Chacko et al. 2003]. Yang et al. [2002a and 2002b] have also isolated retinal progenitors from E17 rats and humans during the 10-13th week of gestation. The rat retinal progenitor cells cultured by Yang et al. (2002a) differentiated into many retinal neurons *in vitro*, but mostly differentiated along the glial lineage when transplanted into host eyes. The cultured human retinal progenitor cells were capable of dividing for multiple generations and differentiating into several types of retinal neurons *in vitro* but have not been tested yet *in vivo* [Yang et al., 2002 b].

Progenitor/Stem Cells Derived from Choroid, Sclera and Corneal Limbal Epithelium (Mammals)

Cells from the iris, cornea limbus area, sclera, and the choroid have all been proposed as potential sources of retinal progenitor cells in mammals [Haruta et al., 2001; Zhao et al., 2002a; Arsenijevic et al., 2003] Fig. (1). Cultured rat iris cells have been induced to differentiate into retinal cells, including photoreceptors when transfected with Crx, a crucial photoreceptor developmental gene [Haruta et al., 2001]. In addition, rat limbal epithelial cells have been shown to express certain neural progenitor markers if grown under certain conditions [Zhao et al., 2002a]. If transplanted into a damaged retina, these neural progenitors will migrate and integrate into different retinal layers and express retinal neural markers [Chacko et al., 2003]. On the other hand, sclera and choroid cells isolated from adult human eyes also have the potential to differentiate towards the neural lineage *in vitro* suggesting these cells may also have the ability to act as retinal progenitors [Arsenijevic et al., 2003] Fig. (4). Although this has not been determined as of yet, it is a possibility worth exploring further.

Retina Regeneration by Differentiation from Non-Ocular Progenitors/Stem Cells

Progenitor/Stem Cells Derived from Adult Hippocampus, Neonatal Brain, Embryonic Spinal Cord and Fetal Cortex (Mammals)

There have been several attempts using neural stem cells (from outside of the eye) to repopulate the damaged retina. Some of the studies included the use of adult hippocampal stem cells which were transplanted into the eye cavity of both adult and neonatal rats with no luck of differentiation into specific neural retinal cells. Despite the failure of hippocampal stem cells to differentiate into neurons with retina-specific proteins, the hippocampus derived neurons acquired appropriate morphological differentiation indicating that cues for neurite extensions and neural polarity are present in the mature, degenerating retina and these morphological cues are not limited to influencing only retinal cells

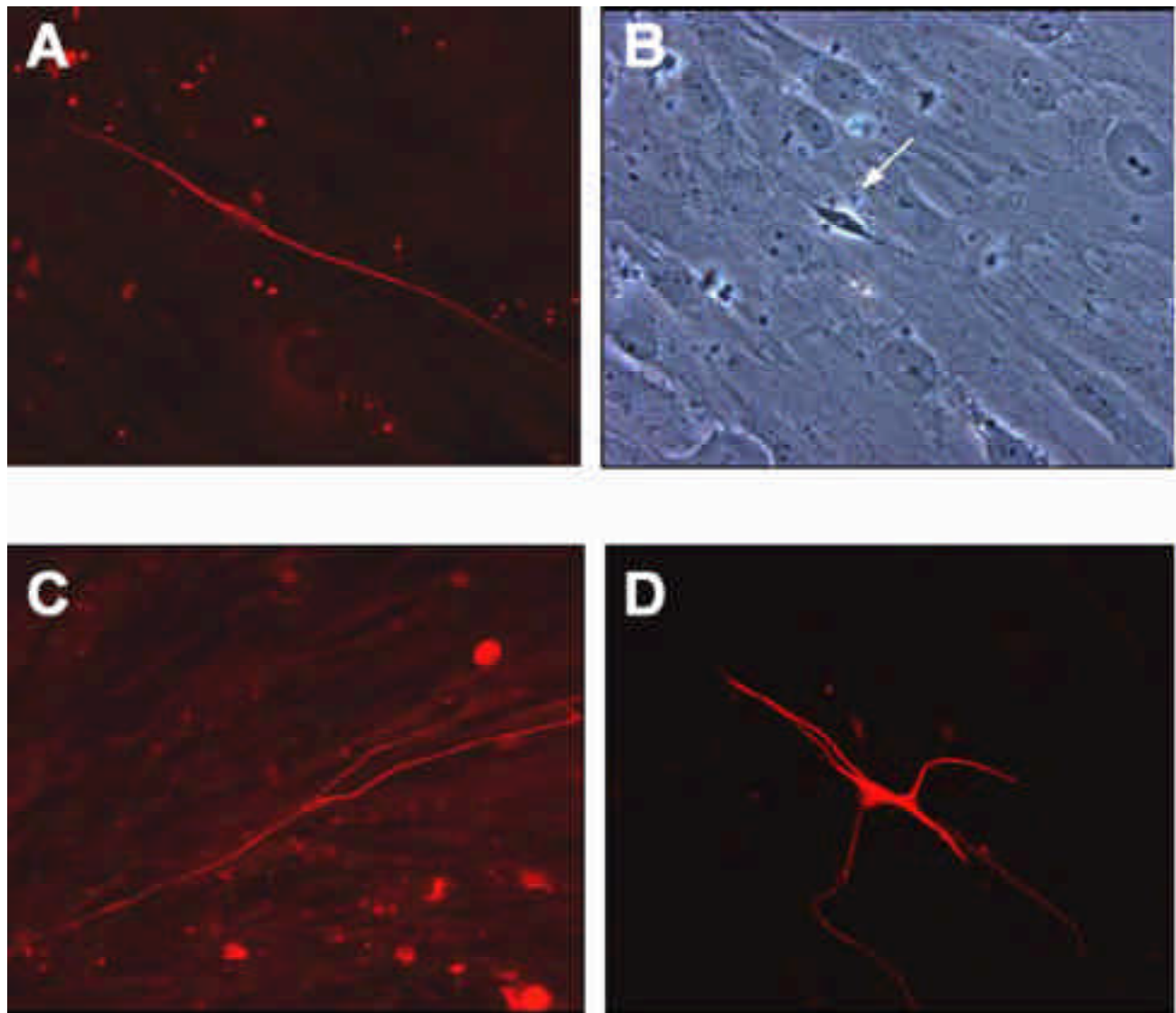


Fig. (4). Sclera and choroid cells were cultured *in vitro* and stimulated to differentiate into neural like cells in the presence of laminin, epidermal growth factor (EGF) and fetal bovine serum (FBS). A subset of these cells expressed α -tubulin-III (A, C, D), an early neuronal marker, and displayed neuronal morphology (arrow in B) as well. B represents a phase contrast image of A. When brain-derived neurotrophic factor (BDNF) was added to the cultures, the cells generated multipolar α -tubulin-III positive cells. (Courtesy: Dr. Yvan Arsenijevic).

[Takahashi *et al.*, 1998; Young *et al.*, 2000; Nishida *et al.*, 2000; Akita *et al.*, 2002]. In some cases these cells even established synaptic connections [Nishida *et al.*, 2000]. Interestingly enough, an injury signal had to be present in the adult host eye for these cells to behave as described [Young *et al.*, 2000; Nishida *et al.*, 2000; Kurimoto *et al.*, 2001]. Some groups are working on finding the optimal retinal differentiation conditions for these stem cells *in vitro* with the idea of implementing such environment during transplantation experiments [Kaneko *et al.*, 2003].

Other reports stress the use of neural progenitors from brain and spinal cord of neonatal and embryonic GFP transgenic mice respectively in transplantation experiments using diseased animal models like the Royal College of Surgeons

(RCS) rats, the retinal degeneration (rd) mice and the beta2/beta1 knock-in mutant mice (displaying photoreceptor apoptotic cell death) as host animals. GFP labeled cells allow for an easier assessment of where the transplanted cells position themselves within the host eye. These studies show that the transplanted cells do survive and migrate within the retina of the different recipient animal models [Lu *et al.*, 2002; Pressmar *et al.*, 2001; Mizumoto *et al.*, 2003] and in some cases they even differentiate into neuronal like cells. However, as in the case of the hippocampal stem cell transplantation experiments, no retinal differentiation was observed [Pressmar *et al.*, 2001; Mizumoto *et al.*, 2003]. In a similar study, GFP labeled murine brain progenitor cells were transplanted into Brazilian opossums, showing survival, differentiation and morphological integration within the

host tissue. This study demonstrated that the younger the retinal host environment used, the higher the percentage of morphological retinal integration. In addition, some of the transplanted GFP expressing cells were also positive for retina neural markers [Sakaguchi *et al.*, 2003; Van Hoffelen *et al.*, 2003].

Recently, human neural stem cells isolated from human fetal cortex were treated *in vitro* with TGF- β 3 and then transplanted into the vitreous cavity of donor rats. These cells migrated and incorporated into the host retina where they differentiated into retinal cells positive for opsin [Dong *et al.*, 2003]. This study not only demonstrates that human neural stem cells can migrate and incorporate into the host retina, but most importantly that these cells can be manipulated to differentiate into retinal cells, particularly photoreceptor-like cells. At this point it is not clear if these cells express the full repertoire of photoreceptor cells and it is too early to know if they can function to transduce light into electrical signals.

Progenitor/Stem Cells Derived from Bone Marrow and Embryonic Cells (ES) (Mammals)

Other reports favor the use of bone marrow stem cells to repopulate damaged retina. Tomita *et al.* (2002) have shown that rat derived bone marrow stem cells injected into the eye cavity of host rats with induced retinal damage do incorporate into the retina and differentiate into retinal-like cells (expressing markers for photoreceptors as well as amacrine and horizontal cells). In another study, marrow stromal cells transplanted subretinally in adult RCS rats integrated into the host retina and expressed the photoreceptor-specific marker opsin [Kicic *et al.* 2003]. Although these results are promising, it should be noted that cell fusion has been reported to occur between bone marrow stem cells and neurons [Alvarez-Dolado *et al.*, 2003]. Therefore, cell fusion between bone marrow stem cells and neurons of the retina may be occurring instead of de novo generation of retinal neurons from bone marrow stem cells. Further experiments are needed to address the mechanism in which bone marrow stem cells contribute to retinal neuron formation.

Lastly, mice embryonic stem cells (ES) cells have been cultured *in vitro* and pushed to differentiate into neural progenitors with the capacity to give rise to cells expressing retinal neural markers that specify photoreceptors and bipolar cells [Zhao *et al.*, 2002b]. These cells also expressed several phototransducing genes suggesting these cells may be capable of functioning as photoreceptors if transplanted. Furthermore, Schraermeyer *et al.* (2001) have taken ES cells that have been induced to differentiate *in vitro* into neuronal precursors and have used them in transplantation experiments using RCS rats demonstrating that photoreceptor cell degeneration in these animals is delayed. All these studies only increase the pool of possibilities for the treatment of retinal degenerative diseases.

CONCLUDING REMARKS

While some organisms, such as the fish and newt, have the intrinsic ability to regenerate damaged retinal tissue during the course of their adult life, most organisms, including mammals, lose this ability after embryogenesis is complete. Even though mammals do not have the intrinsic

ability to regenerate retinal tissue during adulthood, this review shows that there is a great potential for induction of retinal progenitors from a multitude of sources both within and outside of the retina. It has been shown that these retinal progenitor cells can generate cells that express markers characteristic of retinal neurons and in some cases these cells have been shown to display some of the functions of neurons. For example, mammalian retinal progenitor cells derived from embryonic rat retinas have shown to produce cells capable of firing action potentials (Ahmad *et al.*, 1999). Another example includes the use of fetal retinal sheets (that should contain retinal stem/progenitors) as transplants to rescue vision in retinal degeneration animal models where behavioral visual responses have been used to assay for retinal function and photoreceptor rescue/generation [Sagdullaev *et al.*, 2003]. However, proof of communication between the generated cells via neurotransmitters has not been generated yet and would be a definitive test for neuronal function [Reh, 2002]. Promise exists, however, since cultures of neural stem cells from the hippocampus region of the brain produce neurons *in vitro* that meet all the criteria of functioning neurons including communication via neural transmitters [Reh, 2002; Song *et al.*, 2002]. Many labs, including our own, are trying to determine the molecular mechanisms necessary for induction of these retinal progenitors. With so many avenues being pursued, one is likely to lead to production of functional retinal neurons in order to make retinal regeneration a reality for treating retinal degenerative diseases in humans.

ACKNOWLEDGEMENTS

Special thanks to Mayur Madhavan and Natalia Vergara for their contribution of the artwork. We also thank Dr. Yvan Arsenijevic for contributing Figs. (3 and 4). We apologize for works not cited due to space constraints.

This work was supported by NIH grant EY 014197 to KDRT.

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