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REVIEW ARTICLE

Lens and retina regeneration: new perspectives from model organisms

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Comparative studies of lens and retina regeneration have been conducted within a wide variety of animals over the last 100 years. Although amphibians, fish, birds and mammals have all been noted to possess lens- or retina-regenerative properties at specific developmental stages, lens or retina regeneration in adult animals is limited to lower vertebrates. The present review covers the newest perspectives on lens and retina regeneration from these different model organisms with a focus on future trends in regeneration research.

Key words: lens, model organism, regeneration, retina.

LENS REGENERATION

Introduction to lens regeneration

Lens regeneration has been studied for over a century, with the newt being the only adult animal that is capable of completely regenerating this complex organ [1,2]. The sheer unlimited regeneration potential of newt eyes following lens removal, even in older individuals, has established this system as indispensable for the study of organ regeneration and aging [3].

Newt lens regeneration is unique regarding the intrinsic capability to regenerate the whole lens through transdifferentiation of dorsal IPE (iris pigment epithelial) cells without any remaining organ structure information. In contrast, other amphibians such as *Xenopus* can regenerate lenses from the corneal epithelium during developmental stages when certain developmental cues are provided, thus lacking the regeneration potential as adult individuals. In comparison with lower vertebrates, mammals including humans, rodents, cats and dogs are capable of regenerating the lens from LE (lens epithelial) cells when the capsular bag remains intact. The present review outlines and compares the different lens-regeneration processes in amphibians and mammals by delineating the molecular and cellular mechanisms.

Physiology/anatomy of the lens

The lens structure and physiology have been extensively reviewed by Kuszak et al. [4,5] and Al-Ghoul et al. [6]. Lenses are asymmetrical oblate spheroids that form from inverted lens vesicles and grow throughout life [4–6]. During lens development, LE cells differentiate into lens fibre cells that elongate into anterior and posterior directions of the lens equator [4–6]. When elongation is complete, the ends of lens fibres detach from the lens epithelium or capsule, and then lie alongside opposing fibres to form the lens sutures [4–6]. Throughout life, new lens fibre layers are added continuously within the lens periphery, resulting in formation of concentric growth shells [4–6].

The adult lens is composed of an exterior lens capsule with anterior LE cells positioned on the interior of the lens capsule. These epithelial cells expand into an LE/lens fibre cell transition zone towards the lens equator. The posterior part of the lens is free from LE cells. The inner part of the lens is filled with concentrically aligned lens fibre cells and a more dense fibre core within the middle of the lens (Figures 1A and 1B).

Lens regeneration in *Xenopus*

Species of the genus *Xenopus* are the only frogs known to regenerate lenses [7,8]. Of the lens-regenerating frogs, *Xenopus laevis* [9], *Xenopus tropicalis* [10] and *Xenopus borealis* [11] demonstrate lens regeneration at certain larval stages with declining regeneration potential during aging of the tadpole. In general, lens regeneration in *Xenopus* is based on the transdifferentiation of ectodermal central cornea epithelial cells into a lens vesicle that forms a new lens over time, also called CLT (corneal–lens transdifferentiation) [9]. Interestingly, Yoshii et al. [12] demonstrated that *X. laevis* is capable of regenerating the lens post-metamorphosis from the remaining LE cells left behind in the lens capsule [12].

Process of CLT-dependent lens regeneration

Freeman [9] described five distinct phases of CLT in *X. laevis* on the basis of histological analysis. At stage 1 (1–2 days after lentectomy), cells of the inner corneal epithelium layer demonstrate a change from squamous to cuboidal epithelium. During stage 2, cells begin to thicken into a placodal structure with cells characteristic of LE cells. During stage 3 (3 days after lentectomy), a cell aggregate begins to separate from the corneal epithelium by invasion into the vitreous body for formation of a lens vesicle. During stage 4 (5 days after lentectomy), a definitive lens vesicle has formed by separation from the overlying cornea that contains elongated primary lens fibre

Abbreviations used: BMP, bone morphogenetic protein; CB, ciliary body; CE, ciliary epithelium; CGZ, circumferential germinal zone; CLT, corneal–lens transdifferentiation; CMZ, ciliary marginal zone; E, embryonic day; ECM, extracellular matrix; FGF, fibroblast growth factor; HB-EGF, heparin-binding epidermal-like growth factor; INL, inner nuclear layer; IPE, iris pigment epithelial; iPSC, induced pluripotent stem cell; LE, lens epithelial; MAPK, mitogen-activated protein kinase; miRNA, microRNA; MSC, Müller stem cell; NMDA, *N*-methyl-D-aspartate; ONL, outer nuclear layer; RPC, retinal progenitor cell; RPE, retinal pigmented epithelium; Shh, Sonic Hedgehog; TGFβ, transforming growth factor β; Wnt, wingless.

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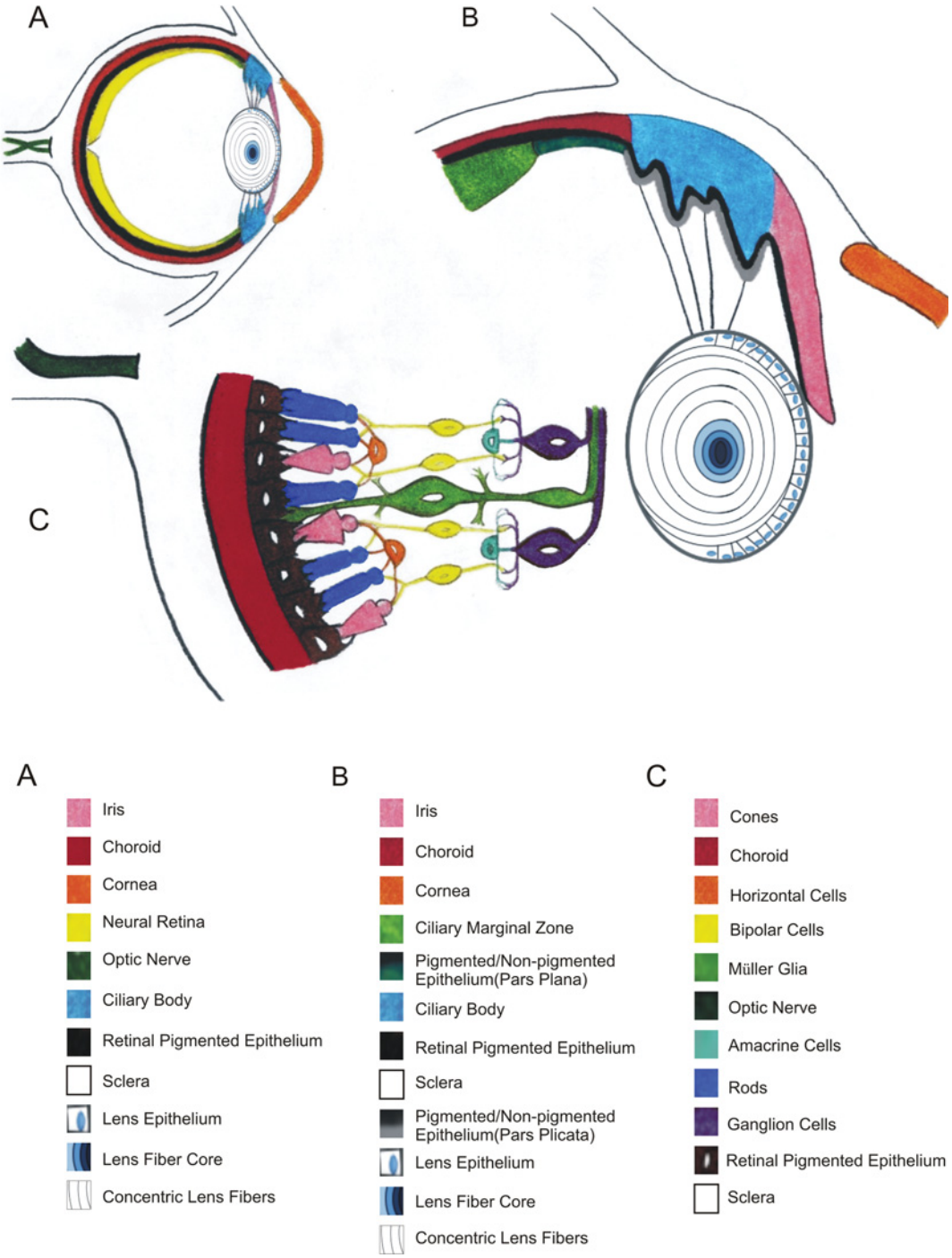

Figure 1 Schematic diagram of the vertebrate eye

(**A**) Basic structures of the vertebrate eye. (**B**) Magnification of the anterior part of the eye, depicting the lens, iris, CE and CMZ. (**C**) Magnification of the posterior part of the eye, depicting mostly the retina and its specific cells. A colour-coded key has been included for each panel to help to identify the eye structures and cells.

cells on the side closest to the vitreous chamber. At stage 5 (10 days after lentectomy), lens formation can be observed with primary and secondary lens fibre formation and visible disappearance of cell nuclei. The cornea has returned to its original squamous epithelial cell composition.

Like other vertebrates, *Xenopus* lenses express high levels of crystallin proteins that are important for lens structure and clarity. There are three major crystallin classes: α-, β- and γ-crystallins [13]. Crystallin accumulation follows a distinct temporal and spatial expression pattern during lens development and regeneration. During lens development, α-, β- and γ-crystallin expression appears simultaneously in the lens placode and later on is limited to lens fibre cells [14]. During lens regeneration, α- and β-crystallins are detected in the posterior vesicle in the middle of Freeman stage 3; however, by stage 4, the expression demonstrates clear restriction to the lens fibres [14–16]. On the other hand, γ-crystallin expression appears at a later time point (early stage 4) and is limited to lens fibres as well [14]. In contrast,

a recent microarray study by Day and Beck [17] demonstrated that α-crystallin expression levels in regenerating or sham-operated corneas remains unchanged, whereas all β- and γ-crystallins identified are up-regulated during CLT. In addition, Day and Beck [17] also suggest similarities in the timing of α-, β- and γ-crystallin expression between regenerating and developing lenses.

Molecular mechanisms of *Xenopus* lens regeneration

The initiation of the CLT process is thought to include the exposure of the cornea to a vitreous humor unknown factor released from the neural retina [18–20]. Growth factors of the BMP (bone morphogenetic protein), FGF (fibroblast growth factor) and Wnt (wingless) signalling pathways were identified as some of the potential candidates for induction of lens regeneration [17,21–23].

In detail, Day and Beck [17] demonstrated that overexpression of the BMP inhibitor Noggin resulted in attenuated corneal cell transdifferentiation into lens vesicles. Day and Beck [17] also suggested that the canonical Wnt signalling pathway is activated during CLT for the induction of the bicoid-related homeobox transcription factors Pitx2, Pitx2a, Pitx1 and Pitx3 [17]. The role of FGF signalling was first implicated by Bosco et al. [22] by demonstrating FGF1-dependent induction of lentoid formation from outer cornea cultures. In a recent study, Fukui and Henry [21] found new evidence for the induction of lens regeneration through FGF family members by demonstrating up-regulation of *fgf1*, *fgf8* and *fgf9* expression in corneal epithelium and retinal tissues before and during the process of lens regeneration. This is supported further by expression of the associated FGF receptors *fgfr1*, *fgfr2* and *fgfr3* within the corneal epithelium throughout the regeneration process and a corresponding inhibition of the lens-regeneration process by the FGF inhibitor SU5402 [21].

Besides the BMP-, FGF- and Wnt-dependent signalling pathways, several transcription factors known to be fundamental for lens development are expressed during the process of CLT including Pax6, Prox1, Otx2 and Sox3 [17,24–28]. For instance, *pax6* is expressed early during development in the anterior embryonic ectoderm and represents a central transcriptional regulator of eye development, lens cell differentiation and the regulation of crystallin expression [25,27,29,30]. Similarly, the lens placode-expressed *prox1* is important for lens fibre cell differentiation and activation of crystallin expression [25,31]. In contrast, *otx2* is expressed in the early head ectoderm and is suggested to participate in lens formation during development through Notch-induced signalling [32].

Other molecules identified to play a role during CLT include target genes of the retinoic acid, Hedgehog, TGFβ (transforming growth factor β) and MAPK (mitogen-activated protein kinase) signalling pathways [17,24–28]. In addition, a huge variety of other lens-regeneration expressed genes have been identified by gene expression profiling including proteins involved in RNA and protein metabolism, and cell transport molecules [17,26].

Role of cellular growth matrix in *Xenopus* lens regeneration

Lentectomy leads to the disruption of the ECM (extracellular matrix) barrier between the eye anterior aqueous chamber and the eye posterior vitreous chamber. The reconstitution of the existing ECM, and, correspondingly, the expression of matrix-associated growth factors, was suggested as an essential requirement for *Xenopus* lens regeneration. For instance, gene expression profiling studies of Day and Beck [17] and Malloch et al. [26] identified changes in matrix metalloproteinase expression and associated changes in matrix modulating molecules such as TGFβ during CLT. In addition, it has been suggested that the early appearance of the matrix-remodelling enzyme gelatinase B [Xmmp-9 (*Xenopus* matrix metalloproteinase 9)] at the peripheral wound site plays a role in induction of wound healing-mediated responses that might also contribute to the induction of CLT [33].

Role of epigenetics and stem cell pluripotency factors in *Xenopus* lens regeneration

In contrast with lens regeneration in newts, Day and Beck [17] suggested that CLT regeneration in *Xenopus* does not include dedifferentiation of corneal cells into cells expressing pluripotency factors. According to Day and Beck [17], *sox2* was up-regulated in differentiated lenses when compared with corneas undergoing CLT. Another pluripotency-associated gene, *fut6*, was significantly up-regulated in sham-operated corneas, but not in corneas undergoing CLT, whereas there was no expression in differentiated lenses. In addition, Day and Beck [17] found evidence of genes associated with chromatin assembly and disassembly during CLT, implying that epigenetic changes may be taking place. Another study, by Perry et al. [34], demonstrated further evidence for changes in lens regeneration-associated chromatin reassembly by identifying elevated histone H3S10P levels following morpholino knockdown of the G-protein-coupled receptor GPR84.

Lens regeneration in newts

To date, adult lens regeneration has been observed only in newts including the species *Notophthalmus viridescens*, *Triturus viridescens* and *Cynops pyrrhogaster*. Lens regeneration of the adult newt was first observed by Collucci in 1891 [2], and independently by Wolff in 1895 [1], after whom the process is often called Wolffian lens regeneration. Wolffian lens regeneration includes the transdifferentiation of neuroectoderm-derived dorsal IPE cells into new lens [35–38].

Process of IPE-dependent lens regeneration

During the first 4 days after lentectomy, IPE cells of both dorsal and ventral regions dedifferentiate, visible by loss of pigmentation and initiation of proliferation [37,39,40]. However, at the mid-dorsal pupillary margin, IPE cells continue to proliferate, and lose pigmentation at approximately 8–10 days post-lentectomy. At this time, cell elongation and synthesis of lens-specific proteins can be observed. Lens vesicle formation appears at day 10 of the regeneration process, which is followed by elongation of the posterior part of the vesicle where lens fibre differentiation is initiated. The anterior cells become lens epithelium [41,42].

Cells in the ventral iris lack the lens transdifferentiation potential. When ventral IPE cells are cultured for approximately 2 weeks and implanted in the eye cavity or the limb blastema, they do not transdifferentiate into lens, whereas implantation of dorsal IPE cell aggregates results in lens formation [43,44]. On the other hand, long-term *in vitro* cultures of dorsal or ventral IPE cells revealed that both cell types are capable of lentoid body formation [45]. This means that the ventral IPE cells have the potential for transdifferentiation; however, this is not permitted *in vivo* or in short-term cultures.

Similar to *Xenopus*, expression of crystallins during newt lens regeneration was demonstrated to adhere to the expression pattern during lens development. For instance, α_A-, β_{B1}- and γ-crystallins appear all simultaneously at the posterior lens vesicle at approximately 10–12 days after lentectomy [40], with appearance of γ-crystallins only in lens fibres [46].

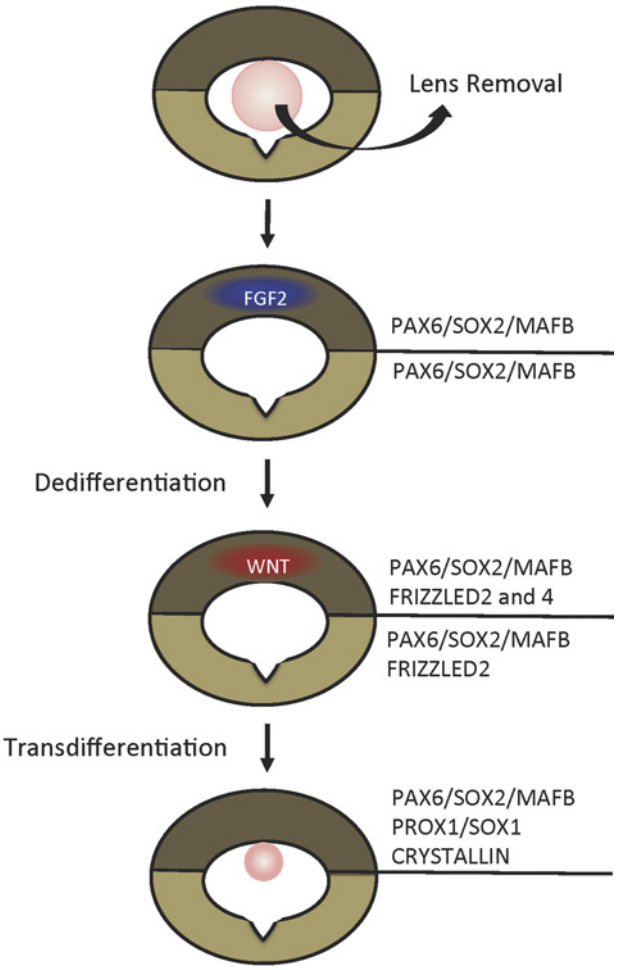

Figure 2 Signalling overview during newt lens regeneration

Within the first days after lentectomy, FGF2 (in blue) and early lens genes such as *pax6*, *sox2* and *mafB* are suggested to promote induction of IPE cell-cycle re-entry and dedifferentiation in both dorsal and ventral halves of the iris (as delineated by the horizontal line) [49–51]. Higher abundance of *fgf2* expression in the dorsal iris is suggested to promote differential expression of Wnt-pathway-associated signalling molecules (in red) such as Wnt2b and Frizzled4 that are activated and confined to the dorsal iris [54]. Following Wnt pathway activation, induction of dorsal iris transdifferentiation, including expression of lens-specific genes such as *prox1* and *sox1* can be observed together with initiation of crystallin expression [28,52,53].

Molecular mechanisms of lens regeneration in newts

Similar to lens regeneration in *Xenopus*, family members of the FGF signalling pathway have been suggested to control urodele lens regeneration [47–49]. In contrast with lens regeneration in *Xenopus*, FGF2 and the FGF receptors FGFR1 and FGFR3 are essential for lens regeneration in the newt [47–49]. Following *fgf2* expression, induction of early lens genes such as *pax6*, *sox2* and *mafB* can be observed within the first few days after lentectomy [49–51] (Figure 2). Induction of late lens genes such as *prox1* and *sox1* occurs shortly before expression of crystallin genes can be observed [28,52,53]. Besides the initial signalling activity of FGF2, Hayashi et al. [54] suggested that a Wnt signal is activated during the second step of the lens-regenerating process. According to Hayashi et al. [54], transcripts for the Wnt ligand and Frizzled receptor families including *wnt2b*, *wnt5a*, *frizzled2* and *frizzled4* can be detected in the iris undergoing regeneration. During early stages of lens removal, *wnt5a* and *frizzled2* are induced in both the dorsal and the ventral halves of the iris following lens removal or FGF2 injection, suggesting that activation of these genes belong to the first-step process [54]. In contrast, *wnt2b* and *frizzled4* are induced only in the dorsal iris, suggesting their direct involvement in the dorsal-limited second step of the lens-regeneration process [54].

In addition, members of the Hedgehog signalling pathway such as Shh (Sonic Hedgehog) and Ihh (Indian Hedgehog) are expressed during lens regeneration [55]. A broad-spectrum analysis of mRNA expression patterns during dedifferentiation of the iris cells revealed further that several cancer and apoptosis-related genes, including signalling factors of the Ras family, p53 tumour suppressor family, TNF (tumour necrosis factor), Rb (retinoblastoma) and Jun transcription factor family might also be involved in the lens-regeneration process [56].

Effect of cellular growth matrix on newt lens regeneration

Following lentectomy in newts, several changes in ECM remodelling that might contribute to IPE cell dedifferentiation can be observed. For instance, higher accumulation of glycosaminoglycans and hyaluronate in combination with increased hyaluronidase activity can be found in the dorsal iris than in the ventral iris [57,58]. In contrast, expression of tissue-remodelling enzymes such as collagenase and cathepsin are up-regulated during lens regeneration in both dorsal and ventral iris [59]. The glycoprotein fibronectin represents another ECM component suggested to support the dedifferentiation of IPE cells [60]. Fibronectin was observed to increase at both the basolateral and apical surface of the dedifferentiating IPE cells and its accumulation continues until the cell surface is completely surrounded. Within differentiating lens fibre cells, fibronectin expression decreases [60]. Correspondingly, Elgert and Zalik [60] suggested that the decrease in fibronectin might be due to an increasing activity in membrane-bound proteases.

A recent study by Godwin et al. [61] proposed that the transmembrane protein TF (tissue factor) is expressed in a patch-like domain of the dorsal iris. Thrombin activation and recruitment of blood cells was suggested to promote formation of a fibrin clot that allows localized expression of growth factors such as FGF2, as the first step for initiation of lens regeneration from the dorsal iris [61]. This idea is supported by prior studies by Imokawa et al. [62,63] that found activation of thrombin at the dorsal margin after lentectomy, and could successfully block lens regeneration by inhibiting thrombin activity. Godwin et al. [61] suggest that the localized FGF2 production is followed by induction of Wnt signalling and delayed activation of FGF expression within the ventral iris. These differences in dorsal and ventral iris signalling are also suggested to contribute to the observed differences in BMP and Wnt signalling between dorsal and ventral iris [44,54,61,64]. Correspondingly, differential BMP signalling in dorsal compared with ventral iris might account for differences in the IPE cell differentiation potential of dorsal compared with ventral iris. This is strongly supported by a study that used inhibition of BMP to induce lens regeneration from the ventral iris, which does not normally regenerate [44]. In addition, potential interspecies differences on the role of BMP regarding Wolffian regeneration in newts and CLT in *Xenopus* might also account for the dedifferentiation potential of IPE cells in the newt.

Role of epigenetics and stem cell pluripotency factors in newt lens regeneration

A recent study of Wolffian lens regeneration using EST (expressed sequence tag) analysis identified several gene transcripts associated with pluripotency reprogramming, such as histone deacetylases and the oncogene c-Myc [56]. That

changes in histone modification and expression are relevant for IPE cell-dependent lens regeneration is supported further by knockdown studies of histone B4 that resulted in alteration of essential lens proteins [65]. In addition, increases in histone H3 methylation and histone H4 acetylation, and a decrease in histone H3 acetylation were observed during IPE cell dedifferentiation [66]. This study also identified differential epigenetic changes in dorsal compared with ventral iris such as persistence of histone H3 methylation in the dorsal iris, and increased histone H3 methylation in the ventral iris [66].

When looking at proteins that play a role in IPE cell dedifferentiation for establishment of multipotency, nucleostemin was observed to accumulate in the nucleoli of dedifferentiating pigmented epithelial cells 2 days before cell cycle re-entry [67]. Furthermore, in contrast with the main pluripotency factors Oct4, Sox2, Klf4 and c-Myc that are required for induction of adult somatic fibroblast dedifferentiation in higher vertebrates [68], expression of only three stem cell factors, e.g. *sox2*, *klf4* and c-*myc* was observed during lens regeneration in the newt [69]. Whereas *sox2* and *klf4* are up-regulated during the very early stages of lens regeneration at day 2 for potential cell reprogramming and initiation of cell cycle re-entry, c-*myc* expression peaked at day 8, together with the establishment of the lens vesicle [69]. However, the pluripotency factors Nanog and Oct4 were non-detectable during lens regeneration [69]. Correspondingly, the lack of Nanog and Oct4 expression during newt lens regeneration might account for the difference between attaining the status of an 'embryonic-like' stem cell [i.e. iPSCs (induced pluripotent stem cells)] and a dedifferentiated cell capable of only re-creating a lens.

Another mechanism for regulation of dedifferentiation includes post-transcriptional regulation of gene expression by miRNA (microRNA). Makarev et al. [70] demonstrated that a number of miRNAs including members of the *miR-124* family, *miR-125b*, *miR-181b*, *miR-133a*, *miR-21* and *let-7b* are expressed within the adult newt eye. In addition, several miRNAs have been identified that play a role during lens regeneration such as members of the *let-7* family and *miR-148* [71,72]. For instance, all of the *let-7* family members are down-regulated in the dorsal iris compared with the ventral iris during the initial dedifferentiation process of lens regeneration [71]. Specifically, *let-7b* that targets *pax3*, *chrd* (chordin) and *tgfβr1* mRNA expression demonstrates a down-regulation in the regenerating dorsal iris compared with ventral iris and the experimental up-regulation was suggested to inhibit proliferation in both dorsal and ventral iris. In addition, *miR-148*, which targets *tgfα*, *nog* (noggin) and *fgf13* mRNA expression, appears to be up-regulated in the ventral iris compared with the dorsal iris [72].

Mammalian lens regeneration

Compared with lens regeneration in *Xenopus* and newts, mammalian lenses do not regenerate completely and are not always of normal shape. Lens regeneration in mammals, including rabbits, dogs, cats, rats and mice, requires the existence of an intact lens capsule [73–79]. In contrast with the IPE cell-driven lens regeneration in newts, lens regeneration in mammals was demonstrated to result from LE cells remaining in the lens capsule [76,79,80]. The lens is sometimes well regenerated with bow regions where active differentiation to lens fibres occurs. In mice, during the initial steps there is expression of inflammatory factors as well as factors involved in tissue remodelling and EMT (epithelial–mesenchymal transition). At later stages, normal expression of crystallin genes is resumed [81]. Research in rats has shown that preservation of the anterior lens epithelium and surgical closure of the lens capsule results in clear lenses with parallel protein expression profiles resembling the ones during lens development [79]. Lens currents also seem to play a critical role in the rat lens-regeneration process [82].

RETINA REGENERATION

Introduction to retina regeneration

Studies conducted over the last 100 years using a wide variety of animals have contributed to the current knowledge on the process of retina regeneration [83]. Amphibians, fish, birds and mammals have all been noted to possess regenerative properties; however, even though most animals are capable of carrying out retina regeneration at specific developmental stages, only a few have been shown to regenerate their retina at maturity [84].

Retina-regeneration strategies used by vertebrates appear to be evolutionarily conserved. The main process of retina repair/regeneration includes transdifferentiation of neural retina-supportive cells such as Müller glia cells or cells from the RPE (retinal pigmented epithelium). Regeneration through the activation of cells located in the CB (ciliary body)/CMZ (ciliary marginal zone) represents the other mode that plays a minor role in most vertebrate animals with regenerative capacity. Regeneration via RPE or Müller glia transdifferentiation generally involves the process of dedifferentiation, where the RPE/Müller glia cells lose their original features, proliferate and eventually differentiate into retina cells. Regeneration through the CB/CMZ involves the activation of stem/progenitor cells residing in this area, and these in turn proliferate and eventually differentiate into retina cells [84,85] (Figure 3).

Physiology/anatomy of the retina

For a better understanding of the retina-regeneration process, we review the anatomy of this sophisticated tissue. The retina is a light-sensitive structure that absorbs light and converts it into nerve impulses that are ultimately deciphered by the brain. The retina is composed of six different types of neural cells, including cone and rod photoreceptors, and horizontal, bipolar, amacrine and ganglion cells (Figure 1C). The photoreceptor cells are distributed in the posterior retina where they capture light and transform it into electrical signals. These cells are distributed in the back of the retina next to the RPE. The cones are utilized for daytime colour vision, whereas rods are used as low-light sensors. The retinal layers are divided into the GCL (ganglion cell layer), the INL (inner nuclear layer), where the cell bodies and nuclei of amacrine, bipolar and horizontal cells are located, and the ONL (outer nuclear layer) where the nuclei of the photoreceptors reside. The OPL (outer plexiform layer) is the site of synaptic contacts between the cones or rods with horizontal and/or bipolar cells. The IPL (inner plexiform layer) is another synaptic region where communication between bipolar and ganglion cells takes place [86].

The RPE that is located between the retina and the choroid consists of a pigmented epithelial cell monolayer. The apical membrane of the RPE faces the photoreceptor layer, whereas its basolateral membrane faces the choroid (Figure 1C) [87]. The RPE supports the retina by carrying out phagocytosis of the photoreceptor outer segments, by maintaining the visual cycle, and by transporting nutrients, ions and fluids to the retina [88]. In addition, Müller glia cells are also essential for retina function and survival and they span the whole retina with their nuclei located in the INL (Figure 1C). The CB is composed of the ciliary muscles

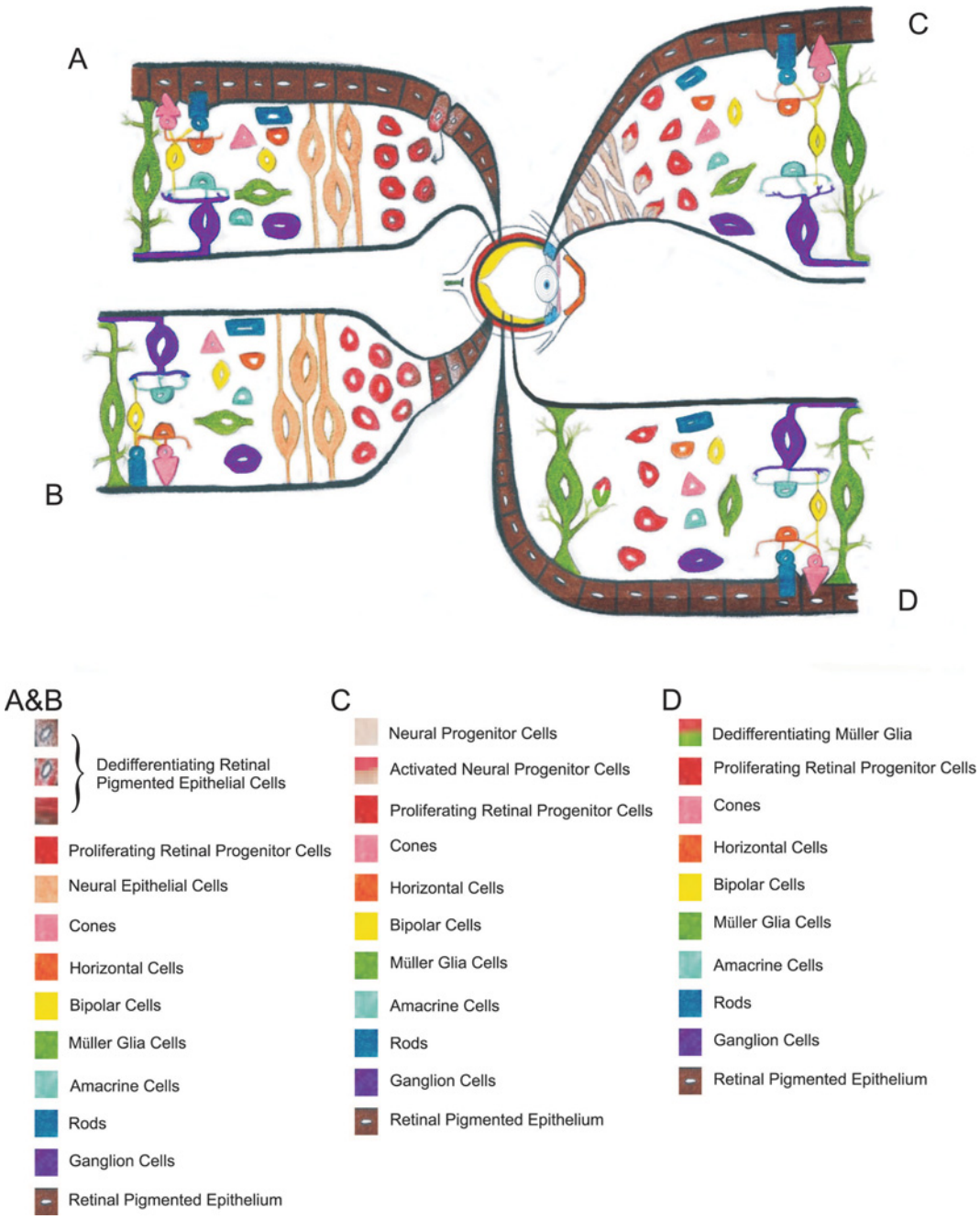

Figure 3 Different modes for retina regeneration in vertebrates

(**A**) The process of RPE transdifferentiation that takes place in amphibians. RPE cells lose their pigment and dedifferentiate to give rise to neuroepithelium that eventually differentiates into the different retinal cells. The RPE also replenish itself, re-establishing the epithelium. (**B**) The RPE transdifferentiation that takes place in embryonic chickens. RPE dedifferentiates as in (**A**); however, the RPE layer is not replenished and the orientation of the regenerated retina is reversed. (**C**) Regeneration via activation of stem/progenitor cells from the CMZ. Progenitor cells are activated and proliferate to give rise to the different retinal cells. (**D**) Transdifferentiation of Müller glia cells that takes place in fish, birds and mammals. Müller glia cells dedifferentiate and proliferate to give rise to the different retina cells. A colour-coded key has been included for each panel (the same key is used for both **A** and **B**) to indicate the different cells.

that are important for lens accommodation, and also contains pigmented and non-pigmented epithelium, including the pars plana and the pars plicata (Figure 1B). The ciliary pigmented epithelium is related ontologically to the RPE, and the non-pigmented epithelium is continuous from the CMZ/retina [89,90]. The CMZ is a small transition zone located between the retina and the ciliary body (Figure 1B) that, in lower vertebrates, represents the main source of proliferative progenitor cells for retina neurogenesis and can be identified with progenitor markers [90–92].

Retina regeneration through the ciliary margin

Amphibians and fish

Retina growth persists until adulthood in amphibians and fish through the contribution of progenitor cells present in the CMZ [93–97] (Figure 1). In fish, this region is called the CGZ (circumferential germinal zone) and contains RPCs (retinal progenitor cells) for the generation of all retinal cells, excluding rod photoreceptors [94,98] (Figure 3C). Rod photoreceptor cells

in fish are produced by specific rod progenitor cells located in the central retina [98–100]. Upon retinal injury in fish and amphibia, the CMZ/CGZ can contribute to retina regeneration by activating progenitor cells to proliferate and eventually differentiate to replace various types of lost retinal cells [100–106] (Figure 3C). In fish, all retinal cells can be regenerated from the CGZ except rod photoreceptors. It is important to note that retina regeneration from the CMZ/CGZ only partially contributes to repair/regeneration since the major contributors of regeneration reside elsewhere in the retina. Whereas retina regeneration in amphibians is mainly based on regenerative cells within the RPE (Figure 3A), Müller glia cells represent the main regenerating cell source in fish [12,98,104–110] (Figure 3D). Recently, Martinez-De Luna et al. [111] proposed a different model of regeneration in pre-metamorphic *X. laevis* that depends on the production of RPCs upon partial retinal resection. To contribute to retinal regeneration, these RPCs not only express retinal progenitor markers, but also depend on the expression of one of these progenitor genes, the homeobox gene *Rx*.

Birds

Similar to fish and amphibians, the CMZ in birds can provide retinal cells for the growth of the retina, but only up to a few weeks after hatching [112,113]. This activity is stimulated by the presence of exogenous factors including FGF, endothelial growth factor, insulin-like growth factor-1, insulin and Shh [112–114]. Although post-hatched chickens cannot regenerate their retina via the activation of the CMZ, Coulombre and Coulombre [115,116] showed that embryonic chicks [stage 23–25 or E (embryonic day) 4–4.5] can regenerate their retinas upon removal from cells of the CMZ (Figure 3C) as long as a piece of retina is present. Park and Hollenberg [117] later identified that FGF1 could induce regeneration from the CMZ. Previous work has shown that FGF2 regenerates a complete retina from E4 chick CMZ via the MAPK pathway [118]. Shh signalling is also sufficient to induce regeneration from this region; however, the Shh and FGF pathways are interdependent on each other, and are required for the proliferation and survival of stem/progenitor cells of the CMZ during regeneration [118,119]. At this stage of development (stage 23–25), the chick eye is not fully differentiated and the CB and CMZ cannot be distinguished, and therefore the regeneration from this region at this stage is referred to occur via the CB/CMZ [118,119]. As a matter of fact, the CMZ is not considered morphologically/molecularly defined until E16, and, as mentioned above, the regenerative properties of the CMZ are lost postnatally [112,120]. Interestingly, a recent study suggests that the early optic cup lip that represents the boundary between pigmented and non-pigmented epithelium contains multi/pluri-potent stem/progenitors that participate during eye morphogenesis and that eventually feed into the CMZ [121].

The survival of stem/progenitor cells is regulated further via another FGF-dependent pathway, the BMP pathway that can induce the CB/CMZ to replace lost retina [122]. BMP induces proliferation of stem/progenitor cells via its canonical pathway (through SMADs) during the early stages of retina regeneration; however, later on, this pathway switches to one of its non-canonical pathways by activating p38 signalling and inducing apoptosis of the newly regenerated retina [122]. Recently, Wnt signalling has been determined to be essential for maintaining the stem cell niche of the CB/CMZ after retina removal and the molecular mechanisms regulating this activity are currently being dissected (K. Del Rio-Tsonis, unpublished work). Changes in these signalling pathways might also explain the decrease in regeneration potential of the chick CB/CMZ as the embryo ages, having marginal regeneration at E5 (K. Del Rio-Tsonis, unpublished work).

Retina regeneration via transdifferentiation

Retina regeneration via RPE transdifferentiation is unique to amphibians; however, several animals have been reported to undergo RPE transdifferentiation during embryonic stages, including mammals and birds. In this process, the RPE cells dedifferentiate, losing their characteristics, proliferate and differentiate into retinal cells (Figures 3A and 3B).

Amphibians

In amphibians, retina regeneration is predominantly achieved via RPE transdifferentiation. Notably, during the process of transdifferentiation in amphibians, the RPE is replenished as it forms a complete retina. This is not the case when RPE transdifferentitation takes place during embryonic stages in birds [115,119] (Figure 3A compared with Figure 3B). Studies have shown that retina regeneration can occur from early developmental stages to post-metamorphic stages [12,123,124].

Anurans. For many years, it was believed that adult post-metamorphic frogs are unable to regenerate their retina via RPE transdifferentiation; however, Yoshii et al. [12] demonstrated that post-metamorphic *X. laevis* are capable of regeneration, even at mature stages. In their studies, the authors showed that if the retina is removed carefully from the eye cup of adult *Xenopus*, leaving the retinal vascular membrane intact, the RPE cells can detach from the RPE monolayer, migrate towards this vascular membrane and, once attached, proliferate to make a neuroepithelium that will eventually differentiate into the different neural cell types [12]. The importance of the vascular membrane for retina regeneration in anuran amphibians was first described by Reh and Nagy [104] in *Rana catesbienna* tadpoles. In that study, the authors described that the association of RPE cells with the vascular membrane is a crucial step in the process of transdifferentiation. In another study, the same group reported the importance of ECM proteins, particularly laminin, as a principal component of the vascular membrane [125]. In addition, *in vitro* studies using RPE explants plated on to different types of extracellular substrates indicated that only laminin is able to induce RPE transdifferentiation. These data suggest that the process of retina regeneration in amphibians is highly regulated by the inductive signals of the ECM [125]. Future studies involving gene manipulations using transgenesis will be very helpful for dissecting the molecular regulation of retina regeneration. To this end, Ueda et al. [126] have recently created transgenic *X. laevis* lines using the *ef1-α* promoter hooked to an eGFP (enhanced green fluorescence protein) gene to mark the tissues undergoing retina regeneration: the RPE and the CMZ.

Vergara and Del Rio-Tsonis [124] developed a new model to analyse retinal regenerative properties in *X. laevis* tadpoles. The retina was completely removed from stage 51–54 tadpoles, leaving only the RPE. Retina regeneration was induced with FGF2 via RPE transdifferentiation. The regenerated retina maintained the proper lamination and the original orientation and also regenerated an optic nerve. The authors also determined that this FGF2-dependent RPE transdifferentiation process requires activation of the MAPK pathway for initiating regeneration. If MAPK inhibitors are added, regeneration is halted.

Kuriyama et al. [127] utilized a three-dimensional Matrigel™ culture to study RPE transdifferentiation in *X. laevis* tadpoles.

The authors cultured both RPE sheets with choroid, and RPE sheets isolated from choroid, in this three-dimensional Matrigel™ system [127]. Their results showed that, in the absence of the choroid which provides contact with the Bruch's membrane (rich in ECM proteins), the RPE is capable of transdifferentiating by first migrating towards the gel matrix, whereas the cultures of RPE sheets with choroid were unable to migrate and therefore unable to transdifferentiate [127]. The authors determined from these results that the loss of cell–cell/cell–ECM interactions triggers the process of transdifferentiation, leading to the up-regulation of Pax6, which is independent of FGF during the first phase of transdifferentiation. However, FGF is a crucial regulator of the overall process of transdifferentiation, maintaining Pax6 and further driving RPE cells into neuronal progenitors.

These studies highlight the importance of environmental cues, cell–cell contact, cell–ECM interactions and their influence to successfully achieve retina regeneration via transdifferentiation.

Urodeles. The adult newt can regenerate its entire retina without induction from exogenous factors or without preserving the vascular membrane. Most importantly, the regenerated retina is fully functional [84,128–132]. The vast majority of newt retina regeneration occurs via RPE transdifferentiation; however, a small domain of the newly regenerated retina is derived from the CMZ (Figures 3A and 3C). Chiba et al. [133] analysed the dynamics of the protein RPE65 present in the RPE, which is involved in the recycling of visual pigments. In their work, the authors documented the presence of RPE65 via immunohistochemistry during the process of retina regeneration, and showed that RPE65 was present even after the RPE cells began to adopt a neural retinal fate [133]. However, their molecular analysis suggested that the presence of this protein during RPE transdifferentiation might represent lingering protein rather than newly synthesized protein. This elegantly marked the domain of RPE transdifferentiation compared with the regeneration domain derived from the CMZ, and a detailed description of the different stages of the transdifferentiation process was suggested to include early stages 1–3 (E1–3; days 10–19), intermediates stages 1–3 (1–3; days 19–23) and late stages (L1–2; days 45–65). Cell-cycle entry takes place at E1 (10 days after retinectomy). In addition, it was also clarified that there is a defined boundary at the CMZ, where no RPE65 protein was found [128,133].

The molecular mechanisms of these two regenerative processes are still being elucidated. A series of gene expression profiles has been performed during the different stages of newt retina regeneration [134–136] and these results are nicely summarized by Chiba and Mitashov [128]. Genes analysed include genes associated with retina stem/progenitor cells such as *pax6*, *chx10/vsx2*, *msi1* and *notch* (expressed early between E1 and E3) as well as with differentiating retina cells such as opsin and voltage-gated Na^+ channel $CpNa_V1$ (expressed from intermediate stage 2 onwards). Further studies were performed with Msi1 (Mushashi-1), a key regulatory molecule expressed in the retina and necessary for photoreceptor survival [137]. *msi1* is expressed in mature newt RPE, stem cells and photoreceptor cells. Upon retinectomy, RPE-transdifferentiating cells express *msi1* in the nucleus and the cytoplasm. However, renewing RPE has less *msi1* expression, as does the differentiated RPE as it transitions into neural retinal cells. Finally, the expression of *msi1* re-establishes in the photoreceptor cells of the newly regenerated retina [138].

Nakamura and Chiba [135] suggested that the Notch signalling pathway plays a role in the process of transdifferentiation. This group analysed and compared the expression patterns of *Notch-1* via *in situ* hybridization in developing retina, as well as during RPE transdifferentiation, concluding that expression patterns in both processes are very similar, localizing to the early forming neuroepithelium, and eventually becoming restricted to the peripheral retina. No expression was found in the adult newt eye. Interestingly, expression of other components of the Notch signalling pathway such as *delta1* and *hes1* were expressed in the adult RPE. Using a pharmacological inhibitor for Notch during the process of RPE transdifferentiation, Nakamura and Chiba [135] were able to detect premature neural differentiation, implicating the Notch pathway in RPC maintenance and inhibition of retinal differentiation.

Na^+ channels have been used to monitor ganglion cell differentiation during RPE transdifferentiation [129,130,139]. In contrast, a study by Vergara et al. [140] reported that the α subunit of the Na^+/K^+-ATPase is up-regulated transiently in the RPE during early transdifferentiation when retinal progenitors form, but is not present in the intact RPE or neural retina. It must be noted that more research is required to elucidate further the role of these channels/transporters during retina regeneration.

In vitro culture systems have been designed by several groups to help to dissect the cellular and molecular mechanisms of RPE transdifferentiation ([141–143], and reviewed in [107]). Mitsuda et al. [144] reported that RPE transdifferentiation *in vitro* is limited to RPE cells/explants that also have a choroid or a source of FGF. However, just separating the RPE from the choroid is sufficient to push the RPE cells to enter the S-phase of the cell cycle [141]. Organ cultures of RPE still connected to the choroid named 'retina-less eye cup' have been used recently to more closely mimic what takes place *in vivo* [142]. Using this system, it was determined that cell-cycle entry is mediated via MAPK signalling and that this activity is modulated by heparin that can affect the overall influence of Wnt-, Shh- and thrombin-mediated pathways. These pathways are able to regulate cell-cycle entry, although more studies must be conducted to determine the precise regulation [142].

Birds

The embryonic chick is able to regenerate its retina via RPE transdifferentiation during a small window of its development between stages 23 and 25 (E3.5–E4.5). A unique feature of this type of transdifferentiation is that the RPE does not replenish itself and the neuroepithelium formed, in consequence, will give rise to a retina with a flipped orientation (Figure 3B). After this stage, the RPE loses its plasticity and is unable to regenerate retina. RPE transdifferentiation is dependent on the presence of exogenous FGF2 [119,145,146]. The molecular mechanism by which FGF2 induces transdifferentiation includes the activation of the MAPK pathway and the up-regulation of Pax6 in the transdifferentiating RPE cells [147]. Furthermore, it has been demonstrated that Shh inhibits this FGF-induced transdifferentation [119]. Recently, the Wnt signalling pathway has been shown to protect the RPE phenotype and, when absent, RPE transdifferentiation can take place (K. Del Rio-Tsonis, unpublished work).

Although there is a restrictive period in which RPE transdifferentiation (up to E4–E4.5) can occur, it has been suggested that this period could be lengthened. Sakami et al. [148] cultured RPE explants from E5 chicks and inhibited activin signalling, extending the time window of transdifferentation *in vitro*. Activin is an integral part of the TGFβ signalling pathway and a key molecule for maintaining the RPE fate. Other studies performed *in vitro* have shown that dissociated RPE cells can be induced to transdifferentiate to retinal neurons, when certain key factors are overexpressed, including *NeuroD*, *Six6*/*Optx2*, *Ash1*, *Sox2*, *neurog1*, *neurog2*, *neurog3* or *Ath5*

[149,150]. For example, RPE reprogramming via neurogenin 1 in embryonic chick cultures primarily generate photoreceptor-like neurons that show the presence of photoreceptor markers as well as key genes in photoreceptor development and components of the phototransduction pathway. These photoreceptor-like cells respond to light exposure in the same way that photoreceptor cells do, and develop inner segments rich in mitochondria, a characteristic of mature photoreceptor cells [151].

The transdifferentiation process in the chick has been studied analysing the dynamic expression of several molecules, and by comparing the differences and similarities between RPE transdifferentiation and regeneration via the activation cells from the CMZ. However, the complexity of these processes makes the analysis of the molecular mechanisms implicated in transdifferentiation difficult. To study the molecular mechanisms of transdifferentiation, it is important to keep in mind the gradual change of cell fates, the timing of cell-cycle entry, the rate of synthesis and degradation of certain proteins, and the time point at which analysis is performed.

Regeneration via Müller glia

Müller glia represent supportive cells localized in the neural retina that function as a structural support and nourishment for neurons. They can also act as neurotransmitter transporters and as immune-response modulators [152–155]. However, Müller glia can also serve as a source of new retina neurons and play a critical role in retina regeneration in several vertebrate species.

Fish

In fish, Müller glia cells are the main source of retina regeneration. Müller glia can give rise to two different progenitor cell populations: rod precursor cells and RPCs in the INL. Rod precursor cells are produced continuously in the adult fish to keep up with the demands of the continuous growth of the retina [95,96]. However, upon injury, Müller glia cells dedifferentiate and proliferate generating multipotent progenitor cells that are ultimately capable of differentiating to all retina cell types (Figure 3D). When rod precursor cells are produced, they migrate into the photoreceptor layer and once they reach the ONL, they will only differentiate into rod cells, whereas the multipotent progenitor cells can differentiate into all retinal cell types [98,108,110,156–159].

Recent work has concentrated on elucidating the molecular mechanism regulating this regenerative process. Some key molecules include Ascl1a, Pax6b, c-Mycb, PCNA (proliferating-cell nuclear antigen), Rx, Chx10/Vsx2, Dkk, Notch, HB-EGF (heparin-binding epidermal-like growth factor) and *Lin-28* via *let-7* miRNA [100,160–165]. Upon retinal injury in zebrafish, HB-EGF is up-regulated in Müller glia. This secreted factor signals via its receptor through a MAPK signalling pathway and in turn regulates the expression of *Ascl1a*, *Lin-28*, *Notch* and *Dkk*, resulting in dedifferentiation and proliferation of Müller glia cells [165]. Thummel et al. [164] sought to study more precisely the role of Pax6 during zebrafish retina regeneration. Knocking down the two isoforms of *Pax6*, they found that, whereas the two isoforms have no effect on Müller glial cell division, the knockdown prevents Müller glia-derived INL neuronal progenitor cell division; specifically, Pax6b regulates the first cell division of neuronal progenitors, and Pax6a regulates later progenitor cell divisions. Interestingly, Ramachandran et al. [162] claim that Müller glia cells express markers of iPSCs, such as *Oct4*, *Klf4* and c-*Myc* [162]. The findings described above demonstrate the need for a cross-talk between several pathways/molecules that activate the molecular switch responsible for the reprogramming of Müller glia to retinal neurons.

Birds

Müller glia cells were first reported to be a source of neuroprogenitor cells in the postnatal chicken [166]. Müller glia-derived neuroprogenitors were observed after retina injury, or via injection of cytotoxic compounds such as NMDA (*N*-methyl-D-aspartate), or even in the presence of exogenous growth factors, such as insulin or FGF injected into the eye cup. Müller glia cells respond to these stimuli by re-entering the cell cycle, dedifferentiating and expressing progenitor markers such as *Pax6*, *Chx10*/*Vsx2* and *Cash1* [166]. Previous studies have tried to elucidate the molecular mechanisms of Müller glia cell activation in birds during retinal damage [167–171]. It is clear that the FGF/MAPK pathway as well as the Notch pathway play a role in the dedifferentiation process as well as in the differentiation of the Müller glia-derived progenitors ([168–171], and reviewed in [167]).

The neurogenic potential of Müller glia is very limited. Upon retinal damage, approximately 10 % of the progenitor cells derived from Müller glia give rise to amacrine and bipolar cells, but, in the presence of FGF or insulin, ganglion-like cells are produced. Interestingly, Müller glia cells have a higher capacity to proliferate and differentiate in the peripheral region of the retina than in the central retina. On the other hand, of the Müller glia cells that enter the cell cycle, only a few express progenitor markers, and over 80 % of the Müller glia-derived progenitors do not differentiate [166,167].

Mammals

The regenerative properties of mammals are restricted in comparison with other animals. For a long time, it was considered that the retina of adult mammals had no regenerative properties. However, strong evidence suggests that Müller glia cells are capable of responding to injury by dedifferentiating and proliferating, leading to retinal neurogenesis. In adult rats, Müller glia cells dedifferentiate and proliferate in response to the neurotransmitter NMDA, producing a limited number of bipolar cells and photoreceptors [172]. Moreover, when homeobox and bHLH (basic helix–loop–helix) genes are overexpressed in retinal explants from postnatal rats, Müller glia cells were able to give rise to several retinal cell types. On the basis of the knowledge that the Wnt signalling pathway is able to regulate stem cell populations, Osakada et al. [173] analysed the role of the Wnt pathway during Müller glia-induced retina regeneration in adult rats. In this study, the activation of the WNT pathway by Wnt3a or GSK3β (glycogen synthase kinase-3β) inhibitors promoted the proliferation of Müller glia [173].

Interestingly, enriched purified rat Müller glia can form neurospheres when induced by FGF2, and these neurospheres have the potential to give rise to retinal neurons and glia. In addition, transplanted enriched Müller glia into postnatal day 1 rat retina, gave rise to lamina-specific retinal neurons [174]. Adult human retinal explants treated with EGF showed that cells from the most anterior region of the retina are able to proliferate and express markers of Müller glia and stem cells, suggesting that cells in this region of the human retina are normally dormant, but have regenerative potential. This region corresponds to the CMZ region present in lower vertebrates [175]. Furthermore, human Müller glia-derived cell lines treated with FGF2 or RA (retinoic acid) are able to express retinal progenitor markers, such as *Pax6*, *Chx10*/*Vsx2* and *Sox-2* [176]. On the other hand, Shh has been

identified as a participant in Müller glia transdifferentiation both *in vivo* and *in vitro* models by up-regulating markers of retinal progenitors. Post-retinal injury studies in rat also demonstrate that Shh promotes Müller glia activation and directs retinal progenitors to differentiate towards the photoreceptor linage [177].

Intriguingly, Bhatia et al. [178] examined the differences between the two cell populations that can carry out neurogenic and proliferative activity in the adult mammalian eye: Müller glia of the neural retina and the CE (ciliary epithelium). The morphology of the two cell populations differed: the non-pigmented CE showed epithelial morphology and MSCs (Müller stem cells) displayed neural morphology. Although both non-pigmented CE and MSCs possessed neural progenitor markers, only MSCs showed the neural stem cell marker Nestin. This study also demonstrated that MSC have a superior proliferative ability when compared with the cells of the CE and this suggests that MSCs could be a potential source for retinal neuron transplantation [178].

SUMMARY

The regenerative processes for lens and retina reviewed in the present paper share some common cellular and molecular mechanisms, including cell signalling pathways, Pax6 transcription factor as a central molecule for the eye and the regenerative process, the transdifferentiation process for tissue regeneration, the role of ECM proteins and finally the evolutionary mechanisms conserved in several animal models.

During the process of lens and retina regeneration, the activation of cell signalling pathways such as FGF/MAPK, Wnt and BMP seems to be conserved, especially the activation of the FGF/MAPK pathway which eventually can regulate Pax6 [49,118,141,147]. Despite the similarities in signalling pathways involved in lens and retina regeneration, the regeneration potential is highly dependent on the sequence and timing of these signalling events and the diverse activation of downstream targets for each pathway that are ultimately defined by the origin and, accordingly, the differentiation state of the cells that will undergo regeneration. For example, during IPE transdifferentiation in the adult newt, the distinct timing and versatile expression of FGF family members is critical, e.g. *fgf2* in dorsal compared with ventral iris [49,179]. In *Xenopus* CLT or chick RPE transdifferentiation, on the other hand, the regenerative ability is limited to certain larval/embryonic stages, even though these processes share an FGF-dependent induction with further participation of the BMP, Shh and Wnt pathways [17,21,22,118,119,147,180].

The activation of some of these signalling pathways converges and eventually targets common downstream effectors such as the Pax6 transcription factor [49,118,141,147]. The activation of Pax6 seems to be a crucial event for both lens and retina regeneration. The *Pax6* gene is highly conserved through the evolution of the animal kingdom from invertebrates to vertebrates, stressing its critical role in eye development [181,182]. The importance of Pax6 in eye development has been well documented; Pax6 has been described as the master regulator of eye development on the basis of gain-of-function experiments or overexpression studies inducing ectopic eyes on appendages [183]. The complex regulation of Pax6 is still being investigated owing to its wide interaction with several gene networks.

It is interesting to note that the contribution of ECM is one more common mechanism for lens and retina regeneration. It is important to highlight that the presence of ECM proteins is critical and sometimes necessary to achieve these regenerative processes, particularly during transdifferentiation [60,104,125]. However, to date, it remains to be determined whether wound healing generated matrix provides the initial signal for lens or retinal regeneration, or if the regeneration of these organs is triggered by a mechanism that is intrinsic to the corresponding tissue of origin.

The transdifferentiation process seems to be one of the common cellular mechanisms that regulates tissue regeneration in lens and retina [12,17,22,115,124,146,147,184]. Understanding the correlation of morphological stages that go along with key molecular changes and their corresponding epigenetic code will help to unravel the molecular complexity of transdifferentiation. The role that the epigenetic code has on controlling the chromatin and DNA status still has to be explored further in the field of tissue regeneration. The current findings in the field of epigenetic regulation have influenced the study of the mechanisms implicated in the maintenance of cell fate and cell reprogramming promoting eye tissue regeneration [66,185].

Finally, research has elucidated that certain animals during their embryonic stages have the potential for lens or retina regeneration; however, this capacity is frequently lost postnatally except in certain lower vertebrates. It is interesting how the mechanisms for lens and retina regeneration are also conserved through evolution in several animals [84,186,187], particularly how lower vertebrates have been able to maintain their regenerative mechanisms, in contrast with higher vertebrates, such as mammals that, at some point during the course of evolution, have reduced their regenerative abilities. In this regard, we should consider that the process of specialization through evolution has contributed to the fact that mammals during this process lost some regenerative capabilities. Moreover, there is the possibility that the regenerative capabilities are still present in mammals, but are repressed. Recent advances in genome and transcriptome expression profiling, the availability of tools to study epigenetic regulation, as well as the application of siRNA (small interfering RNA) technology and morpholino knockdown represent promising tools to study the complex mechanism of lens and retina regeneration in vertebrates. Continued research in the field of eye tissue regeneration needs to be conducted to elucidate further the various mechanisms and molecules involved, in order to eventually lead to therapies to cure human lens and retina degenerative diseases. The knowledge of the regenerative processes in lower vertebrates may lead us to understand or provide the knowledge to activate the regenerative properties lost in other organisms.

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