Regeneration of the amphibian retina: Role of tissue interaction and related signaling molecules on RPE transdifferentiation

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Regeneration of eye tissue is one of the classic subjects in developmental biology and it is now being vigorously studied to reveal the cellular and molecular mechanisms involved. Although many experimental animal models have been studied, there may be a common basic mechanism that governs retinal regeneration. This can also control ocular development, suggesting the existence of a common principle between the development and regeneration of eye tissues. This notion is now becoming more widely accepted by recent studies on the genetic regulation of ocular development. Retinal regeneration can take place in a variety of vertebrates including fish, amphibians and birds. The newt, however, has been considered to be the sole animal that can regenerate the whole retina after the complete removal of the retina. We recently discovered that the anuran amphibian also retains a similar ability in the mature stage, suggesting the possibility that such a potential could be found in other animal species. In the present review article, retinal regeneration of amphibians (the newt and *Xenopus laevis*) and avian embryos are described, with a particular focus on transdifferentiation of retinal pigmented epithelium. One of the recent progresses in this field is the availability of tissue culture methods to analyze the initial process of transdifferentiation, and this enables us to compare the proliferation and neural differentiation of retinal pigmented epithelial cells from various animal species under the same conditions. It was revealed that tissue interactions between the retinal pigmented epithelium and underlying connective tissues (the choroid) play a substantial role in transdifferentiation and that this is mediated by a diffusible signal such as fibroblast growth factor 2. We propose that tissue interaction, particularly mesenchyme–neuroepithelial interaction, is considered to play a fundamental role both in retinal development and regeneration.

**Key words:** newt, retina regeneration, transdifferentiation, *Xenopus laevis*.

Brief overview of retinal development, histogenesis and cell differentiation

Eye development has been one of the most extensively investigated subjects in developmental biology over the last century. Regeneration of eye tissues, including the lens or the retina, has continuously drawn interest from embryologists, in parallel with ocular developmental studies. For a better understanding of the mechanisms underlying the processes of retinal regeneration, a brief description of eye development will be given first.

Recent progress in the genetic analysis of vertebrate eye development has elucidated a number of genes involved in ocular development and their ordered expression patterns (Chow & Lang 2001; Bailey *et al.* 2004; Hatakeyama & Kageyama 2004). Retinal development is one of the most intensely studied subjects among various organogenesis phenomena and includes many topics such as fate determination, cell proliferation, precursor cell differentiation, organized layer formation, projection mapping and so on (Adler 2000; Kondoh 2002).

The retina initially develops as a lateral protrusion of the forebrain called the optic vesicle, which further develops to form a double-layered optic cup by invagination (Fig. 1). Subsequently, the optic cup
develops into two different regions; the neural retina (NR) and the retinal pigment epithelium (RPE), by inductive signals from neighboring tissues (Saha et al. 1992; Chow & Lang 2001). At the same time, the invagination of the optic vesicle extends from the distal to the proximal direction at the ventral part to form the future optic fissure in the ventral optic stalk, through which the retinal ganglion axons would pass into the optic tectum. Thus, the early optic vesicle appears to consist of at least two discrete dorsal and ventral compartments that show different developmental fates. This is also suggested by the different expression patterns of various transcription factors (Shulte et al. 1999; Koshiba-Takeuchi et al. 2000) and by reverse transplantation along the dorsoventral polarity (Uemonsa et al. 2002; Kagiyama et al. 2005). These studies indicate that the establishment of D-V polarity in the optic vesicle plays an essential role in optic cup formation, the patterning and differentiation of NR and RPE. The optic vesicle makes contact with various tissues, such as the overlying surface ectoderm and pericellular mesenchyme, and it has been assumed that the specification of the NR and RPE is influenced by signals from the surface ectoderm and mesenchyme, respectively (Fig. 1) (Dragomirov 1937; Lopashov 1963; Johnston et al. 1979; Hyer et al. 1998; Fuhrmann et al. 2000).

Recent studies have elucidated numerous signaling molecules that have critical roles both in retinal development and regeneration (Moshiri et al. 2004). In the present paper, we describe how these molecules are involved in the processes of retinal development and regeneration, with particular emphasis on tissue interactions and related signaling molecules.

**Regeneration of vertebrate retina**

Retinal regeneration can take place in a variety of vertebrates including fish, amphibians, birds and mammals, although it is restricted to certain stages of development in most animals (Reh & Fisher 2001; Del Rio-Tsonis & Tsonis 2003; Okada 2004). Only urodelian amphibians have been known to possess the ability, even in adulthood, to regenerate the whole retina after the complete removal of the retina; the cellular sources of regenerating retina in urodeles are the retinal pigment epithelial cells (RPE) which undergo transdifferentiation and differentiate into retinal progenitor cells (Fig. 2). Stem cells in the ciliary marginal zone (CMZ) that contribute to the ongoing growth of the retina also participate to some degree in retinal regeneration in these animals. Retinal regeneration after the removal of the whole retina occurs in embryonic stages of annuran amphibians and also in embryonic avian embryos. In the latter case, however, exogenous administration of growth factors is inevitable (Park & Hollenberg 1989).

Partial retina regeneration has been described in many adult animal species, such as fish, amphibians and birds. Cells sequestered in the retinal tissue, including rod precursors or Müller glial cells, are the source of regenerating retina in addition to retinal stem or precursor cells in the CMZ. In teleost fish such as goldfish, for instance, retinal regeneration occurs either after surgical removal of a portion of the central part of the retina or pharmacological lesioning by ouabain that destroys the neural retina but leaves rod cells intact (Hitchcock & Raymond 1992). The rod precursors, in addition to the stem-like cells located within the retina (in the inner nuclear layer), appear to be the origin of retinal regeneration. RPE cells, however, appear not to play any substantial role in these animals. Mammals also possess cells that can proliferate and differentiate into various types of retinal cells under certain conditions of cell cultures; these are iris epithelial cells or ciliary pigmented epithelial cells. Such an approach opens up the possibility of a clinically oriented autologous graft for retinal regeneration (Ahmad et al. 1999; Haruta et al. 2001; Fischer et al. 2002).
From these features it is apparent that retinal regeneration takes place by means of several different mechanisms and cell sources depending on the animal species. Retina can be replaced or repaired by RPE cells via the transdifferentiation process or by stem-like cells in the CMZ and/or within the retina. Different animals use different strategies and regeneration also depends on the age of the animal, although potent cells are retained in a wide variety of animals. In the following part of this review, I will focus on the transdifferentiation process of RPE cells that are the main source of retinal regeneration in the newt. Interestingly, we recently found for the first time that *X. laevis* retains the ability to regenerate the retina and lens following the surgical removal of these tissues even in mature animals (Fig. 5) (Yoshii et al. 2007). Because we still have very little information on the cellular and molecular mechanisms involved in amphibian RPE transdifferentiation (Eguchi 2004; Okada 2004), the findings of complete retinal regeneration in a different amphibian species provide a new tool for the molecular analysis of regulatory mechanisms involved in retinal regeneration.

**Amphibian retinal regeneration**

Complete retinal regeneration in adult animals following surgical removal of whole retinal tissue had been considered to take place only in certain urodele amphibians (Figs 2, 7(b)). This has been best studied in the newt, with over a century since the time when experimental studies of this phenomenon were initiated at the end of the 19th century (see reviews by Okada 1991; Mitashov 1997; Hitchcock et al. 2004). In contrast, it was considered that anuran amphibian species retain the ability only in the larval stages and that adult anurans in the postmetamorphic state lose it, retaining the ability to repair partially damaged retinal tissue (Lombardo 1969). Contrary to this generally accepted notion, we for the first time showed that *X. laevis* retains the ability to regenerate the retina and lens following the surgical removal of these tissues even in mature animals (Fig. 5) (Yoshii et al. 2007). Because we still have very little information on the cellular and molecular mechanisms involved in amphibian RPE transdifferentiation (Eguchi 2004; Okada 2004), the findings of complete retinal regeneration in a different amphibian species provide a new tool for the molecular analysis of regulatory mechanisms involved in retinal regeneration.

**Newt (urodele) retinal regeneration**

In the newt, RPE cells undergo transdifferentiation to produce all cell types constituting the neural retina.
Newt retinal regeneration has been fully documented in numerous studies (Stone 1950a,b; Hasegawa 1958; Reyer 1971, 1977). Recent studies have also suggested that many common features exist between the development and regeneration of the retina in gene expression of several transcription factors necessary for ocular development (Kaneko et al. 1999, 2001). The mode for cell-cell communication of RPE cells appears to alter during the early phase of regeneration (Haruta et al. 2001; Umino & Saito 2002), suggesting that cell adhesion molecule-mediated cell communication between RPE cells may play a role in regenerating events.

The fact that RPE cells become labeled with BrdU almost simultaneously at an interval of 4–5 days after surgical removal of the retina also indicates the significance of RPE cell communication (Fig. 2IJ) (Ikegami et al. 2002). Once they initiate cell division, RPE cells produce retinal stem cells. Therefore, the initial period of 4–5 days is a sort of ‘latent period’, in which several genes must be activated for the sequential events of regeneration. An attempt to find early activated genes has been made and several candidate genes are described, although none have yet been identified as master control genes for retinal regeneration (Goto et al. 2006).

In vitro culture studies are definitely essential to elucidate the mechanisms involved in newt RPE transdifferentiation. RPE cells appear to occasionally differentiate into cells with a certain neural phenotype such as a neuron with Na ion channel under a dissociate cell culture condition (Sakai & Saito 1994). We have developed a new culture method suitable for the analysis of cell and molecular mechanisms, in which RPE epithelial sheets were cultured on the culture membrane filter (Fig. 3) (Ikegami et al. 2002). Under the organotypic culture condition (a tissue consisting of RPE and choroid was used), newt RPE cells were shown to undergo transdifferentiation. RPE cells in the epithelial sheet proliferated, migrated out of the tissue explant onto the filter membrane and finally differentiated into those that have neuronal morphology with several different types of retinal phenotypes. This work was significant in that it enabled us to compare cell properties of RPE from various animal models under the same in vitro conditions, and it was found that fibroblast growth factor 2 (FGF2) is a potent factor in stimulating RPE transdifferentiation in the newt as well as in embryonic avian tissues (Araki et al. 1998).

Interestingly, RPE transdifferentiation into neural cells can be seen only when they are cultured with the underlying connective tissue (the choroid). RPE cells do not proliferate or differentiate into neural cells when cultured alone (Fig. 3) (Mitsuda et al. 2005). The results indicate that the presence of the choroid is essential for RPE cells to proliferate and differentiate.
into neural cells, and suggest the possibility that the choroid plays an essential role in newt retinal regeneration probably by means of diffusible substances. An in vitro pharmacological approach indicates that FGF signaling is actually most critical in RPE cell transdifferentiation. Insulin-like growth factor (IGF)1 plays some supplementary role in the newt, as has been shown in the case of chick retinal regeneration (Fisher & Reh 2001); IGF1 alone has no effect on RPE cells but it has a strong synergistic effect when added together with FGF2. Reverse transcription-polymerase chain reaction (RT-PCR) of in vivo regenerating newt eyes shows that expression of both FGF2 and IGF1 are upregulated in the choroid after retinal removal (Mitsuda et al. 2005).

Whether or not in vitro cultured RPE cells have the potency to reconstitute the retinal structure has not yet been shown, but the intraocular transplantation of cultured RPE tissues suggest they have that potency. When RPE cells cultured with the choroid were transplanted into the posterior eye chamber, they regenerated the neural retinal layer in a similar manner as they do in the normal regeneration process (Fig. 4) (Mitsuda et al. 2005). When cultured RPE sheets alone were transplanted in the same way, they did not change but still remained pigmented (Mitsuda et al. 2005). These results indicate that cultured RPE cells retain the capability to develop the retinal structure under a culture condition. Further studies are still necessary to reveal what substances play roles in retinal regeneration, which includes RPE transdifferentiation followed by the formation of a multilayered structure.

Although the most important question has not yet been answered (why the removal of the retina from the newt eye triggers RPE cells to initiate transdifferentiation), the above studies revealed for the first time that the tissue interaction between the RPE and the choroid plays an essential role in newt RPE transdifferentiation. There are several possibilities to address the question. One preferable possibility is that the persistence of the intact retina–RPE tissue interaction may inhibit RPE cells from responding to the choroid-derived factors by suppressing the FGF signal pathway within RPE cells. Alternatively, the removal of the retina may cause the choroid cells to initiate FGF gene expression. Although there is no information to address these theories, the latter possibility may not be involved in the present case, because the retina is a rich source of FGFs and there must be some repressive mechanism in RPE cells not to respond to FGFs produced by the retina. It is noteworthy to mention once again that there is a latent period (4–5 days) before RPE cells initiate DNA synthesis (BrdU uptake) during both in vivo regeneration and in vitro transdifferentiation. Cultured RPE cells also become susceptible to FGF2 only after day 5 of culturing (Mitsuda et al. 2005). These data suggest that RPE cells need some extra time before they become ready to start regeneration only after the onset of necessary gene expression such as FGF receptors. It is of interest to determine which step in the FGF signaling pathway is the crucial one and how signal transduction might be suppressed under normal conditions.

Anuran amphibian retinal regeneration

It has long been considered that anurans are unable to regenerate the retina after metamorphosis when the whole retina is surgically removed. Although studies on anurans are not as conclusive, in tadpoles of Bombinator, Bufo or some species of Rana, regeneration does not occur (review by Reyer 1977; Del Rio-Tsonis & Tsonis 2003; Hitchcock et al. 2004). Ocular transplantation studies, however, showed that RPE sheets grafted in the posterior eye chamber undergo metaplasia and form a new retina in some species of Rana or Xenopus (Sologub 1977; Bosco 1988). In vitro studies revealed that RPE from X. laevis larva does transdifferentiate under the effect of FGF2 (Sakaguchi et al. 1997). When the retina of Rana tadpoles is devascularized by complete removal of the eyeball and restored into the orbit, new retina

![Fig. 4. Intraocular transplantation of cultured retinal pigmented epithelium (RPE). RPE with the adhering tissues (the choroid and the sclera) was cultured for 10 days and then transplanted into the newt eye chamber. A part of Figure 5A is shown at higher magnification in (B). Well defined structures of RPE (black arrow) and developing retina are observed. The yellow arrow indicates the Bruch membrane. Ch, choroids; S, sclera (from Mitsuda et al. 2005).](image-url)
Regenerates following retina degeneration (Reh & Nagy 1987). A partial retinal regeneration was reported in adult *Rana* when a radial incision or partial retinal removal was made (Lombardo 1969). These studies indicate that some anurans retain the ability to regenerate the retina under certain conditions and that this ability is mostly lost after metamorphosis.

While we were culturing *Xenopus laevis* RPE to see if RPE cells of mature *X. laevis* transdifferentiate into neural cells as we had observed in the newt, we found that *X. laevis* RPE cells differentiate into neural cells similarly to the newt RPE. When RPE sheets were isolated from the choroid and cultured, RPE cells did not differentiate into neural cells and remained epithelial although they were proliferating. FGF2 administration induced neural cell differentiation in singly isolated RPE cultures. These observations led us to re-examine the ability of retinal regeneration in *X. laevis* after metamorphosis. We found that regeneration can take place in mature animals following surgical removal of the whole retina (Figs 5, 7(a)) (Yoshii et al. 2007). This was the first conclusive result that vertebrate species other than the urodelian amphibians have regeneration ability at the mature stage following the complete loss of the retina. Under the same methodological conditions of the surgical operation for retinal removal and of organ cultures between the two animals, the newt (*Cynops pyrrhogaster*) and the *X. laevis*, results can be now significantly evaluated by comparing them with each other.

In *in vitro* studies revealed that *Xenopus* RPE cells can transdifferentiate into neural cells under the effect of the choroids, as was confirmed in the newt. A question then arises as to whether or not transdifferentiation of RPE cells also plays the main role in *Xenopus* retina regeneration. We surgically removed the retina from *X. laevis* of 1–8 months after metamorphosis to see how retina regenerates; whether in the same mode as seen in the newt or through a different process (Fig. 5). Between day 7 and day 12 after the operation, a newly formed pigmented cell layer was always found in the vitreous space, facing the RPE layer or a little distance apart from the RPE layer. Subsequently, this newly formed layer appeared to become thicker and less pigmented, although some cells in the layer were still pigmented. These pigmented...
cells in the new epithelial layer are derived from RPE cells because of their positive staining for RPE65 antibody (Fig. 6) (retinal pigmented cell-specific marker). They were also immunoreactive to Pax6 antibody. BrdU uptake and proliferative cell nuclear antigen (PCNA) immunoreactivity also suggest they were proliferative. How can RPE cells form a new epithelial layer in the eye chamber? Because numerous single pigmented cells were observed in the space between the newly formed and the original RPE layers, we speculate that some RPE cells detach from the RPE layer which then migrate inwardly. A retinal vascular membrane (RVM) consisting of the inner limiting membrane (basement membrane) and capillaries will supply an anchoring base for migrating RPE cells, because in cases when the RVM was intentionally removed, no pigmented layer was formed, resulting in no regeneration. The RVM is laminin-immunoreactive, but for the moment, we do not know about the molecular nature characteristic of RVM. An important question remains to be solved as to why RPE cells at the original RPE layer do not transdifferentiate in situ and instead only do so after migrating to the RVM. Our culture study indicates that some extracellular matrix (ECM) components are needed for RPE cell transdifferentiation and formation of a retinal stratified structure.

Cells in the CMZ also contribute to the regenerating retina in X. laevis by adding retinal precursor cells at the periphery of the RVM. At the early stage of regeneration (about day 10), the RVM is covered by non-pigmented multilayered epithelium at the periphery (close to the CMZ) and pigmented single epithelium at the central part. Although the extension of CMZ-derived epithelium differs from animal to animal, this suggests that retinal progenitors in CMZ migrate on the RVM and form a substantial part of the regenerating retina.

Finally, it is noteworthy that the lens also regenerates in X. laevis after metamorphosis, although more precise experiments are needed (Yoshii et al. 2007). When the lens is surgically removed, a new lens is regenerated 20–30 days later. The retina appears to have stimulative effects for lens regeneration because removal of both the lens and retina delays lens regeneration. In tadpoles, it has been reported that the lens can be regenerated from the cornea (Filoni et al. 1997), but in the present cases, the corneal tissue appears to be intact and no morphological alteration can be observed. We speculate that the remaining lens epithelial cells, which are very small in number, that attach to the lens capsule are the source of regenerating lens.

Transdifferentiation of retinal pigmented epithelium into retinal fate in developing chick embryos: Avian model of retinal regeneration

A similar retinal regeneration from RPE cells through transdifferentiation also occurs in some avian species, as already mentioned, but the regenerative capacity
is strictly limited to the early stages of embryonic development and also require exogenously administered factors such as FGFs for the onset of transdifferentiation (Park & Hollenberg 1989; Araki et al. 1998; Del Rio-Tsonis & Tsonis 2003). No RPE layer develops again and all RPE cells transdifferentiate into the retina. Thus, there are many fundamental differences between amphibians and birds.

The developing chick embryo has been studied as a model for retinal regeneration; by removing neural retina in ovo from early embryos, RPE cells start to proliferate and enter into neural retinal fate. There are several fundamental differences in the phenomena between the amphibian and the chick; all of the RPE cells finally transform into a retinal layer with reverse polarity (photoreceptor layer is located at the innermost position facing the vitreous fluid and ganglion cells are in contact with the choroid) and they do not transform into RPE again (Coulombre & Coulombre 1965, 1970). This process of transdifferentiation can be seen only in early embryos (up to about 5 days of incubation) and requires some neural derivatives like a fragment of retinal tissue to be left in the eye chamber. The molecular nature of substances that activate RPE cells to initiate transdifferentiation was unknown for a long time until Park and Hollenberg (1989, 1991) reported that intraocularly administered FGF2 actually triggers RPE transdifferentiation. In vitro studies also revealed that FGF2 causes RPE cells to proliferate and redifferentiate into neurons (Pittack et al. 1997). All of the RPE cells differentiate into neural cells in vitro as seen in vivo, and this contrasts with cultures of amphibian RPE where some RPE cells remain as RPE cells and others migrate and transdifferentiate into neural cells (Araki et al. 1998). FGF2-induced RPE transdifferentiation into retinal tissue was also reported in Xenopus embryonic RPE cultures (Sakaguchi et al. 1997), suggesting that FGF2 is a substance widely functioning in RPE transdifferentiation. This is not surprising if we consider that FGFs serve as inductive signals for NR development in the embryonic optic vesicle (Hyer et al. 1998; Zhao et al. 2001; Spence et al. 2004).
RPE transdifferentiation is also reported in mammalian embryonic cultures, but this occurs only in the very early stage of mouse development (E13 to E14, the optic cup stage) under the effect of FGFs (Zhao et al. 1995). Because the outer layer of the optic cup at this stage has not yet expressed RPE properties, this is a phenomenon of change in fate determination. Whether or not mammalian RPE retains its transdifferentiation ability in more advanced developmental stages is still not clear, but several in vitro studies have indicated that RPE at later stages quickly becomes unable to fully transdifferentiate (Neill & Barnstable 1990).

The regulatory mechanism involved in RPE transdifferentiation has been studied intensively at the molecular level. Mitf, a basic helix-loop-helix-leucine-zipper (bHLHzip) protein, plays a substantial role in the fate determination of RPE cells. Retrovirus-mediated overexpression of Mitf inhibits FGF2 induced transdifferentiation of cultured chick RPE cells which would otherwise transdifferentiate into both the lens and neural retina, indicating that the downregulation of Mitf expression is essential for RPE transdifferentiation (Mochii et al. 1998a). A link between the intracellular FGF signaling pathway and Mitf has been studied in the developing eye (Nguyen & Arnheiter 2000; Zhao et al. 2001), and developmental conversion of RPE into neural retina can be mimicked by the activation of downstream intracellular targets of FGF receptors that regulate Mitf expression (Galy et al. 2002).

While tissue interaction between the mesenchymal tissue (the choroid) and RPE plays an important role in amphibian RPE transdifferentiation, it is unknown if a similar tissue interaction plays any role in the RPE transdifferentiation of avian embryos. Under a culture condition of RPE sheets purely isolated from 4-day-old chick embryos, RPE cells are much less susceptible to FGF2 than those combined with the underlying periocular connective tissues (Araki et al. 1998). A study using a unique avian mutant, Silver quail homozygote, shows that such a tissue interaction also has a significant role in RPE transdifferentiation.

**Avian mutant model for retinal pigmented epithelium transdifferentiation**

Another example of a choroid–RPE tissue interaction which plays a role in RPE transdifferentiation comes from a quail mutant, Silver (B/B) (Fig. 8) (Homma et al. 1969; Fuji & Wakasugi 1993). The Silver homozygote mutant has a clear phenotype of plumage color, indicating that melanogenesis in feather melanocytes is affected. Interestingly, the Silver homozygote also has a unique eye defect; during embryonic development, RPE cells autonomously transdifferentiate to neural retina in the homozygous mutant, resulting in a double layered NR at the posterior region of the eye (one original and the other newly formed retinal layer). This transdifferentiation begins at about 4 days of incubation and occurs only at the posterior central region of the eye (Fig. 8) (Araki et al. 1998). The newly formed retinal layer has a reverse polarity and the photoreceptor cells are located at the innermost position, as seen in the experimentally transdifferentiated NR. Mitf from Silver homozygote has an amino acid substitution in the basic region and is truncated in the C-terminal region (Mochii et al. 1998b). Because Mitf is expressed in melanocytes, neural crest-derived cells, feather color is also affected. Mitf expression is found in several different tissues including RPE, neural crest cells, osteoclasts and mast cells. The fact that transdifferentiation occurs only in a limited portion of the RPE indicates that some specific tissue interactions between RPE and other neighboring tissues must take place in that area. This is supported by an in vitro experiment where the RPE layer from Silver mutant embryos was purely isolated from the choroid, a connective tissue, and cultured alone; RPE cells do not transdifferentiate into neural cells but they actually do when combined again with the remaining choroid (Araki et al. 1998).

In embryonic transplantation experiments, optic vesicles were excised from a donor quail mutant and then transplanted into the right place of the iso-chronic chick embryo from which the optic vesicle had been removed (Araki et al. 2002). This creates a chick embryo with one eye whose corneal epithelium, lens, neural retina and RPE are all derived from a donor quail optic vesicle of Silver homozygotes. Interestingly, the grafted optic vesicle developed normally without any RPE transdifferentiation. It was found that most of the cells in the choroid (fibroblastic cells and a few melanocytes) were those of the chick, indicating that choroid cells are responsible for the transdifferentiation phenomenon. The choroid develops from neural crest cells migrating towards the optic vesicle. In the Silver homozygote, neural crest migration was also affected and the periocular neural crest cells did not differentiate to the cartilaginous tissue (the sclera) (Araki et al. 2002). These results indicate that the choroid cells surrounding the RPE play a principal role in the fate determination of RPE cells and that periocular neural crest cells are affected by mutation and have some defect in conducting the development and maintenance of RPE.

It is unknown by what the tissue interaction is mediated; a diffusible substance or a kind of ECM.
suggested that soluble molecules like FGF2 is a critical mediator. This will be also the case in the Silver mutant quails as shown by culture studies. A question then arises as to whether the underlying connective tissue (the choroid) is just a supplier of FGF2 or has any other function in transdifferentiation. The basement membrane has a crucial role in the maintenance of cell properties and Bruch’s membrane forms an interface between RPE and the choroid. In the amphibian, retinal regeneration detachment from Bruch's membrane appears to be a prerequisite for transdifferentiation. An important problem to be solved is what substances in the ECM have the key role in this step.

Fig. 8. Transdifferentiation of retinal pigmented epithelium (RPE) into the retinal tissue in Silver quail homozygote. (A) Eye cups of day 15 homozygote embryos. At the left hand side of the figure is an eye of a Silver embryo and at the right is a wild type embryo. Both are left eyes. A circular non-pigmented area is seen in the Silver embryo at the nasal side from the pectene. (B) Micrograph of 8-day embryonic homozygote eye. The region indicated by an arrow shows a thickening of RPE epithelium and has already transdifferentiated to the retinal fate. (C) In a 13-day embryo, transdifferentiated neural retina shows well-organized cell layers with a reversed polarity. The original retina begins to degenerate. An arrowhead shows scleral cartilage. (D, E) Culture of RPE sheets from Silver homozygote embryos. Tissues were removed from day 3 embryos and the RPE sheet was separated from the neighboring tissue, the choroid and then cultured for 9–10 days on filter membranes in the absence (D) or presence of fibroblast growth factor (FGF)2 (E). In (D) the epithelial sheet remains densely pigmented and does not transdifferentiate. In the presence of FGF2 (E), RPE cells proliferate, depigment and then transdifferentiate into iodopsin-immunoreactive cone cells.
Conclusion and perspectives

We can find many common features in retinal regeneration of urodele and anuran amphibians, although RPE cells behave seemingly differently at the initial stage of regeneration (Fig. 7). The availability of culture methods enables us to evaluate the RPE transdifferentiation of both animal species under the same conditions. With this approach we can also compare the results from different species. Our observations of cultured RPE cells from both amphibian species indicate that there are no fundamental differences in the cellular processes, particularly in the initial step of transdifferentiation. The seemingly different cell behavior between the newt and Xenopus RPE cells is not due to cell intrinsic properties but reflects some differences in the extrinsic cues such as ECM components and/or neighboring cells. The avian model of RPE transdifferentiation also indicates the significance of tissue interaction between the RPE and the connective tissue (the choroid) underlying RPE. In contrast to numerous molecular biological studies on the development of ocular tissues, particularly on tissue interaction, only a few studies have been carried out at the gene expression level and in regulatory mechanisms of retinal regeneration. There are some common features in tissue interactions that control both retinal development and regeneration. Accumulating evidence, including both in vitro cultures and gene expression patterns, now makes it possible to compare various aspects of retinal development with those of regeneration. It is also becoming increasingly important to compare the aspects among a variety of animal models. This will make it possible to address the important question as to what mechanisms are involved in those vertebrates, including humans, which cannot regenerate the retina.

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