General discussion

bFGF and photoreceptor regeneration

Steinberg: In addition to Müller cells in the mammalian retina there is another support cell, the retinal pigment epithelial cell or RPE cell, about which we have heard from Professor Lopashov. This is a multipotential cell and it is also a multifunctional cell, in the mature retina. The RPE cell is a transporting cell, carrying metabolites to and from the subretinal space, which is the primitive third ventricle space. The RPE cell recycles vitamin A and isomerizes it during dark adaptation. This cell also functions as a macrophage, phagocytosing the shed tips of the outer segments from rods and cones, which replenish these discs on a daily basis.

That the outer segment grows every day also provides for its ability to regenerate. This is not regeneration of the whole photoreceptor, just of the outer segment. We can lose the outer segment, as occurs in retinal detachment when the neural retina becomes displaced from the RPE. The outer segment can regrow, as long as the inner segment and soma region survive. We don't know the physiological characteristics of this survival, or the degree of injury that will prevent this regeneration.

I want now to tell you about some recent research that I have been engaged in on the role of the basic fibroblast growth factor in photoreceptor regeneration and survival in the rat. This research was done in full collaboration with Dr Matthew M. LaVail and with a medical student at UCSF, Ella G. Faktorovich. Douglas Yasumura and Michael T. Matthes also contributed to these experiments*.

The retinal pigment epithelium contains basic fibroblast growth factor (bFGF) (Schweigerer et al 1987, Sternfeld et al 1989), which has been shown to act as a mitogen and differentiation-promoting factor in many systems. For instance, bFGF induces retinal regeneration from the RPE in the chick embryo (Park & Hollenberg 1989). Moreover, bFGF has been shown to act as a neurotrophic agent in several regions of the central nervous system (Sievers et al 1987, Anderson et al 1988, Otto et al 1989). To investigate its possible role as a survival factor in the vertebrate retina, we studied its effect in two conditions that lead to profound degeneration of photoreceptors in the rat—inherited retinal

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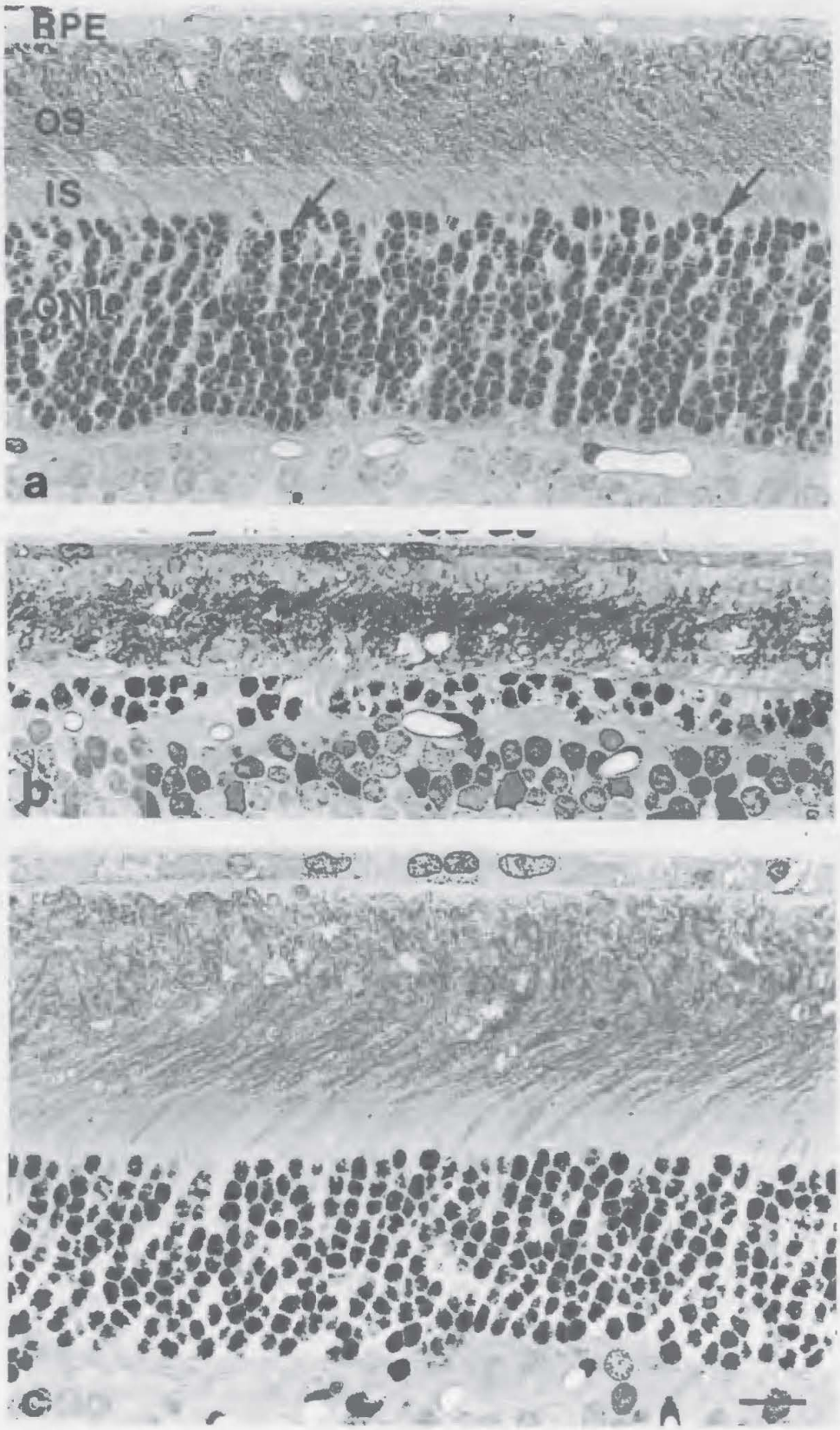
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dystrophy in the Royal College of Surgeons (RCS) rat (Mullen & LaVail 1976), and exposure to constant light in the albino rat (Noell et al 1966).

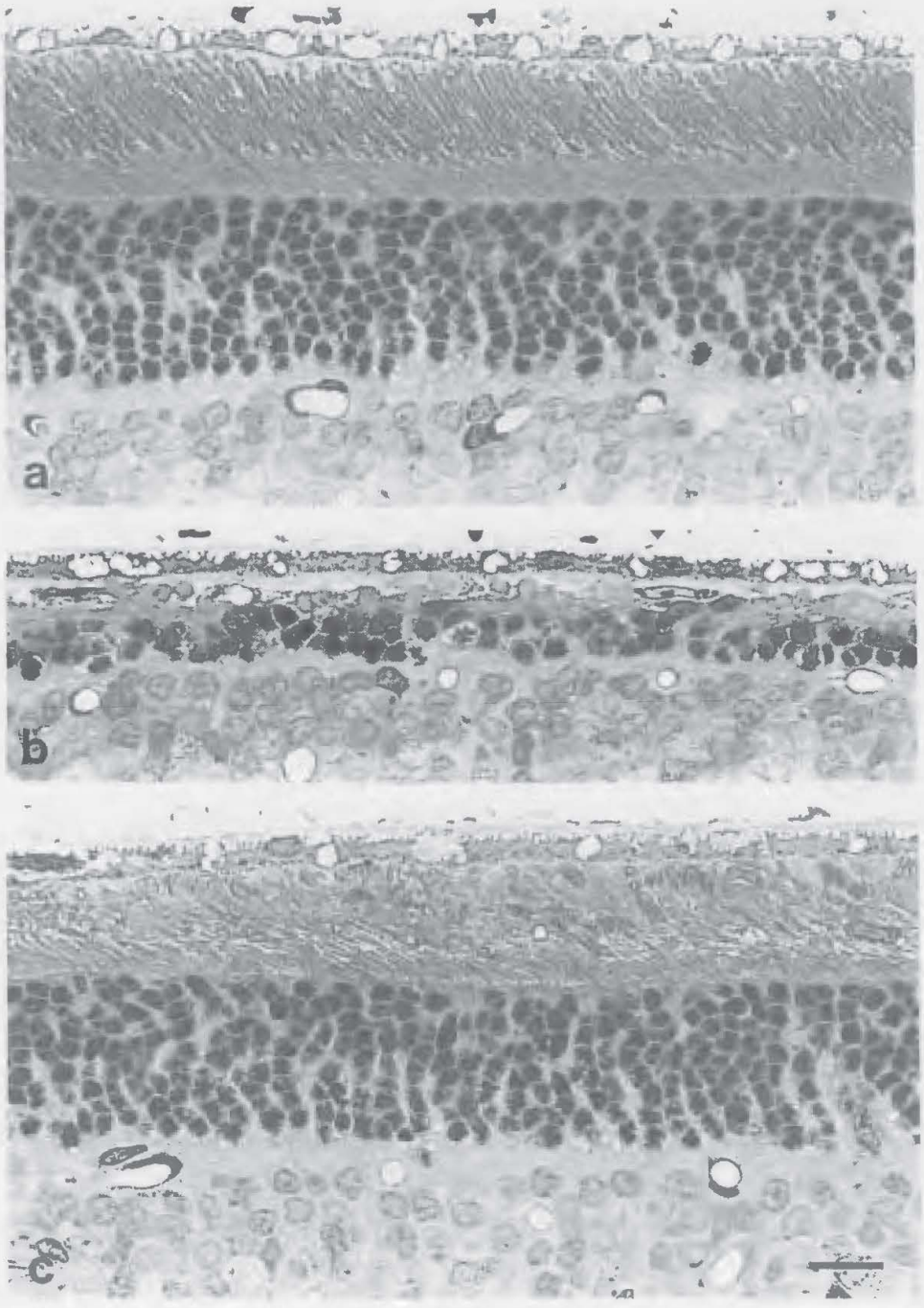
In the RCS rat (Faktorovich et al 1990) we transsclerally injected bFGF dissolved in phosphate-buffered saline (PBS) (500 or 820 ng in 1 μl) into the interphotoreceptor space or subretinal space of the superior hemisphere of the eye at postnatal day 23 (P23), when photoreceptors have just started to degenerate. We removed the eyes after one or two months and and examined them histologically in plastic sections or polyester wax serial sections. For comparison and as controls we injected acidic fibroblast growth factor (aFGF) (500 ng in 1 μl) dissolved in PBS, or PBS alone. We also did the 'mechanical' control of penetrating the interphotoreceptor space with a needle with no injection. To obtain wider dissemination of bFGF, we also injected it into the vitreous body and did intravitreal controls.

When bFGF was injected into the interphotoreceptor space we saw remarkable preservation of many photoreceptors in the superior hemisphere of the injected eye. After one month (P53), the retina appeared practically intact compared to the extensive photoreceptor degeneration observed in uninjected controls, as judged by the thickness of the outer nuclear layer (ONL) and the presence of inner and outer segments (Fig. 1). After two months (P83), bFGF-injected retinas still showed substantial photoreceptor rescue, although less than at one month. Remarkably, all the subretinal controls, including PBS, aFGF and the dry needle, showed rescue that was *qualitatively* similar to the rescue produced by bFGF, in terms of preservation of photoreceptors. Rescue in the controls, however, was much more limited in extent, usually being localized to the site of injection. Intravitreal injection of bFGF produced a greater extent of rescue than subretinal bFGF, encompassing almost the entire retina. It should be noted that bFGF did not reverse the phagocytosis defect known to occur in the RCS rat, from the absence of phagosomes in the RPE and the presence of a debris zone in the rescued areas (see Faktorovich et al 1990).

Similar experiments were repeated in albino rats exposed to constant light. This causes photoreceptor degeneration (Noell et al 1966). We injected bFGF

FIG. 1 (*Steinberg*) Plastic-embedded sections of RCS rat retinas. (a) P23, at the time of injections; cell death had just begun and some photoreceptor nuclei in the ONL were pyknotic (arrows). Photoreceptor inner segments (IS) and outer segments (OS) are present, as well as some accumulated outer segment debris membranes near the apical surface of the RPE. (b) P53, an uninjected control animal; the ONL is reduced to 1–2 rows in thickness, no photoreceptor inner or outer segments are present, and the outer segment zone is composed only of debris membranes. (c) P53, a region of optimal photoreceptor rescue one month after subretinal bFGF injection; photoreceptor inner and outer segments are present and the retina generally appears unchanged from the time of injection. The retina in the opposite hemisphere of the same eye as in (c) was fully degenerated and indistinguishable from that shown in (b). Toluidine blue stain. Scale bar, 10 μm (From Faktorovich et al 1990, with the permission of Macmillan Magazines Ltd 1990.)

intravitreally 48 hours before placing 3–4-month-old Fischer 344 or Sprague Dawley albino rats in constant light for seven days (LaVail et al 1991). One week of exposure to fluorescent lights (115–130 ft-c) reduced the thickness of the ONL from the normal 8–10 rows of photoreceptor nuclei to 1–2 rows in the most degenerated region of the eye in uninjected or PBS-injected controls



(Fig. 2). There was also a profound loss of most photoreceptor inner and outer segments in this region.

We saw extensive photoreceptor rescue in the bFGF-injected rats: the most degenerated regions of the ONL had 4–5 (Fischer 344) or 6–8 (Sprague Dawley, Fig. 2) rows of nuclei, and many photoreceptor inner and outer segments remained. When bFGF-injected and control rats were allowed to recover in cyclic light for 10 days, after the week of exposure to constant light, the bFGF-injected retinas showed much greater regeneration of photoreceptor inner and outer segments than did the control retinas. The insertion of a dry needle in the subretinal space, 48 hours before exposure to constant light, also greatly reduced the degree of photoreceptor degeneration.

These results show that bFGF can *delay* photoreceptor degeneration in an inherited retinal degeneration and can *prevent* the degeneration of photoreceptors that occurs in light damage. The mechanism for these rescue effects is not known, nor is it known whether bFGF acts directly on photoreceptors or through other cells or other factors. The rescue produced in the subretinally injected controls may result from the release of endogenous bFGF when RPE cells or other cells are damaged during injection. We caution about generalizing these results to humans, especially because of the potentially harmful side-effects of bFGF, given its mitogenic and angiogenic properties.

Farbman: You conclude that the lesion in the RCS rats is in the pigment epithelium; but is it also in the photoreceptors?

Steinberg: So far as we know, there's no lesion in the photoreceptor. One defect in the RCS rat is the failure of phagocytosis. By giving this growth factor, we can delay the death of the photoreceptors. I don't know whether there is a defect in the production of growth factor by the RPE or a problem in access of that growth factor to the photoreceptors through the debris zone. The interphotoreceptor matrix might be sufficiently disturbed in this region that FGF, which might reach the photoreceptors normally, as a survival factor, is lost.

Farbman: When you inject FGF into the vitreous and get survival, how does this large molecule, which is 16 000 Da in molecular mass, get to the photoreceptor?

FIG. 2 (*Steinberg*) Plastic-embedded sections of retinas from three-month-old Sprague Dawley albino rats. (a) Normal retina from a rat maintained in a 12 hour on:12 hour off cyclic light environment (15 ft-c). Photoreceptor inner segments and outer segments are present and the ONL is 8–10 rows of photoreceptor nuclei in thickness. (b) Retina from an uninjected control, killed at the end of a seven-day period in a constant light environment (115–130 ft-c). The ONL is reduced to 1–2 rows in thickness, and there are no inner segments and no normally appearing photoreceptor outer segments. The RPE appears intact. (c) A region of photoreceptor rescue from an animal injected intravitreally with 1000 ng of bFGF in 1 µl of PBS 48 hours before being placed in the constant light environment for seven days. The ONL is 6–8 rows of photoreceptor nuclei in thickness. Many inner segments are present, and the outer segment zone is filled mostly with swollen disorganized outer segment profiles. Toluidine blue stain. Scale bar, 10 µm.

Steinberg: It could get there by diffusion, if it works directly on the photoreceptor; we don't know that for sure.

Calof: FGF is known for its ability to bind to molecules of the extracellular matrix, such as glycosaminoglycans; it's a heparin-binding molecule. Since it binds well to extracellular matrix, how do you know it can diffuse rapidly in the eye? Also, do you know anything about the half-life of FGF in this situation?

Steinberg: We can work out the answer to many of these questions in the light-damage model. We are looking at the half-life by giving it various times before the continuous light period. The effect of FGF has decreased considerably after about eight days.

Calof: So is there an immediate effect of FGF that results in the rescue effect?

Steinberg: To investigate the immediacy of its effect we would need to give FGF, then put the light on and look within hours; we are doing that.

Calof: Do you see any effect of FGF on the pigment epithelium itself, in the RCS rat degenerative model?

Steinberg: There's no evidence yet of any change in the RPE due to the growth factor, itself.

Calof: So histologically it looks normal, but there could be functional differences?

Steinberg: Yes.

Reh: You compared the effect of injecting FGF with a saline injection and inserting a dry needle. It is known that certain growth factors, TGF-β and FGF for example, injected under the skin, cause excessive inflammation. Therefore one explanation for your results is that if a small, sham injection causes a little bit of inflammation, injecting a growth factor into that same space causes a lot of inflammation. Could that be an explanation for the long-term rescue?

Steinberg: One hypothesis for the rescue seen in the dry-needle controls would be release of FGF from the pigment epithelium by the insertion of the needle through it. Regarding inflammation, TGF-β, alone, does not rescue the photoreceptors, nor do any of the other growth factors that we have tested that are involved in the inflammatory response. With vitreous injections, we see an increase in macrophages in the retina, so macrophages coming in as part of an inflammatory response may be very intimately related to the rescue. Ultimately, we are interested in what molecules and cells cause the rescue, and how they are part of a sequence that begins with FGF.

Raymond: You are injecting FGF shortly after the rods have been born, postnatally, in rats. We (unpublished work) have seen in goldfish that after stab wounds of the eye there is a massive up-regulation in the rate of rod precursor proliferation, so potentially instead of rescuing cells, you may be augmenting proliferation of the rod progenitor cells so that new rods are being generated.

Steinberg: One way of dealing with that question was to try the constant light model, in an adult rat, where there are no precursor cells.

Rubel: Is there any differential effect on the outer segments? That is, if you

wait until the outer segments have pretty much disappeared, and inject FGF, do you get regrowth of the outer segments?

Steinberg: That is a very good question, and we don't have the results. It's a difficult experiment to do in terms of the timing, because while the outer segments are degenerating, some photoreceptor cells are dying as well. It is hard to define a time point when only outer segments have been injured.

Powers: In the RCS rats, how long does the FGF effect last, once you have given the injection?

Steinberg: After the one injection of growth factor, by three months the rescue effects have diminished. So we are delaying the degeneration; whereas in the light-damaged retina we are preventing it.

Reh: Does the small amount of 'saving' that you get in the 'dry needle' sham control show the same time course as FGF, in the RCS rat or in the other model?

Steinberg: We have not analysed the data for that, but I would expect it to be the same.

Powers: You said phagocytosis is not normal, but you didn't say how normal the rod outer segments are.

Steinberg: They look normal; as to whether they are functioning or not, we are looking at that with electroretinograms.

Powers: Are they shed normally?

Steinberg: Yes, because the debris zone builds up.

Pujol: What causes the degeneration in the light damage?

Steinberg: One hypothesis is lipid peroxidation due to a build-up of superoxide radicals. Ascorbate injections, before the continuous light, also retarded degeneration in this model (Organisciak et al 1985, Li et al 1985).

Pujol: Do you see swelling of the neurites?

Steinberg: In the light-damage model the photoreceptor outer segments swell a great deal.

Fernald: If the process that you have uncovered is part of a continuum that goes from overexposure to light to real damage, is it possible to find an up-regulation of something like FGF in slightly brighter light, if what you have found is a natural response of the eye, designed to protect it?

Steinberg: We do not have data, yet, on whether there's an up-regulation of FGF in the light-damaged retina. That would be a good experiment.

Fernald: The question of the time course becomes interesting, because one might wonder why you get a 'protective' response with FGF given before photic damage.

Steinberg: We think it takes time for bFGF to affect the photoreceptor cell and turn genes on or off and produce new proteins. The exact timing of the effect is still under investigation.

Fernald: Is there any chance that the RPE is itself responsive to light? If there is photoreceptive capability in the RPE, then very bright lights might activate RPE cells directly, to produce growth factors.

Steinberg: Dr K. T. Brown obtained electrical responses of the RPE cells of

frogs when driven by very intense light. This is a response coming from a direct effect of light absorbed by melanin granules. The light used to produce damage in our second model is not very intense light; it's normal room lighting, and that intensity has never been shown to produce a direct electrical response from the RPE.

Reh: We have been studying the neurogenesis of the retina in the rat, and the response of neuroepithelial cells to various growth factors. We have looked at FGF in terms of the question that Pamela Raymond addressed earlier—whether it would stimulate the production of new rods in cultures and what effects it has on the rods. Others have looked in culture models to see if FGF has survival effects on rods.

There is evidence that the retina has many growth factors in it, during its development: mRNA for FGF and its receptor; for EGF and TGF-α, message and receptor; and NGF and the NGF receptor. So there are enough growth factors to regulate the whole process of neurogenesis. But in our culture models, so far, none of these factors selectively influences the production of one type or another of retinal cell; but the factors can, in combination or alone, promote the proliferation of neuroepithelial cells to produce more neurons and glia overall. So from our experiments we would not conclude that any of these factors is a controlling element that regulates the production of different types of cells, but rather that they may act together, in concert—in survival, in axon and dendrite development, and in proliferation—to regulate those processes. They don't seem to be (at least in culture models) what determines what a cell becomes.

Rubel: To follow that up in the ear, I can't talk about normal development because we don't fully understand the expression of the receptors for these factors, but during regeneration in the chick we have looked at the expression of RNA for receptors for FGF, EGF and NGF. The results with at least two of these probes are negative; we don't see an increase in the expression of these receptors during regeneration.

Lewis: Dr Steinberg, can you show any defect in FGF production in any of the retinal degenerative disorders?

Steinberg: We are in the process of investigating this, in the RCS rat. We have looked at some other rodent mutants, but that study is not completed yet.

Rubel: Is there any evidence that retinal precursor cell types such as the rod precursor cell remain after birth, and has anybody tried to induce light damage or any other kind of damage to see if anything happens?

Raymond: We (Raymond et al 1988) tried to produce light damage in goldfish. Unless we use very high, photically damaging, levels of light, we don't get the loss of photoreceptors that Roy Steinberg sees in his albino rat model. Potentially this is because in these retinas there is a continual replenishment of rods. We did not assess whether rods were being killed by light, and subsequently replaced; we only scored the number of rods that were present after various intervals. It's possible that there was actual turnover—that is, loss and replacement. After one year of constant light exposure, from hatching, there was a 30% loss of

rods and a concominant loss in sensitivity, and no loss of cones (Raymond et al 1988, Powers et al 1988).

With regard to the persistence of neuroepithelial cell precursors in retinas that have differentiated, there are reports that in frog retinas, in *Xenopus* for example, cells that incorporate thymidine persist in the differentiated retina (Taylor et al 1989). Some of these cells are probably analogous to the cells that we have shown in the fish retina to give rise to rod precursors. There are no rod precursors in amphibians, because there is not continuous addition of rods in these retinas, but there is evidence of a population of resting stem cells, potential precursor cells, that do not normally divide, or do so very slowly. Under certain conditions perhaps they could be induced to proliferate. In my paper I described only rod precursors in the fish retina because that is what we understand best, but even in the teleost retina we suspect that there might be other cells in the inner nuclear layer in differentiated regions of the retina that do not divide under normal circumstances but that can be induced to proliferate under conditions of damage.

Rubel: Are you suggesting that, unlike normal development, different precursors exist for different cell types?

Raymond: No; I am suggesting that it is possible that there are small numbers of neuroepithelial cells, which do not differentiate but remain in a resting phase, undifferentiated, and have the potential to proliferate. That doesn't mean that they are a different kind of cell from the primitive neuroepithelial cells of the embryonic retina. We have no markers, however, for recognizing an undifferentiated cell that is not dividing. So there could be lurking in all of these differentiated sensory tissues, particularly those tissues that are continuing to grow, potential proliferative cells that we are not aware of.

Reh: In the rat, studies have been made of the regenerative capability of the retina in the neonatal period, after a crush lesion of the optic nerve to eliminate all the ganglion cells, or earlier, in embryogenesis, using X-irradiation to get rid of developing ganglion cells. After X-irradiation at a stage in embryogenesis where neuroepithelial cells are still present and generating all cell types, histological examination of the adult showed that the retinas are fairly normal (Rugh & Wolf 1955).

By contrast, after a neonatal optic nerve lesion which destroys virtually all the ganglion cells, there is no recovery of ganglion cells, even though neurogenesis is still going on in the retina. The neuroepithelial cells in neonatal rats are still producing rods, bipolar cells and Müller cells, yet when ganglion cells are deleted they don't make new ganglion cells.

Rubel: As if those precursors have lost the ability to make ganglion cells?

Reh: Or they are just too far away to receive the signal that the ganglion cells are gone. We could think of many other explanations.

A more recent study with kainate in the rabbit shows that there appears to be some reorganization of postnatal rabbit retina after damage, although it is not clear what the final outcome of the recovery process is (Messersmith & Redburn 1990).

Raymond: Those authors saw a change in the ratio of photoreceptors, but they have not demonstrated any change in overall cell number.

Pujol: The effect of light damage is very surprising. Is there any other mammalian species besides the albino rat in which normal light (continuous) causes complete degeneration of the retina?

Calof: In albino humans, there is retinal degeneration.

Rubel: There are medical reports of light blindness in humans. It was common in the early explorers of the West.

Steinberg: One can damage the retina in man with light, but not with normal intensities, as far as I know. Light damage has been produced during the removal of cataracts, but at levels above the normal light level.

Pujol: Is there another experimental animal?

Steinberg: There are some light-damage models, but usually with much more intense light.

Raymond: Premature babies exposed to ultraviolet light to treat jaundice were found to have damaged retinas (Fulton et al 1986). Now their eyes are shielded during these treatments.

Steinberg: The light-damage model in the rat has been extensively described (Noell 1980). There's an explanation for light damage that involves the rat regulating the amount of rhodopsin in its eyes, depending upon the lighting environment in which it lives, and age is also a factor (Penn et al 1985, Rapp et al 1990). It will down-regulate when it goes into brighter light, and increased rhodopsin levels are found when rats are kept in the dark.

Margolis: Is it a possible extension of your hypothesis that FGF protects against normal damage in these animals?

Steinberg: There's no evidence yet that FGF protects normally against damage.

Reasner: Dr Raymond, in your light experiment with goldfish, did you use a full spectrum light?

Raymond: It was daylight fluorescent light, at levels typical of normal room illumination.

Jørgensen: In your fish with normal retinas, do you see cells similar to the dark cells that we find in the inner ear?

Raymond: Yes, we see pyknotic (dying) cells during normal development.

Jørgensen: Do you have any idea of their function? Are you sure they are dying?

Raymond: We presume so, from their appearance in the electron microscope; they look similar to those you are showing, with very condensed chromatin. We have seen such cells mostly in developing retinas and at the margins of the adult retina, so we believe that cell death is associated with cell proliferation. We presume that the cells that die are the ones that have failed to act on the differentiation signals that they have received, or those in which a fatal error has occurred in the mitotic process.

Jørgensen: How about macrophages in the retina? They have been described by Braekevelt (1980). Do you see them?

Raymond: There are macrophages, both within the neural retina itself and also in the subretinal space (between the neural retina and pigmented epithelium). In goldfish, in addition to the involvement of RPE cells in the phagocytosis of photoreceptor outer segments, there are wandering phatocytic cells in the subretinal space that also serve this function.

Rubel: One of the major differences between what has been presented in the retina and what we presented in the ear is that in the retina you have a fairly good idea at least of the precursors for the rod cells, and, in the case of pigment epithelium, where the regeneration is occurring.

We have been interested in the nature of the signals that stimulate cells to enter into mitotic activity. Do any of the those who study retina want to comment?

Raymond: I have tried hard to make the fish like a frog! In other words, I have devascularized the fish retina, thinking that our failure to get the RPE to take part in retinal regeneration might be due to differences in the way we produce degeneration of the retina. We typically use neurotoxins, ouabain or 6-hydroxydopamine, to destroy the retina, but I have also ligated the ophthalmic artery, and then released the ligature after three to 24 hours to allow blood back into the retina. Under those conditions the goldfish retina degenerates as it does in the amphibian, and it regenerates, but with the same mechanism that we showed with ouabain. Again the RPE cells do not seem to be directly involved. Tom Reh has looked at some of my retinal sections and confirms that they look different from the frog, in terms of the pattern of cell proliferation. We did not see RPE cells detaching and migrating into the retina, touching the vascular membrane and initiating the whole sequence of events that he has described for the frog.

Reh: It just appears that frogs are a lot better than goldfish at this!

Raymond: Or that the frog RPE is more able to regenerate a totally intact, nicely laminated structure than are the foci of proliferating cells which originate from rod precursors in the goldfish.

Reh: Also, the frog RPE often appears to spread out on the vitreal vascular membrane, before undergoing the switch to neuroepithelial cells. So there seems to be a continuous layer of cells of approximately the same developmental stage. Whereas with the foci generating from rod precursors in the fish, the cells are at various developmental stages. In the frog there is always a discontinuity at the point where the retina regenerated from the marginal zone meets retina regenerated from the pigmented epithelium.

Powers: Can you obtain regeneration in adult frogs?

Reh: We never have, but Lopashov & Sologub (1972) transplanted the pigment epithelium of adult frogs into the eye of a tadpole. It appeared to give rise to new retina and pigment epithelium; so the potential seems to be there, though we have never observed it in the adult.

Powers: It strikes me that, comparing these two sets of studies (tadpole and goldfish), you have in the frog a larval tissue which may be able to produce new cells in a more orderly fashion than an adult tissue, which has to go back and do things over again, as it were.

Raymond: I think it may be more a function of the mechanics of the situation, and the fact that in the amphibian case there is a continuous sheet of dividing cells, more or less synchronized, whereas in the fish retina regenerating from rod precursors there are isolated foci. As Dr Reh suggests, the less-than-perfect result in the regenerated fish retina may simply be a function of the asynchrony of the process.

Rubel: How good is the mosaic within one of your foci? How normal is it?

Raymond: When you talk about a mosaic, retinal aficionados think of a cone mosaic, because the most regularly arrayed cells in the fish retina (and most other retinas as well) are the cone photoreceptors. The cone mosaic in the regenerated retina appears to be quite regular, but the inner layers of the retina are much more disorganized.

Rubel: Is the whole retina in the frog pluripotent in terms of its ability to regenerate?

Reh: Nobody has looked carefully to see whether one area of frog retina is particularly good at regenerating, but we have never seen any evidence of that. Generally one classifies the regeneration as coming either from the marginal zone or from the back of the eye.

Fernald: On the question of what might be regulating cell proliferation, we have shown a robust circadian rhythm in the teleost *Haplochromis burtoni*; we find that cell generation in the temporal region of the eye can be enhanced when that in central retina is not, so the rod progenitors are out of phase with the marginal zone (Fernald 1991). So I would suspect that there may be a circadian regulation of a number of these factors, and, when we look at regeneration, circadian features may play distinct roles at different times of day.

Rubel: Do we know anything about the signals that up-regulate the amount of mitotic activity in the retina after damage?

Raymond: We are now starting to learn what growth factors regulate proliferation *in vitro*, but *in vivo* we do not know.

Powers: We are beginning to investigate what happens to visual function during retinal regeneration, using the model of ouabain destruction that Dr Raymond presented earlier. In the ouabain-treated fish (*Carassius auratus*) the retina degenerates, and connections to the brain also degenerate. We have to consider therefore not only the regeneration of the receptors themselves; regeneration of the connections has to be appropriate to mediate the behaviour.

I have done two preliminary experiments. In one we use the optokinetic nystagmus response, which consists of the smooth pursuit response of the eye to a moving object, followed by a rapid saccadic re-set in the direction opposite to the movement that is being followed. We put a fish in a drum and rotate

stripes around the fish. We then record eye movements. We have tried to measure visual acuity. As shown in Pamela Raymond's results, the photoreceptors come back; the question was when vision comes back. It returns shortly after the photoreceptors are regenerated, which suggests that some neural connections must be made.

There is a flaw in this experiment, namely that there is a ring of normally developed cells around the eye, so we don't know whether the fish were using that ring of normal cells or the regenerated cells to follow the stripes. We have therefore turned to an electrophysiological measure, recording electroretinograms in fish injected with ouabain and allowed to recover. We inject one eye with ouabain and leave the other eye uninjected; then periodically, after the injection, we record from injected and control eyes. Because ouabain causes the lens to become cloudy, we slice the cornea off, remove the lens, and have the fish lying on its side; then we stimulate with a tiny fibre optic probe that contains only seven fibres. I am pretty sure it is stimulating only the part of the retina that was destroyed and then is regenerating. In this initial experiment I am looking at rod function by examining b-wave responses in dark-adapted retinas. Although the results are incomplete, I find that at about the time that rods appear in the regenerating retina, I can record an ERG in the ouabain-treated eye. I can't yet say whether the amplitude is the same as in the control eye when the ERG first comes back, or what the spectral sensitivity of the response is. However, it looks as if at least the first levels of retinal function are wired up as soon as the rod photoreceptors come back, because the b-wave response is known to reflect the activity of bipolar cells.

We plan to look also at the output of the eye by recording the whole optic nerve response with a suction electrode, to see whether signals are getting out of the retina, and, if so, how they compare to normals. Then we want to address the issue of spatial vision; we plan to record at the level of the optic tectum from single ganglion cell fibres. There is a map of the visual world—the retinal field—onto the tectum. By looking at the nature of this map and how it changes during retinal regeneration, we can determine what connectivities exist after regeneration and develop hypotheses about the nature of the animal's spatial vision which we can ultimately test psychophysically.

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