Apoptosis in the Retina: The Silent Death of Vision

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Pathogenetic mechanisms of retinal degeneration include cell loss by apoptosis. This gene-regulated mode of single-cell death occurs in a number of widespread human diseases such as neurodegeneration. The knowledge of genes and signaling in retinal apoptosis is expanding and opens up therapeutic strategies to ameliorate blinding retinal diseases.

The photoreceptors of the retina present a puzzling phenomenon: they can be injured or even destroyed by light, the very element they are designed to detect. Light-induced damage is a widespread occurrence from fly to man and represents a suitable model system to study retinal degeneration (11). Equally widespread are animal models of human inherited retinal dystrophies. Both degeneration and dystrophy have recently experienced a breakthrough of insight, in that apoptotic cell death was discovered as the correlate underlying the observed cell loss (8) (Fig. 1). The phenomenon of light-induced apoptosis revives the longstanding discussion about whether lifelong exposure to bright light or other exogenous factors are contributory elements to retinal diseases, especially age-related macular degeneration (AMD), which is a leading cause of severe visual impairment in elderly people in developed countries.

Whereas recent years have seen considerable progress in the elucidation of gene mutations leading to retinal dystrophies, the steps from a gene mutation toward functional deficits within the cell and the signaling that finally leads to the decision to die are much less understood.

The phenomenon of regulated cell death

Apoptosis is a gene-regulated death of single cells that does not significantly affect neighboring cells and tissues (2). A loss of tissue homeostasis induced by a variety of stimuli, i.e., the regulated balance between cell proliferation, cell life, and cell death, may underlie several significant disease groups, including cancer, neurodegeneration, and immune diseases, as well as malformations during development. For example, in cancer the loss of apoptotic cell death mechanisms prevails, whereas in neurodegeneration the balance is shifted towards apoptosis. In contrast to apoptosis is the unregulated and devastating necrotic cell death destroying larger tissue areas. Different from the demise of entire cells is autophagic bulk degradation of cytoplasmic constituents by the lysosomal system and proteolysis within the ubiquitin-proteasome system. Both autophagy and the proteasomes represent a regulated mode of disposal which may serve adaptive functions and may even interact with apoptotic mechanisms.

Apoptosis can be divided into four major phases: the induction by a multitude of endogenous and/or exogenous stimuli linked to the effector phase by signaling systems, the initiation and effector phase consisting of a proteolytic cascade involving a family of proteases named caspases, the execution phase, again involving proteolysis, which completes the death program, and finally the phagocytosis of cell remnants, called apoptotic bodies, by neighboring cells or invading phagocytic cells. Morphological changes of apoptotic cells include the condensation of cytoplasm and nuclear chromatin and a decay into apoptotic bodies. The biochemical hallmark of apoptosis is the cleavage of chromosomal DNA into nucleosomal fractions, which marks the final blow in the death process.

The multitude of apoptosis-inducing stimuli that may activate cell- and tissue-specific signals has also been termed the "private pathways" of apoptosis. They converge to common pathways of proteolysis with caspase activation, cleavage of cellular substrates, and DNA degradation. Caspases function both as initiators of proteolysis by responding to apoptotic signals as effectors and by performing the disassembly of cell constituents as executers. They are highly specific, with a requirement of cleavage after aspartic acid, and in many tissues they may be constitutively present as precursors. The long-sought DNase that cleaves nuclear chromatin has now been identified and called caspase-activated DNase (CAD), which is present in the cytoplasm as an inactive complex bound to the inhibitor ICAD (1).

The mechanisms by which phagocytes recognize, ingest, and degrade apoptotic cell bodies are not well understood. An important recognition signal appears to be the exposure of phosphatidylserine at the cell surface (15). Similarly, the consequences of phagocytic failure remain to be elucidated. For example, would such a failure induce a secondary tissue necrosis or an immune reaction or both? Recent articles presenting current knowledge of apoptosis are gathered in Ref. 6.

Regulative genes and transcription factors

Considerable evidence suggests a role for altered gene expression during apoptosis. Inhibition of both RNA and protein synthesis impairs the onset of apoptosis in a wide variety of systems, suggesting that specific genes need to be induced

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and that transcription factors are thus components of the regulatory mechanisms. On the other hand, several cell types express the cell death machinery constitutively. For example, on removal of survival signals that seem to suppress the intrinsic death program, such cells die by apoptotic mechanisms without de novo gene expression (9).

Among the transcription factors identified in apoptosis in at least some systems are c-*myc*, p53, oncoprotein E2F, Nurr77, and activator protein AP-1. However, apoptosis can also occur in their absence. For example, p53 knockout mice apparently show normal programmed cell death (PCD) during development, and p53-independent pathways have been described in mature tissues as well. Similar observations have been made in connection with the transcription factor AP-1. Both AP-1-dependent and AP-1-independent pathways have been described. Thus it is not yet clear whether or not a given cell dies by a specific transcription factor activation. It might be possible to elucidate the role of these transcription factors in detail once their target genes have been identified and analyzed.

One of the most frequently investigated candidates is the Bcl-2 family of pro- and antiapoptotic proteins. Recent studies imply a cytosolic and a mitochondrial location of the Bcl-2 protein, further supporting an important role of mitochondria in the death program. Bcl-2-sensitive and -insensitive apoptotic pathways may exist. The latter observations may explain the failure of protection against apoptosis by Bcl-2 overexpression in some animal models of retinal degeneration.

The pivotal role of mitochondria in apoptosis

Recent thinking attributes a key role to mitochondria in vertebrate apoptosis (13). Proapoptotic factors such as ultraviolet light, prooxidants, viral infections, chemotherapeutic agents, ischemia, inflammatory cytokines, neurotoxins, and many others can induce a variety of intracellular changes, such as increased calcium levels, NO release, ceramide activation, and antioxidant depletion. Those alterations cause damage to mitochondrial inner and outer membranes with the activation of caspases and/or the release of cytochrome *c* and the apoptosis-inducing factor (AIF), which may represent another caspase, followed by a proteolytic cascade performed by downstream caspases (5). An alternative pathway may induce swelling and rupture of mitochondrial membranes, which may lead not only to caspase activation and apoptosis but also to necrosis due to a breakdown of ATP production and the creation of reactive oxygen species. A crucial step toward irreversible death commitment in many systems is the release of cytochrome *c*. Thus, in view of a central role of mitochondria in the death program, a three-step model of apoptosis is proposed: a premitochondrial phase during which damage pathways and/or signal transduction cascades are induced, a mitochondrial phase with the loss of membrane function, and a postmitochondrial phase during which the release of cytochrome *c* and other proteins causes the activation of proteases (caspases) and endonucleases that degrade the cellular components lamin, fodrin, actin, and poly(ADP-ribose) polymerase and nuclear chromatin, leading to cellular disintegration.

Apoptosis in the retina: genes and mechanisms

The mammalian eye is one of the organs showing the largest number of genetic diseases. Genetic eye diseases are one of the leading causes of blindness in developed countries. Furthermore, there are over 100 genetic syndromes, which include one type of retinal dystrophy (Gregory-Evans and Bhattacharya, *Trends Genet.* 14: 103–108, 1998).

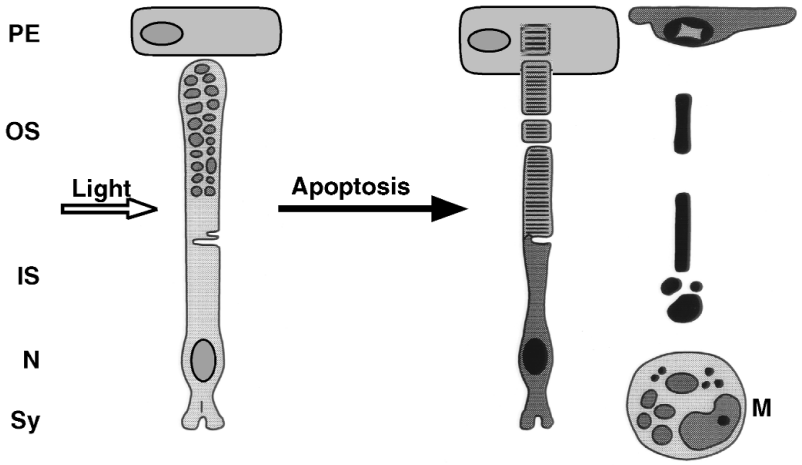

FIGURE 1. Schematic drawing illustrating photoreceptor and pigment epithelium (PE) apoptosis. Exposure to diffuse white fluorescent light or to monochromatic blue light (403 nm) via a system providing a ganzfeld illumination of the eye induces apoptosis in photoreceptors and PE, with exception of mouse, in which PE appears to be protected. In threshold light damage, photoreceptor outer segments display vesiculations and disruptions of disk membranes. At higher light doses, photoreceptors show condensed inner segment cytoplasm and nuclear chromatin and condensed and/or disrupted outer segments. At later stages of apoptosis, visual cells decay into apoptotic bodies, which are removed by phagocytic cells. PE displays chromatin condensation at nuclear margin and condensed cytoplasm. In rat retina, PE apoptosis follows that of photoreceptors with a time lag of ~6 h. OS, outer segment; IS, inner segment; N, nucleus; Sy, synaptic body; M, phagocytic cell.

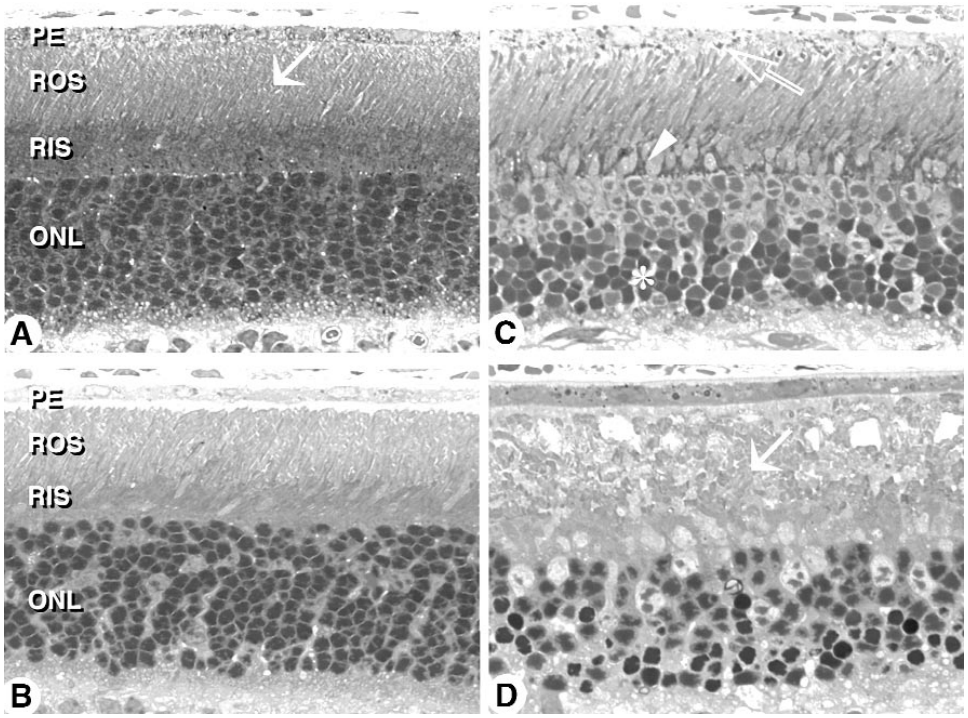

FIGURE 2. Light micrographs from retinas of c-*fos* knockout mice and wild-type littermates. Exposure to high-intensity (15,000 lx) diffuse white fluorescent light creates distinct cell loss by apoptosis in wild-type animals, whereas lack of functional c-*fos* completely protects against cell death even after extended postexposure periods (10 days). *A*: retina from a c-*fos* knockout mouse immediately after exposure to 15,000 lx for 2 h. Except for minor disruptions of outer segments (ROS, arrow), no lesions are apparent. *B*: retina from a c-*fos* knockout mouse 24 h after a 2-h exposure to 15,000 lx. Again, no lesions are visible. *C*: retina from a wild-type littermate immediately after exposure to 15,000 lx for 2 h. Distinct apoptotic nuclei appear in outer nuclear layer (ONL) (*). Condensed inner segments (RIS) are visible (arrowhead). Note the phagosomes (arrow) evoked by light exposure in the PE. *D*: retina from a wild-type littermate 24 h after a 2-h exposure to 15,000 lux. Massive decay of ROS (arrow) and condensed apoptotic nuclei in the ONL are visible. Original magnification of all pictures = 40× oil immersion.

Apoptosis has recently entered eye research as a result of several reports that photoreceptors die by apoptosis in animal models of retinal dystrophies and degeneration and in human diseases. Apoptosis is considered the final common death pathway converging from a variety of primary defects. The underlying cellular lesions are rather multifarious and mostly involve vital elements of photoreception: the visual pigment molecule rhodopsin, altered components of the photoreceptor cytoskeleton, disturbed steps in the phototransduction cascade, and inhibited phagocytic activity of pigment epithelial cells. It is largely unknown how any of those disturbances initiates apoptosis. Following the first observations of apoptosis in the retina, several other ocular conditions were described in which apoptosis eliminates diseased cells (for review see Ref. 10).

The genes involved in cell death in the retina are not very well elucidated. In the central nervous system, the proto-oncogenes c-*fos* and c-*jun* participate in the regulation of apoptosis. c-Fos and c-Jun and their protein family members (Fra-1, Fra-2, FosB, JunB, JunD) constitute the transcription factor AP-1, which is considered the molecular mediator of cell death. AP-1 proteins may not be directly involved in the basic death program but may participate in its activation.

Indirect evidence suggests that c-*fos* may regulate important physiological functions in the retina, perhaps including some of those related to phototransduction. There is a diurnal rhythm of c-*fos* expression in specific retinal neurons (7, 14). Moreover, light exposure stimulates c-*fos* expression in a dose-dependent manner, with a rapid induction in the range of minutes to a few hours, indicating that the c-*fos* protein may also function as the mediator of a general stress response. A continuous c-*fos* expression was observed in mouse models of retinal dystrophies during the period of enhanced apoptotic cell death (12).

The role of c-*fos* in retinal apoptosis

In view of the above studies, our laboratory investigated the role of c-*fos* in light-induced apoptosis. Acute exposure to bright light in mice lacking c-*fos* function and in wild-type control littermates resulted in striking differences: there was only marginal apoptotic cell death in c-*fos* knockout mice, whereas distinct apoptosis occurred in wild-type animals (4). Even after exposure to very high light doses (15,000 lx) and extended postexposure periods (11 days), the c-*fos* knockout mice were protected (Fig. 2). Mitochondria in c-*fos* knockout mice were remarkably well preserved in contrast to wild-type littermates, which displayed heavily injured organelles (Wenzel et al., *J. Neurosci.* 20: 81–88, 2000). It remains to be seen where within the apoptotic death cascade c-*fos* interacts in wild-type mice. In contrast, retinal development appears to proceed normally because normal morphology was found in c-*fos* knockout mice. However, their electroretinograms showed a moderate reduction in light sensitivity and the rhodopsin content of their retinas was reduced by 25%. Those moderate changes, however, cannot explain the complete

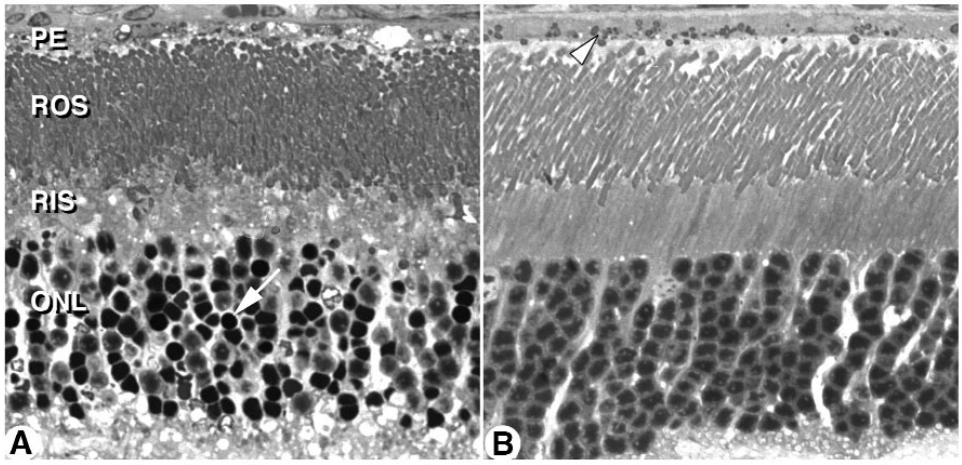

FIGURE 3. Light micrographs from rat retinas exposed to 3.1 mW/cm^2 of blue light (403 nm; *A*) or 8.7 mW/cm^2 green light (550 nm; *B*), respectively, for 60 min. *A*: exposure to blue light induces massive apoptotic cell death in photoreceptors and PE. Condensed nuclei appear in the ONL (arrow). *B*: exposure to green light does not affect retinal morphology. However, a burst of phagosomes (arrowhead), a physiological light response, is seen in PE. Original magnification of all pictures = 40× oil immersion.

protection against light-induced apoptosis (Kueng-Hitz et al., *Invest. Opthamol. Vis. Sci.* 41: 909–916, 2000).

The identification of c-*fos* as an essential component in light-induced apoptosis raises the question of whether c-*fos* is a key regulator of retinal apoptosis in general. Alternatively, c-*fos* may be involved in the private pathway of light-induced cell death. We used *N*-methyl-*N*-nitrosourea, which is a potent inducer of retinal apoptosis, and demonstrated that apoptosis did occur in both knockout and control animals (Wenzel et al., 2000). When mice bearing a mutation that leads to apoptotic photoreceptor cell death with concomitant upregulation of c-*fos* (*rd* mouse) were crossed with *c-fos* knockout mice (*rd/rd* / c-*fos*–/–) , the offspring showed apoptotic cell death that was indistinguishable from rd/rd littermates (3). Our data thus indicate that c-*fos* may be an essential component in the private pathway of light-induced apoptosis but not a universal regulator of retinal apoptosis in general. Light-induced apoptosis, however, gains widespread importance in retinal dystrophies of humans and animal models: several gene mutations have recently been identified that distinctly increase the susceptibility of the retina to light-induced lesions (see, for example, Cideciyan et al., *Proc. Natl. Acad. Sci. USA* 95: 7103–7108, 1998).

Other genes in retinal apoptosis

The tumor-suppressor gene p53 plays an important role in the regulation of apoptosis in a variety of tissues. p53-dependent and -independent apoptosis has been observed. To investigate the function of p53 in retinal apoptosis, we have illuminated mice deficient for functional p53. Morphological, histochemical, and electrophysiological data revealed no difference in the degree of photoreceptor damage between p53-deficient and -sufficient mice, indicating that light-induced apoptosis is not under control of p53.

The role of the Bcl-2 family of proteins in regulating photoreceptor apoptosis has not been fully clarified to date. Although overexpression of Bcl-2 delayed apoptotic photoreceptor death in animal models, the transgene was unable to prevent the final outcome of the degeneration.

Is there a spectral dependence in light-induced apoptosis?

An intriguing question is whether certain wavelengths of the visible spectrum may preferentially induce apoptosis in the retina. Linked to this is the inquiry about the chromophore(s) and the death pathways mediating light-induced apoptosis. We obtained a striking all-or-none response when albino rats were exposed to either monochromatic blue light of 403 nm (3.1 mW/cm^2) or monochromatic green light of 550 nm (8.7 mW/cm^2) with a light-exposure regimen providing a homogeneous illumination on the anesthetized animal's retina with a ganzfeld device. No apoptosis and no other light-induced lesions could be found in green light-exposed eyes, whereas massive apoptotic cell death occurred after illumination with blue light (Fig. 3). When the kinetics of bleaching and regeneration of rhodopsin were analyzed, green light exposure caused a rapid and almost complete bleach. Blue light exposure, however, did not result in a complete bleach. Instead, ~20–30% of visual pigment remained. Further analysis revealed that blue light photoregenerated rhodopsin from a bleaching intermediate (probably metarhodopsin II) and thus provided chromophore during the periods of light exposure (Fig. 4). Is the rod visual pigment rhodopsin the chromophore for light-induced retinal apoptosis? In a recent study we obtained the definitive answer using a knockout mouse model (*Rpe*65$^{-/-}$) that lacks the functional visual pigment rhodopsin but shows normal retinal structure. Whereas control mice revealed distinct apoptotic cell death with consecutive retinal degeneration after light exposure, the knockout mice were completely protected and no increase in the transcription factor AP-1 was seen (Grimm et al., *Nat. Genet.* 24: 1–4, 2000). Further support comes from our study in which we used a variety of mouse strains that show distinctly different regeneration kinetics of the visual pigment rhodopsin. Mice with slow regeneration after exposure to bright light were the least susceptible to light-induced apoptosis, whereas mice with fast regeneration revealed damage after only 10–20 min of light exposure. Against the currently held dogma, we must conclude that the availability of rhodopsin during light exposure

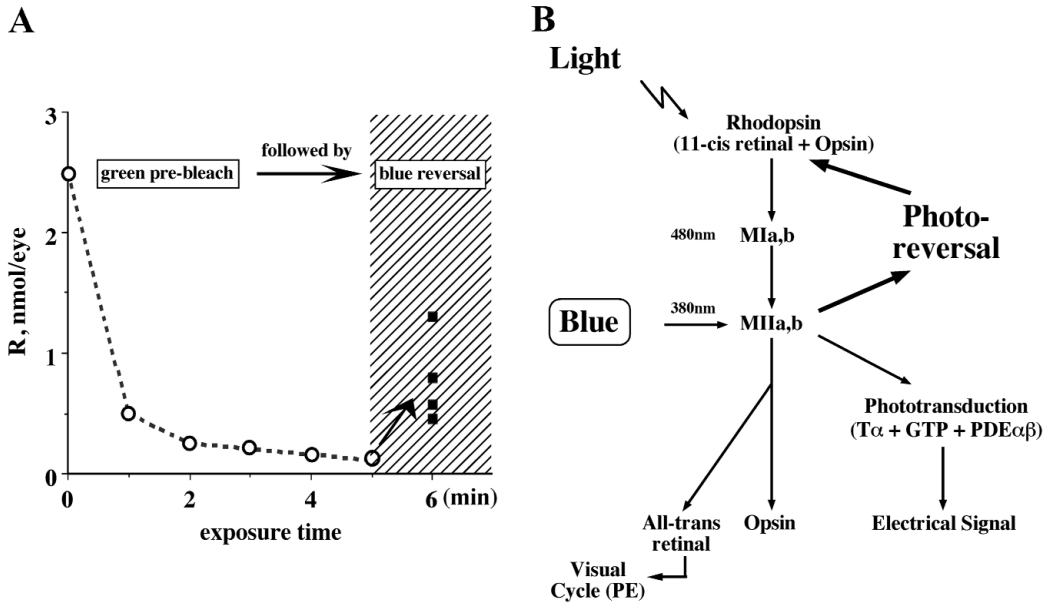

FIGURE 4. *A*: diagram demonstrating photoreversal of rhodopsin (R) bleached by green light (550 nm). High-energy monochromatic blue light (403 nm) reverses ~20–30% of bleached pigment into unbleached chromophore. *B*: simplified scheme indicating process of photoreversal of bleached rhodopsin. When a visual pigment molecule is excited by photon absorption, rhodopsin rapidly decays (1 ms) through bleaching intermediates to metarhodopsin I and II (MI and MII). High-energy blue light is absorbed by MII, which is photoreversed back to original chromophore 11-*cis* retinal attached to its apoprotein opsin. In high-photon fluxes, 1 molecule of rhodopsin can be reversed many times (>50 times within 30 min). Molecules that are not photoreversed are funneled into physiological phototransduction cascade. MII binds G protein transducin, which separates into Tα and Tβγ, with Tα performing the exchange of GTP for GDP. The latter complex then triggers further steps in the signal transduction cascade. After separation of chromophore from opsin, all-*trans* retinal is reduced (detoxified) to retinol and funneled into visual cycle in PE for regeneration and transport back for insertion into photoreceptor outer segment membrane opsin.

determines the sensitivity to light-induced apoptotic cell death (unpublished observations).

Therapeutic strategies: light at the end of the tunnel?

There are several therapeutic alleys to rescue retinal photoreceptors and pigment epithelium from death by apoptosis. To restore vision, transplantation of tissue or implantation of a retinal "prosthesis" are under experimental trial. Once the genes and their regulatory functions in apoptosis will be better understood, their manipulation to prevent apoptotic cell death might be an approach to saving dying cells. This has been attempted by overexpression of the antiapoptotic gene Bcl-2. The survival of retinal ganglion cells in a model for glaucomatous lesions was increased, whereas the rescue of photoreceptors in animal models and in light-induced apoptosis was incomplete. Similarly, cell death in the rd mouse could not be prevented by eliminating the influence of c-*fos*, and light-induced apoptosis was independent of p53. Future approaches might employ inhibitors of caspases and/or endonucleases, once specific caspases and their actions have been elucidated for the retina. For the retina it is not yet known whether apoptosis may be induced through the family of inflammatory cytokines and their death receptors, such as Fas or tumor necrosis factor. At least in the case of light-induced apoptosis, cytokines may well be released either directly or stimulated by potent lipid mediators that are evoked by light (11). The pigment epithelium, which is intimately connected to the retina structurally and functionally, is known to release various cytokines upon stimulation. Furthermore, the application of growth factors alone or in conjunction with antiapoptotic drugs appears to be a promising future direction.

A different angle of therapeutic approach implies gene therapy of those mutations known to cause retinal degeneration and dystrophy. The "reason" for a cell to enter apoptosis would be eliminated by acting upstream of any apoptotic stimulus or message. However, restoring defective gene function without altering the mutation might clarify a puzzle on the level of cellular decision making: when apoptosis is prevented with the damaging genetic mutation still present, would cells just die by a mechanism other than apoptosis? But there are other intriguing questions to be solved. For example, how are rods and cones interacting in retinal degeneration and dystrophy? Recent studies reveal that rods might send survival signals to cones under physiological conditions. Would the reverse also be true? Might the pigment epithelium directly or indirectly participate in this mutual dialogue? Many of the degenerative and dystrophic diseases in humans progress slowly over decades. This poses a significant therapeutic problem, namely the right judgment about when to begin with gene therapy or the inhibition of apoptosis. Such a decision might be easier in acute diseases and/or in the case of ocular tumors, and those might be the first ones to be treated.

Conclusion

In view of recent progress in the elucidation of gene mutations as well as exogenous factors initiating or promoting cell death, there may indeed be light at the end of the tunnel. The silent death of vision might be reduced or even prevented

within the next decade. However, it is equally true that an enormous amount of research is still to be done before human therapy can be attempted. An essential side effect of developing therapies in animal models will be a further understanding of retinal cell and molecular biology. For example, what is/are the role(s) of c-*fos*, does photoregeneration of rhodopsin by blue light occur under natural conditions in mammals, and do autophagy, the proteasome system, and apoptosis represent means of adaptation to changing metabolic and environmental conditions? In this latter context, the apparent paradox of light eliminating photoreceptors might be resolved into a meaningful measure in those cases in which adaptation to bright light is required. The death of single cells might help the remaining ones to survive through the reduction of overall light sensitivity or photon absorption, respectively.

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