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Apoptotic Cell Death in Retinal Degenerations

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Abstract—Apoptosis is a regulated mode of single cell death that involves gene expression in many instances and occurs under physiological and pathological conditions in a large variety of systems. We briefly summarize major features of apoptosis in general and describe the occurrence of apoptosis in the retina in different situations that comprise animal models of retinitis pigmentosa, light - induced lesions, histogenesis during development, and others. Apoptosis can be separated into several phases: the induction by a multitude of stimuli, the effector phase in which the apoptotic signal is transmitted to the cellular death machinery, the execution period when proteolytic cascades are activated, and the phagocytic removal of cellular remnants. Control mechanisms for retinal apoptosis are only beginning to be clarified. Potential apoptotic signal transducers were investigated in our laboratory, including metabolites of arachidonic acid and downstream mediators of signaling molecules such as transcription factors. Work in our laboratory revealed an essential role of the immediate-early gene product c-Fos in light-induced apoptosis. c-Fos is a member of the AP-1 family of transcription factors and, together with other members of this family, it may regulate apoptosis in the central nervous system. Expression of the *c-fos* gene in the retina can be evoked by light exposure and follows a diurnal rhythm. Future studies will have to clarify how light can control the expression of specific genes, and specifically, the role of *c-fos* and other genes of retinal apoptosis including potential target genes and signaling pathways. © 1998 Published by Elsevier Science Ltd. All rights reserved

1. INTRODUCTION

The study of apoptotic cell death is one of the most rapidly expanding research fields and there are not many areas that span such a wide range of biological and medical disciplines. Despite the considerable increase in knowledge, several questions remain to be answered. This fact, together with the diversity of systems in which apoptosis occurs, makes it nearly impossible to draw a coherent picture that can portray completely the molecular mechanisms underlying the morphology of apoptosis. Apoptosis is often used synonymously with programmed cell death (PCD). However, programmed cell death implies death at a specific, genetically determined period in the lifetime of a given cell, whereas apoptosis can be induced by a variety of exogenous as well as endogenous stimuli at random within the lifespan of an organism. Both PCD and apoptosis often but not always depend on gene expression (Martins and Earnshaw, 1997; Schwartzman and Cidlowski, 1993a; Nagata, 1997; Vaux and Strasser, 1996; Steller, 1995).

PCD and apoptosis subserve a variety of specialized functions to maintain tissue homeostasis and to perform tissue remodeling. They remove unwanted cells which may be injured, misplaced or surplus in development and in mature tissues. There is a growing body of clinical diseases which show impaired apoptosis (such as cancer, virus infections, autoimmune diseases) or excessive apoptotic cell death (such as neurodegenerations, ischemia-reperfusion, toxin induced cell death or diseases of the bone marrow) (e.g. Thompson, 1995; Gorman *et al.*, 1996).

Apoptosis is structurally and functionally distinct from necrosis. Whereas apoptosis represents a highly controlled and complex system of effector cascades that converge to a common death execution program of single cells, necrosis displays a random mode of cell death that includes damage of larger groups of cells.

1.1. Apoptosis Versus Necrosis: Morphological Features

One of the earliest morphological signs of apoptosis is membrane blebbing that is followed by nuclear chromatin condensation and condensation of the cytoplasm. Cellular organelles remain relatively well preserved. Cells shrink and are fragmented into apoptotic bodies, which are removed by macrophages or neighboring cells (Wyllie, 1980). In most but not all cases, the nuclear DNA is cleaved endonucleolytically into multimers of 180 bp. Therefore, gel electrophoresis of DNA from apoptotic cells shows the characteristic ladder formation. Immunostaining of DNA nick ends by the TUNEL method (terminal transferase-mediated dUTP nick end labeling; Gavrieli *et al.*, 1992) stains apoptotic cells, making it possible to distinguish them from normal cells. But nick ends resulting from single strand DNA breaks during necrosis may also be labeled (Charriaut Marlangue and Ben Ari, 1995).

Necrotic cell death is characterized by swelling and lysis of cells and their cytoplasmic organelles.

The release of lysosomal enzymes leads to the destruction of surrounding cells and tissues accompanied by an inflammatory response. Nuclear DNA is degraded in an unspecific manner and reveals a diffuse smear when analyzed by gel electrophoresis.

1.2. Phases of Apoptotic Cell Death

Apoptosis can be divided into several phases. The induction phase leads to the onset of the apoptotic program, the effector phase is marked by the transduction of the signal with delivery of the message to the cell death machinery and activation of effector molecules. The following execution phase coordinates and executes the death signal. Finally, the cell disintegrates into apoptotic bodies which are removed by macrophages and/or neighboring cells.

1.2.1. *Induction/Effector phase: multiple stimuli and signal delivery*

The list of compounds and diverse stimuli that can induce apoptosis is growing rapidly. Apart from developmental signals, which are poorly understood, experimental apoptosis is initiated by cytokines such as tumor necrosis factor (TNF) and Fas-ligand (FasL), UV or γ-irradiation, heat shock, oxidative stress, viral infection, chemotherapeutic drugs, growth factor depletion and detachment from the extracellular matrix (anoikis) (Wertz and Hanley, 1996).

The initial biochemical events are dependent on the nature of the various agents and may vary from cell type to cell type. In that sense, the induction phase may also be called the private pathway. The initial events are subsequently linked in a largely unknown manner to downstream effectors. One of the best known private pathways is mediated by transmembrane receptors like Fas/APO1 and TNF receptor type 1. Upon binding of the ligand (FasL or TNF), the receptors are activated through oligomerization and recruit adaptor proteins to the cytosolic domains of the receptors such as FADD or TRADD. The adaptor proteins themselves bind other proteins and thereby link early events at the receptor level to downstream effector events (Nagata, 1997). Activation of the ICE protease and the generation of ceramide, arachidonic acid and other secondary messenger systems may still be part of the private pathway because these molecules do not play a role in all forms of apoptosis and their activation may occur through a variety of mechanisms.

The effector events of apoptosis may be viewed as a rather early phase of the common apoptotic pathway, that is still subject to regulatory mechanisms. It was proposed by Kroemer and coworkers that the mitochondria may play an essential role in the effector phase (Zamzami *et al.*, 1996; Susin *et al.*, 1997). Signals generated during the induction phase, such as activation of caspase-1 seem to converge and contribute to a disruption of the mitochondrial transmembrane potential ($\Delta\psi_m$). Disruption of $\Delta\psi_m$ occurs concomitantly with the opening of a megachannel. It appears that through the open pore the mitochondria release potent apoptosis inducing factors such as cytochrome C (Liu *et al.*, 1996) and a protease AIF (Zamzami *et al.*, 1996) which then irreversibly initiate the execution phase of cell death.

1.2.2. *Execution phase: catabolic cascades*

Genetic studies in the nematode *Caenorhabditis elegans* (*C. elegans*) have identified the pro-apoptotic genes CED-3 and CED-4 and the anti-apoptotic gene CED-9 as the key players in the control and execution of apoptosis (Hengartner, 1996). The identification of mammalian homologs to all of these genes underscores the phylogenetic conservation of the apoptotic process. The apoptosis activating factor 1 (APAF-1) has been characterized as the mammalian homolog of CED-4. APAF-1 interacts with and activates CPP32 (caspase-3, the homolog of CED-3; Yuan *et al.*, 1993) upon binding of cytochrome C, dATP and another factor called APAF-3 (Zou *et al.*, 1997; Vaux, 1997). CPP32 itself is a member of the growing ICE family of cysteine proteases (caspases). It appears that caspase activity forms a novel, tightly regulated proteolytic network

within the cell to orchestrate execution of apoptosis (reviewed in White, 1996; Alnemri, 1997). These proteases can cleave a variety of substrates like PARP, lamins and U1–70kD, linking them to the execution phase of apoptosis (Martins and Earnshaw, 1997).

CED-9 is homologous to the mammalian cell death suppressor gene Bcl-2 (Hengartner and Horvitz, 1994), a member of a second group of proteins involved in the regulation of apoptosis (Reed, 1997; Kroemer, 1997). Both death antagonists (e.g. Bcl-2, Bcl-X_L, Bcl-w, Bfl-1, Bag-1, Mcl-1, A1) and -agonists (e.g. Bax, Bak, Bcl-X_S, Bad, Bid, Bik, Hrk) belong to the Bcl-2 family. Most of the proteins contain a transmembrane region at the carboxyterminus which localizes them predominantly to the outer mitochondrial membrane (Kroemer *et al.*, 1997). These data and reports that Bcl-2 might also be involved in the regulation of the mitochondrial transmembrane potential have placed mitochondria into the focus of apoptosis research (see Section 4.6.1).

A classical feature of apoptosis is the endonucleolytic cleavage of chromosomal DNA (Wyllie, 1980). As a late event, nuclear DNA is cleaved first into large fragments of 50 to 300 kb (Oberhammer *et al.*, 1993) which are then cleaved endonucleolytically into multimers of 180 bp that correspond to nucleosomal DNA. It has to be emphasized, however, that the occurrence of a DNA ladder is not obligatory to all apoptotic cell death (Walker and Sikorska, 1994; Brown *et al.*, 1993). Nevertheless, cleavage of the DNA into the high-molecular weight DNA fragments occurs also in those cases and can be detected by pulsed-field electrophoresis. The enzyme(s) responsible for the DNA fragmentation during apoptosis have not yet been identified definitively, but several candidates are being discussed in a recent review (Montague and Cidlowski, 1996).

1.2.3. *Degradation phase: removal of apoptotic bodies*

Remnants of dead cells are removed, mostly without an accompanying inflammation, by phagocytic macrophages or neighboring cells. Transglutaminase-catalyzed cross-linking of cellular proteins (Piacentini, 1995; Nemes *et al.*, 1996) ensures the inactivation of potentially harmful molecules. Studies in *C. elegans* have revealed at least 8 genes (*ced-1*, *ced-2*, *ced-5*, *ced-6*, *ced-7*, *ced-8*, *ced-10*, *nuc-1*) involved in phagocytosis and final degradation of apoptotic bodies (Ellis *et al.*, 1991; White, 1993). The ATP binding cassette transporter (ABC1) and the class A scavenger receptor (SR-A) are involved in the phagocytosis of apoptotic bodies (Luciani and Chimini, 1996; Platt *et al.*, 1996). A structural homologue of ABC1 that maps closely to the ced-7 locus exists in *C. elegans*. Since animals defective in ced-7 are impaired in the engulfment of cell particles, ABC1 might be the mammalian homologue to the nematode gene (Luciani and Chimini, 1996).

1.3. Gene Transcription and Transcription Factors

Considerable evidence suggests a role for altered gene expression during apoptosis. Inhibition of both RNA- and protein synthesis inhibits the onset of apoptosis in a wide variety of systems (Naora *et al.*, 1995; Yonish-Rouach *et al.*, 1995), suggesting that specific genes need to be induced and that transcription factors are thus components of the regulatory mechanisms. On the other hand, several cell types can express the cell death machinery constitutively. Upon removal of survival signals that seem to suppress the intrinsic death program, such cells die by apoptotic mechanisms without *de novo* gene expression (Raff *et al.*, 1993).

Among the transcription factors identified in apoptosis in at least some systems are c-Myc, p53, E2F, Nurr77 and AP-1 (Shi *et al.*, 1992; Evan *et al.*, 1992; Wu and Levine, 1994; Field *et al.*, 1996; Liu *et al.*, 1994; Woronicz *et al.*, 1994; Estus *et al.*, 1994; Preston *et al.*, 1996; Colotta *et al.*, 1992; Hafezi *et al.*, 1997a; Ko and Prives, 1996). However, apoptosis can also occur in their absence. For example, *p53* knock-out mice apparently show normal PCD during development (Donehower *et al.*, 1992) and *p53*-independent pathways have been described for other systems as well (Liebermann *et al.*, 1995; Marti *et al.*, 1998). Similar observations have been made in

connection with the transcription factor AP-1. Both AP-1 dependent (e.g. Colotta *et al.*, 1992; Hafezi *et al.*, 1997a) and AP-1 independent pathways (Lenczowski *et al.*, 1997) have been described. Thus it is not clear yet 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.

2. PROGRAMMED CELL DEATH (PCD) IN RETINAL DEVELOPMENT

Several unsolved yet fundamental problems stimulate research in the field of development. Basic questions concern the molecular mechanisms of morphogenesis, the commitment of cells to a particular fate including the decision to die or to divide, and the role of cell-to-cell signaling (reviewed in Barinaga, 1996). An essential part of these complex events is PCD.

2.1. Functions of PCD in General

Developmental cell death mostly subserves the function of removing unwanted cells, for example in phylogenesis when larval structures are eliminated. Morphogenesis or the sculpting of organs requires PCD for changing shape and form, when ducts or cavities are needed, or, in the eye, the optic vesicle, cup and fissure. Histogenetic cell death during the differentiation of tissues controls cell numbers and eliminates abnormal, misplaced cells in addition to non-functional or harmful cells (Young, 1984; Jacobson *et al.*, 1997). The regulation of these events is not understood and may vary from one tissue to another. For example, the balance between survival and death signals or the competition of neurons for a limited quantity of survival signals may constitute important components.

For ocular development, retinoic acid may play a crucial role as a transcriptional regulator (Dräger and McCafferty, 1997; Hyatt and Dowling, 1997). Thus it is conceivable that PCD may be regulated, at least in part, by the retinoic acid system.

2.2. PCD in the Retina

PCD in the developing retina has been described in several species (review: Cowan *et al.*, 1984), such as cat (Stone and Rapaport, 1986), mouse (Young, 1984) and human (Provis and van Driel, 1985; Penfold and Provis, 1986).

In the mouse retina PCD is described and quantified in detail, revealing different periods of apoptotic waves in different retinal layers (Young, 1984). Ganglion- and amacrine cells die at postnatal days 3–7, followed by bipolar and Müller cells from postnatal days 8–13. Inner rods are observed to die at days 6–8, whereas outer rods follow from day 11–17. Developmental cell death is completed by day 30. Those morphological findings analyzing pyknotic nuclei were later confirmed by immunostaining with the TUNEL method (Chang *et al.*, 1993; Portera Cailliau *et al.*, 1994; Wong, 1994, 1997). In the developing rat retina, PCD reduces the number of ganglion cells from 3×10^5 to 10^5 (Galli-Resta and Ensini, 1996).

Macrophages play an important role in the removal of the hyaloid vessels and the pupillary membrane in normal mice; in transgenic animals with disrupted macrophage populations these structures persist (Lang and Bishop, 1993). Apoptosis is observed in the vascular endothelial cells and appears to be induced by macrophages which possess apoptosis-inducing activities. The molecular mediators of macrophage killing capacity include TNF α, nitric oxide (NO) and reactive oxygen species (Lang, 1997).

The signaling pathways and genes leading to cell death in the developing retina have yet to be elucidated. Members of the AP-1 family of transcription factors (Curran and Franza, 1988) may be involved. *c-fos* expression precedes apoptosis in several other tissues (e.g. Smeyne *et al.*, 1993; Marti *et al.*, 1994). In the developing human retina, expression of *c-fos* is monitored in different layers to different degrees from week 15 to 40 of gestation (Yu *et al.*, 1994) roughly coinciding

with morphological cell death (Penfold and Provis, 1986). In the *rd*-mouse, the expression of *c-fos* precedes cell death (Rich and Zhan, 1997; Rich *et al.*, 1997). Because retinal morphogenesis apparently proceeds normally in *c-fos* knock out mice, there may be redundancy for members of the AP1 family during development (Hafezi *et al.*, 1997a). On the other hand, developmental cell death did occur in neurons of mouse embryos lacking both *c-fos* and *c-jun* (Roffler Tarlov *et al.*, 1996).

The histogenetic PCD raises questions related to the transplantation of developing retinal cells in animal models of retinitis pigmentosa (RP). Will cell death occur in those transplanted cells according to their predetermined intrinsic clock? The signals to die or to live may not function properly in the retina of a host bearing the retinal degeneration, leading to aberrant histogenesis. Thus, it may be crucial in transplantation to choose developmental periods where PCD is mostly completed.

A different aspect of PCD may concern the photostatic regulation of light sensitivity (Penn and Williams, 1986; see also Section 5). In the adult rat retina, the light history of an animal affects retinal sensitivity by inducing up- or down-regulation of rhodopsin levels, rod outer segment length and transduction proteins. In the developing retina, this may be performed by light-induced up- or down-regulation of the extent of histogenetic PCD in the inner rod layer. This notion is supported by the observation that light-induced apoptosis predominantly occurs in the innermost rods of the mature retina (see for example Fig. 1e in Hafezi *et al.*, 1997a; or Fig. 1c in Hafezi *et al.*, 1997b; or Fig. 1a in Remé *et al.*, 1995).

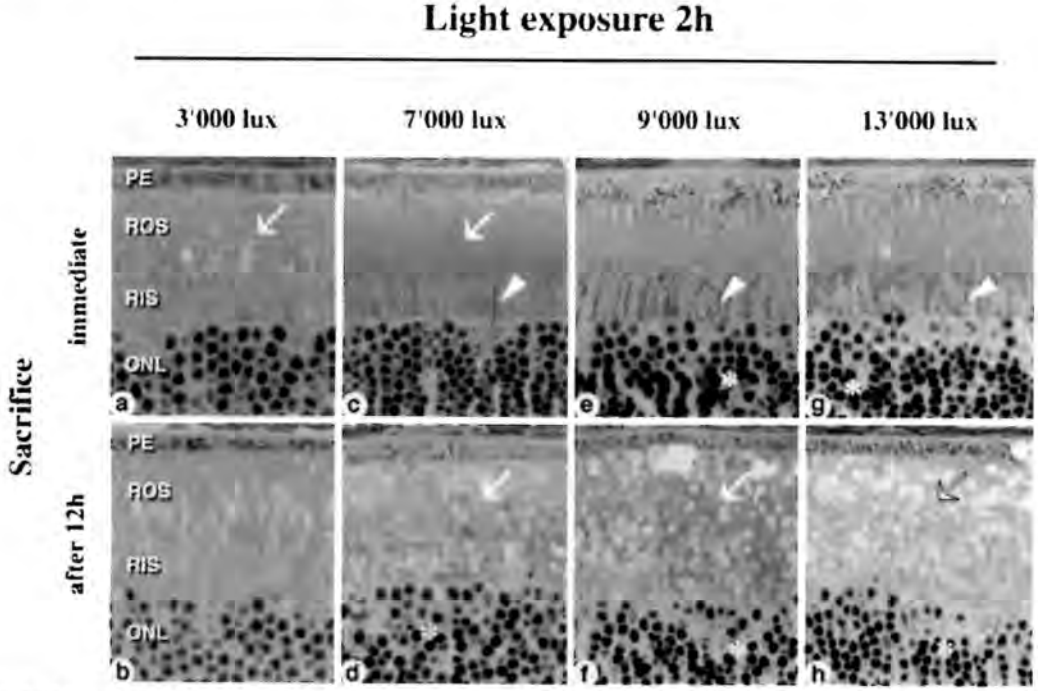

Fig. 1. Light micrographs illustrating photoreceptor lesions as a function of illuminance levels. Free moving mice were exposed to diffuse, white fluorescent light of increasing illuminance levels for 2 h and killed immediately after the exposure (0 h, top panel) or 12 h (12 h, bottom panel) after the end of the exposure. a,b) Exposure to 3000 lx resulted in minor disruptions of ROS (↑) at 0 h, with no increase in lesions at 12 h. c,d) Exposure to 7000 lx created moderate disruptions of ROS (↑) and few apoptotic cells (▽) at 0 h, whereas a distinct destruction of ROS (↑) and numerous apoptotic nuclei appeared after 12 h. e,f) Exposure to 9000 lx led to a distinct immediate apoptotic response with condensed rod inner segments (RIS) (▽) and nuclei (*) at 0 h and an extensive destruction of ROS (↑) with abundant apoptotic nuclei (*) at 12 h. g,h) Exposure to 13 000 lx produced qualitatively similar changes but more pronounced as those seen for 9000 lx. Original photographic magnification 800×.

3. APOPTOSIS IN RETINAL PATHOLOGIES

Cell loss via apoptosis is increasingly recognized as a frequent component in the course of different retinal diseases and has been considered as a final common pathway resulting from a variety of primary defects. Thus, in some instances apoptosis may represent an epiphenomenon, whereas in others, regulated cell death may be the primary pathogenic event. Table 1 summarizes, with references, the main conditions where apoptosis has been observed.

3.1. Animal Models of Retinitis Pigmentosa (RP)

Apoptosis has recently entered the thinking of retinal research as a result of several reports that photoreceptors die by apoptosis in animal models of RP and in the human disease. In several different mouse models, aberrant photoreceptor apoptosis is demonstrated or suggested during developmental periods or in the mature retina. The underlying cellular lesions are rather multifarious and could involve one of the following: the rhodopsin molecule, altered photoreceptor cytoskeletal elements, disturbed phototransduction cascade, inhibited phagocytosis of pigment epithelial cells, inhibition of the Fas/FasL system, or others (Table 1, see references there). It is mostly unknown how any of those disturbances initiate apoptosis.

3.2. Other Retinal Pathologies

Following the first observations of apoptosis in RP animal models, several other conditions were described where apoptosis eliminates diseased cells. The DNA-damaging substance N-Methyl-N-Nitrosourea (MNU) induces a rapid apoptotic degeneration of photoreceptors. Several clinical situations, such as retinal detachment, proliferative vitreo-retinopathy (PVR) and diabetes, imply cell death by apoptosis. It has been shown that experimental axotomy, human anterior ischemic optic neuropathy (AION), and experimentally raised intraocular pressure all resulted in the death of retinal ganglion cells by apoptosis. Apoptosis was also induced by ischemia-reperfusion, insulin and insulin like growth factors, SV40 T antigen and the lack of signaling from the interphotoreceptor matrix (Table 1).

3.3. Photoreceptor Rescue

The occurrence of apoptosis in numerous animal models of human diseases stimulates different concepts of both photoreceptor and retinal rescue strategies.

An interesting aspect in view of the transplantation paradigm, is the immune privilege of important ocular tissues which may protect transplanted retinal tissues from systemic immune attack. One essential component of this immune privilege is provided by apoptotic death of invading inflammatory cells (Griffith and Ferguson, 1997; Griffith *et al.*, 1995; see Section 5.2).

Of increasing importance in the rescue of retinal cells, at present in animal models but anticipated in humans for the future is the prevention or reduction of apoptotic cell death. This has been attempted by overexpressing the anti-apoptotic gene *Bcl-2* or by adding neuroprotectors such as nerve growth factor or ciliary neurotrophic factor. Overexpression of *Bcl-2* increased the survival of ganglion cells after optic nerve transsection (Cenni *et al.*, 1996; Bonfanti *et al.*, 1996), whereas rescue of photoreceptors in light-induced lesions and in RP animal models was incomplete (Tsang *et al.*, 1997; Joseph and Li, 1996; Chen *et al.*, 1996). A partial rescue of photoreceptors was achieved by reintroducing the wildtype gene into affected animal models of RP (Bennett *et al.*, 1996; Lem *et al.*, 1992).

In general, for an efficient reduction of cell death it is necessary to understand mechanisms of gene regulation and signaling systems. Therefore, we adapted our model of acute retinal light damage in mice and rats (e.g. Bush *et al.*, 1991; Remé *et al.*, 1994) for the study of such events.

Table 1. Apoptosis in retinal pathologies

Condition	Species	Cell type	Authors
RP			
	Human	Photoreceptors	Li and Milam, 1995
	rd Mouse	,,	Chang ***et al.***, 1993
	rds, rd, Q 344ter Mouse	,,	Portera Cailliau ***et al.***, 1994
	rd Mouse	,,	Lolley ***et al.***, 1994
	RCS rat	,,	Tso ***et al.***, 1994
	vitiligo Mouse	,,	Smith ***et al.***, 1995
	rd Mouse	,,	Jomary ***et al.***, 1995
	C3H Mouse, Fas negative	,,	Nambu ***et al.***, 1996
	Mouse, Ser-6	,,	Chang ***et al.***, 1997
	Mouse, Pro23His	,,	Naash ***et al.***, 1996
RP: Functional testing			
ERG	Mouse, rh -/-	Photoreceptors	Humphries *et al.*, 1997
ERG	Mouse opsin gene	,,	Goto *et al.*, 1996
ERG, morphology	Mouse, oncogene expression	,,	Peachey *et al.*, 1995
Single rod response, morphology	Q 344ter	,,	Sung *et al.*, 1994
Light-induced			
Light	Rat	Photoreceptors	Shahinfar *et al.*, 1991
Light	Rat	,,	Remé *et al.*, 1995; Hafezi *et al.*, 1997b
Light	Mouse	,,	Hafezi *et al.*, 1997a
Toxins			
MNU	Rat	Photoreceptors	Nakajima *et al.*, 1996a
MNU	Rat	,,	Nakajima *et al.*, 1996b
MNU	Mouse	,,	Yuge *et al.*, 1996
Clinical			
Retinal detachment	Human	Photoreceptors	Chang *et al.*, 1995
,,	Cat	,,	Cook *et al.*, 1995
,,	Rabbit	,,	Berglin *et al.*, 1997
PVR	Human	Vitreous cells	Esser *et al.*, 1996
Diabetes	Rat	Ganglion cells Müller cells	Hammes *et al.*, 1995
Glaucoma			
Axotomy	Neonatal rat	Ganglion cells	Rabacchi *et al.*, 1994
Axotomy	Rat	,,	Berkelaar *et al.*, 1994
Exp. glaucoma	Rat	,,	Garcia Valenzuela *et al.*, 1995
AiON	Human	,,	Levin and Louhab, 1996
Axotomy	Mouse	,,	Porciatti *et al.*, 1996
Axotomy	Monkey	,,	Quigley *et al.*, 1995
Exp. glaucoma	Rabbit	,,	Quigley *et al.*, 1995
Various			
Reperfusion, ATA	Rat	Photoreceptors	Lam *et al.*, 1995
AMOG -/-	Mouse	,,	Molthagen *et al.*, 1996
SV40T-antigen	Mouse	,,	Al-Ubaidi *et al.*, 1997
IGF's, Insulin L-glutamine Ø	Chick-embryo	,,	Calvaruso *et al.*, 1997

Immune tolerance			
	Mouse	Lymphoid cells Inflammatory cells	Griffith *et al.*, 1995; Griffith *et al.*, 1996 Griffith and Ferguson, 1997
Rescue			
Anti- apoptotic measures			
NGF	Neonatal rat	Ganglion cells	Rabacchi *et al.*, 1994
Bcl 2	Rat	,,	Porciatti *et al.*, 1996
Bcl 2	rd Mouse	Photoreceptors	Joseph and Li, 1996
Bcl 2	rd Mouse	,,	Chen *et al.*, 1996
Bcl 2	γ-PDE Mouse	,,	Tsang *et al.*, 1997
CNTF	rd Mouse	,,	Cayouette and Gravel, 1997
Reviews			
Animal models		Photoreceptors	Wong, 1994; Wong, 1997
,,		,,	Lolley, 1994
,,		,,	Gregory and Bird, 1995
,,		,,	Lem and Makino, 1996
,,		,,	Papermaster, 1997
Light	Rat, mouse	,,	Abler *et al.*, 1996
Light	Rat, mouse, squirrel	,,	Remé *et al.*, in press 1998b

4. THE MODEL OF LIGHT-INDUCED APOPTOSIS IN THE RETINA

4.1. The Experimental System

Our light exposure system consists of metal cages with reflective interiors, where free moving animals are exposed to white fluorescent light coming from a light source mounted above the cages. UV is blocked by a diffusing screen shielding the fluorescent tubes. Illuminance levels can be adjusted by a rheostat and by varying the distance from the exposed animals (e.g. Remé *et al.*, 1994). The pupils are dilated in pigmented animals during light exposure. Particular care is taken that the animals keep their eyes exposed and do not hide their heads. For most experiments, the exposure duration is kept constant at 2 h. Animals are sacrificed at intervals ranging from 0 h to 2 weeks after the end of exposure.

The magnitude of the response of disk-shedding and autophagy to acute bright light exposure follows an endogenous rhythm (Remé *et al.*, 1985; Remé *et al.*, 1986, 1998a). In light damage experiments, a burst of phagosomes (e.g. see Fig. 3c) and autophagic vacuoles is readily observed. The susceptibility to light-induced lesions varies with strain and species and, in some species, with time of day of the damaging light exposure (White and Fisher, 1987). Furthermore, there is a multitude of damage-promoting and -reducing factors (review: Remé *et al.*, 1998b).

4.2. Characteristics of Acute Light Damage and the Induction of Apoptosis

Our model system induces a synchronized burst of apoptosis and provides the possibility to investigate defined time points after the induction. Furthermore, the extent of an apoptotic lesion can be adjusted to the actual experimental questions. Apart from the induction by diffuse white light, retinal apoptosis is also induced by exposure to diffuse broad spectrum green light (Shahinfar *et al.*, 1991; Wong *et al.*, 1994; Abler *et al.*, 1996) or by monochromatic light of 403 nm (Remé *et al.*, 1998b).

In our model, a distinct dose-response relationship is observed when illuminance levels from 500 to 15 000 lx are chosen. In albino rats, up to 1000 lx for 2 h create disturbance of rod outer segments (ROS). At higher intensities, a burst of apoptotic cell death followed by photoreceptor loss and scar formation is seen (Szczesny *et al.*, 1995; Remé *et al.*, 1995; Remé *et al.*, 1998b; Hafezi *et al.*, 1997b). In the pigmented mouse ret-

ina, similar phenomena are seen but require higher illuminance levels (Fig. 1). Apoptosis is confined to the central retina at lower light levels and spreads towards the periphery when high illuminance is chosen. Apoptosis is verified morphologically by light- and electron-microscopy, by a modified TUNEL staining *in situ* and by biochemical analysis of DNA fragmentation.

Both rats and mice show an immediate response after light exposure in photoreceptors, followed by a gradual loss of visual cells, invasion of macrophages and a final scar formation with an intact inner retina. Positive TUNEL-staining and DNA-ladder formation are seen in photoreceptors 5–6 h later than morphological apoptosis. This observation raises the question of the molecular correlates of chromatin- and cytoplasmic-condensation which appear to precede the biochemical markers (Hafezi *et al.*, 1997b).

By investigating the time course and morphological characteristics of light-induced apoptosis in rats and mice, some potentially important species differences emerge. In rats, the PE also undergoes distinct apoptosis, however with a time lag of 5–6 h after that of photoreceptors. Interestingly, in the mouse there is no apoptosis of the PE after light exposure and the mouse PE appears to survive without photoreceptors, at least for 1–2 weeks. This suggests that the mouse retina/PE can provide protective mechanisms which are lacking in the rat (Fig. 2).

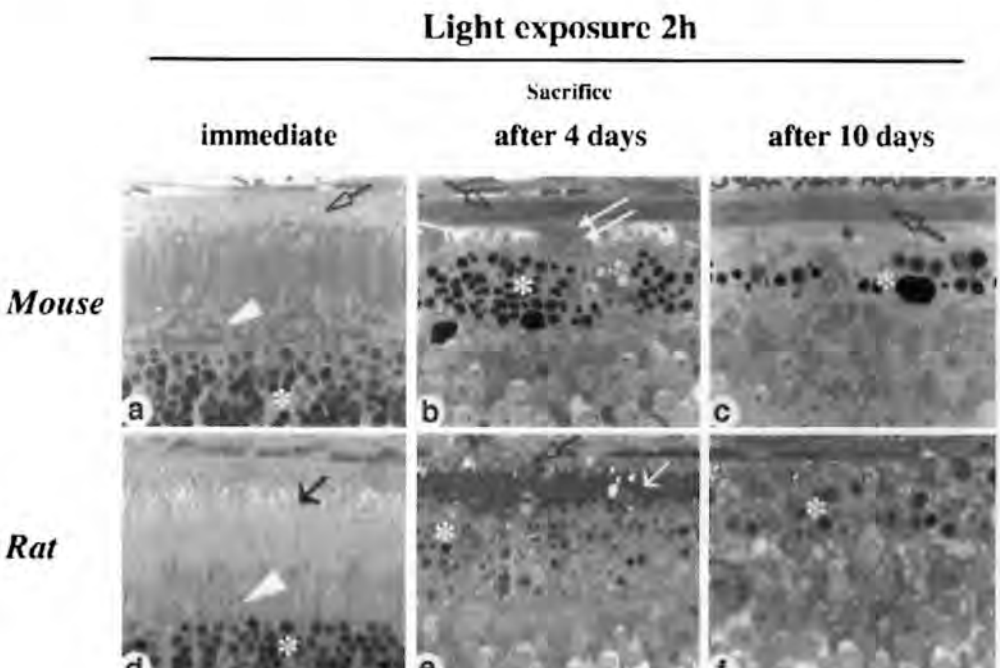

Fig. 2. Light micrographs illustrating the time course of photoreceptor and PE changes in the mouse (upper panel) in comparison to the rat (lower panel) after light exposure. Albino mice were exposed to 5800 lx of diffuse, white fluorescent light for 2 h and killed at 0 h, 4 days and 10 days, respectively, after the end of the light exposure. Albino rats were exposed to 3000 lx of diffuse, white fluorescent light for 2 h and killed at 0 h, 4 days and 10 days, respectively, after the end of the light exposure. a) A distinct apoptotic response with condensed nuclei (*) and RIS (∇) appears as well as slight swelling of the PE (⇓). b) Four days after the exposure, ROS have disappeared, most photoreceptor nuclei are heavily condensed (*), macrophages appear in the subretinal space (⇊), PE cells are slightly condensed (⇓). c) 10 days after the exposure, most photoreceptor nuclei have disappeared, apoptotic bodies are distinct (*), PE cells appear normal (⇓). d) An apoptotic response consisting of condensed RIS (∇) and nuclei (*) and disruptions of ROS (⇓) is seen. The PE appears normal. e) ROS are shortened and disrupted (⇓), several photoreceptor nuclei are transformed into apoptotic bodies (*), PE cells are extremely flattened (⇓). f) The photoreceptor layer is almost completely removed leaving few scattered nuclei (*) and apoptotic bodies, the PE has disappeared. Original photographic magnification 1250×.

Molecular mechanisms of apoptosis induction in the retina are poorly understood. For example it is unclear which cellular signaling pathways mediate the death of defective photoreceptors in RP. Which signaling pathways are triggered by light and could induce apoptosis *via* the induction of gene expression and/or the activation of the death cascades?

Work in our laboratory has demonstrated that light exposure activates phospholipases and induces the release of arachidonic acid and docosahexaenoic acid and their metabolites in the retina, the lipid mediators of important cellular functions (Jung *et al.*, 1993; Jung and Remé, 1994; Reinboth *et al.*, 1995; Reinboth *et al.*, 1996; review: Remé *et al.*, 1998b). Several of those mediators, especially peroxidized intermediates (Tang *et al.*, 1996), were found to induce apoptosis in other tissues. Moreover, molecules of oxidative stress are potent inducers of apoptosis (Buttke and Sandstrom, 1994; Gorman *et al.*, 1996) and may play the same role under conditions of photochemical lesions in the retina. Preliminary studies in our laboratory have shown an induction of apoptosis in the retina *in vitro* by lipid mediators (Hafezi *et al.*, 1998).

There is a multitude of exogenous and endogenous factors that can induce apoptosis in several tissues (e.g. Schwartzman and Cidlowski, 1993b) and many of these factors, such as the exposure to cytokines or the deprivation of protective signals, could affect the retina as well. Furthermore, the alteration and/or the lack of matrix molecules is a stimulus for epithelial cells or neurons to initiate the death pathway (Ruoslahti and Reed, 1994). This was indeed observed in retinal photoreceptors (Molthagen *et al.*, 1996) and the PE (Tezel and Del Priore, 1997).

4.3. Gene Regulation of Apoptosis: Potential Role of c-Fos

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 (Morgan and Curran, 1995; Soares *et al.*, 1994). Death signals can activate transcription factors in the nucleus, at the cell membrane or in the cytoplasm through different pathways (Hill and Treisman, 1995). Fos and Jun are members of the leucine zipper family, which form homo- or heterodimers and bind to the nuclear DNA comparable to ligands and their receptors and regulate gene expression (Curran and Franza, 1988; Karin *et al.*, 1997). The different AP-1 complexes may regulate different genes and can therefore fulfill various biological functions. AP-1 proteins may not be directly involved in the basic death program but may participate in its activation (Karin *et al.*, 1997).

The potential role of *c-fos* in apoptosis may vary from one tissue to another. Continuous expression of *c-fos* precedes apoptosis in several tissues (Smeyne *et al.*, 1993) and apoptosis is induced by the c-Fos protein *in vitro* (Preston *et al.*, 1996). On the other hand, *c-fos* was not essential to induce apoptosis in lymphoid tissues *in vitro* (Gajate *et al.*, 1996) and even protected against UV induced cell death in mouse fibroblasts (Schreiber *et al.*, 1995; Haas and Kaina, 1995).

c-fos may also regulate important functions in the retina, perhaps including some of those related to phototransduction. In this context, *c-fos* expression was monitored in different experimental conditions as summarized in Table 2. Importantly, there is a diurnal rhythm of *c-fos* expression in specific retinal neurons (Nir and Agarwal, 1993; Yoshida *et al.*, 1993), further suggesting important regulatory functions of *c-fos*. Moreover, light exposure stimulated *c-fos* expression in a manner similar to that of mechanical injury or axotomoy of nerve fibers. Most of those studies found a rapid induction of *c-fos* expression 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 the *rd* and *rds* mouse during the period of enhanced apoptotic cell death (Rich *et al.*, 1997; Agarwal *et al.*, 1995). This might be observed

Table 2. c-Fos expression in the retina

Condition	Species	Cell type	Stimulus	Authors
LD-cycle light pulse	Mouse	Whole retina	White light	Nir and Agarwal, 1993
LD, DD light pulse	Rat	Retinal neurons	White light	Yoshida *et al.*, 1993
LD cycle	Rat	Retinal neurons	UVA, UVB	Gillardon *et al.*, 1995
LD cycle	Rat	Whole retina	White light	Gudehithlu *et al.*, 1993
LD cycle	Mouse	Retinal neurons cones	White light	Masana *et al.*, 1996
LD cycle	rd Mouse	Retinal neurons	,,	Rich *et al.*, 1997
LD cycle	rds Mouse	Retinal neurons	,,	Agarwal *et al.*, 1995
LD cycle	Rat	Ganglion cells	Axotomy	Robinson, 1994
LD cycle	Rat	Retinal neurons	Flashing light Sustained light	Yoshida *et al.*, 1996
LD cycle	Rat	Retinal neurons	Development	Ohki *et al.*, 1996
LD cycle	Rat	Retinal neurons Müller cells	Retinal injury	Yoshida *et al.*, 1995
LD cycle	Mouse	Retinal neurons	Light pulse	Hafezi *et al.*, 1997a

before or during the period of cell loss in other animal models of RP.

4.4. The Role of c-Fos in Light-Induced Apoptosis

In view of the above studies our laboratory investigated the role of *c-fos* in light-induced apoptosis. Acute light exposure in mice lacking *c-fos* function and in wildtype control littermates resulted in striking differences: there was practically no apoptotic cell death in *c-fos* -/- mice whereas distinct apoptosis occurred in wild-type animals (Hafezi *et al.*, 1997a). Even after exposure to high light doses (15 000 lx) and extendet post-exposure periods (11 days) the *c-fos* -/- mice were protected (Fig. 3). By contrast, retinal development in *c-fos* -/- mice appears to proceed normally; normal morphology and 20% reduced rhodopsin content were found in *c-fos* -/- mice and their electroretinograms did not differ significantly from that of wild-type mice even though a distinct variability was found (Niemeyer, personal communication). These and other studies (Rich *et al.*, 1997) support an essential role of *c-fos* in retinal apoptosis.

The identification of *c-fos* as an essential component in light-induced apoptosis raises the questions whether *de novo* gene expression is required to trigger apoptotic cell death of photoreceptors, and if so, how photon absorption can modulate such gene expression. Retinoic acid is synthesized in small quantities after light exposure by oxidation of all trans-retinal. Retinoic acid subserves important messenger functions, including the regulation of gene expression in development, and may also do so in mature tissues by binding to nuclear receptors (Dräger and McCafferty, 1997). In view of those observations it will be interesting to study potential effects of retinoic acid on the expression of *c-fos*.

4.5. Other Regulatory Genes

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 (e.g. Donehower *et al.*, 1992; Clarke *et al.*, 1993; Papermaster, 1997). To investigate the role 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* (Marti *et al.*, 1998).

The role of the Bcl-2 family of proteins in regulating photoreceptor apoptosis has not been clarified to date. Although overexpression of

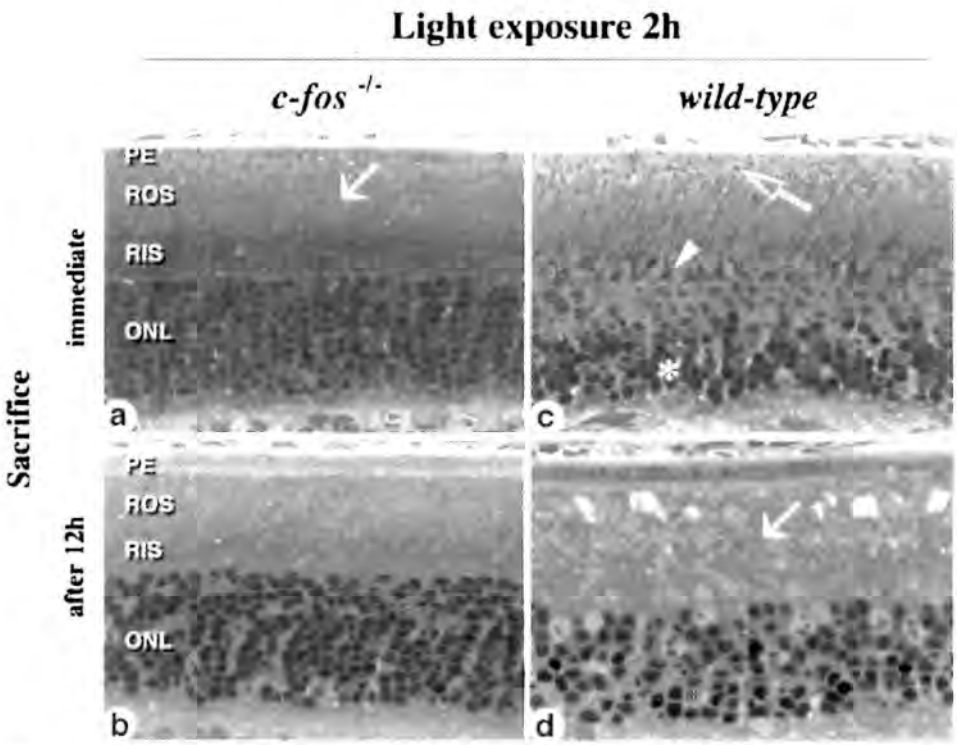

Fig. 3. Light micrographs depicting retinae from a *c-fos* -/- mouse and a wildtype littermate after light exposure. Free moving mice were exposed to 15 000 lx of diffuse, white fluorescent light for 2 h and killed immediately and 12 h after the exposure. a) Retina from a *c-fos* knock out mouse immediately after light exposure. Apart from mild disruptions of ROS (↓), no changes are observed. b) Retina from a c-fos knock out mouse 12 h after the end of the light exposure. Again, no alterations appear. c) Retina from a wildtype littermate immediately after the light exposure depicting a distinct acute apoptotic response with condensed RIS (▽) and nuclei (*). Note the burst of disk-shedding in the PE (⇓), which is absent in the knock-out animals. d) Retina from a wildtype littermate 12 h after the light exposure depicting severe lesions of ROS (↓) and numerous condensed photoreceptor nuclei (*). Original photographic magnification 800×.

Bcl-2 delayed apoptotic photoreceptor death in RP animal models, the transgene was unable to prevent the final outcome of the degeneration (Chen *et al.*, 1996; Tsang *et al.*, 1997). On the other hand, Joseph and Li found an increased resistance to light damage which was attributed to a reduction in rhodopsin level rather than to an antiapoptotic effect of the overexpression of Bcl-2 (Joseph and Li, 1996).

4.6. Approaches to Mechanisms

4.6.1. *The role of mitochondria*

Recent studies implicate an essential role of mitochondria in apoptosis (Kroemer *et al.*, 1997). Mitochondria appear to release cytochrome C and AIF (Kluck *et al.*, 1997) or cytochrome C (Yang *et al.*, 1997) which can trigger apoptosis. Other studies indicate that a critical early event in apoptosis is a change in mitochondrial membrane potential ($\Delta\psi_m$) leading to a permeability transition. This can be initiated by exogenous damaging agents or by receptor mediated signals and leads to the release of AIF and cytochrome C. While the initial induction phase of apoptosis may vary in different cell systems and tissues, the effector phase is thought to converge to common mechanisms including mitochondrial changes and the activation of proteases (Kroemer *et al.*, 1997; Zamzami *et al.*, 1996).

Proteins of the Bcl-2 family are located in the membranes of the cell nucleus, the endoplasmic

reticulum and, importantly, the outer mitochondrial membrane. Depending on specific ratios of pro- or antiapoptotic members of the family, the death pathway is supported or inhibited. Bcl-2 was found to inhibit the release of cytochrome C, thereby blocking apoptosis (Kluck *et al.*, 1997; Yang *et al.*, 1997). Bcl-2 also suppressed the mitochondrial membrane permeability transition and the release of AIF (Kroemer, 1997; Susin *et al.*, 1996).

When the ultrastructure of illuminated retinae in wildtype and *c-fos* -/- mice was analyzed, a prominent difference in the preservation of photoreceptor mitochondria was observed. While the mitochondria in *c-fos* deficient mice showed normal morphology, those of wildtype mice revealed distinct lesions with swelling and rupture of cristae (Fig. 4).

In view of light-induced apoptosis, mitochondrial damage as an early effector event appears as a plausible mechanism induced by photochemical lesions that produce reactive oxygen species and other molecules that can injure mitochondria. UV induced lesions or toxic agents (e.g. elevated levels of cGMP) may exert similar effects.

To date it is unclear why mitochondria in *c-fos* -/- mice are protected against light-induced lesions. *c-fos* may alter the equilibrium of pro- and anti-apoptotic Bcl-2 family members in favor of apoptosis, and the lack of *c-fos* would be anti-apoptotic. Further studies will have to clarify the relation of *c-fos* and mitochondria in light-induced apoptosis.

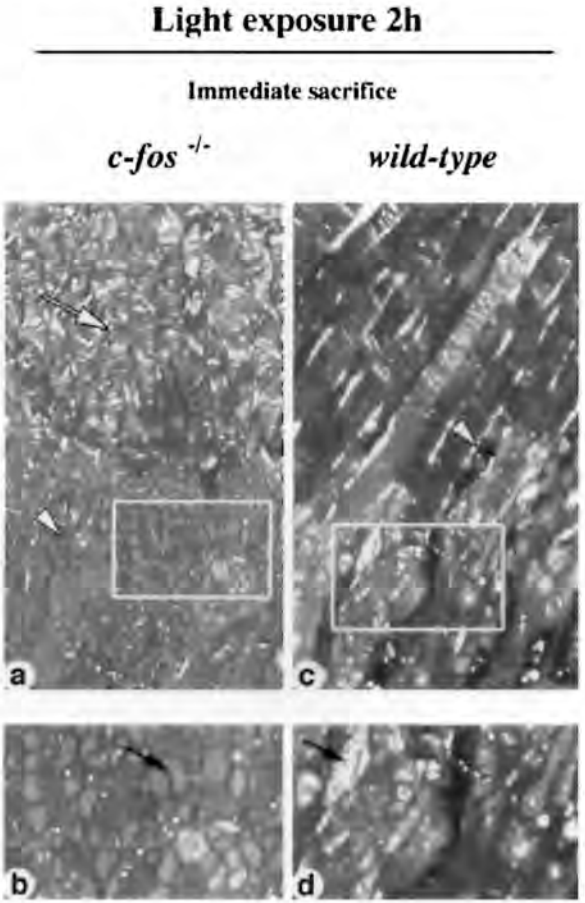

Fig. 4. Electron micrographs showing retinae from a *c-fos* knock out mouse and a wildtype littermate sacrificed immediately after 2 h exposure to 15 000 lx of diffuse, white fluorescent light. a) ROS and RIS from a *c-fos* knock out mouse. Disruptions of ROS (↓) are visible, RIS (▽) appear well preserved. b) The same specimen illustrating well preserved mitochondria (↓). c) ROS and RIS from a wildtype littermate. Disruptions of ROS are visible as well as condensed inner segments (▽) indicating apoptosis. d) The same specimen depicting lesioned mitochondria with disrupted cristae (↓). Original photographic magnifications 2500× (a and c) and 10 000× (b and d).

4.6.2. *Apoptosis as a major correlate of light damage*

Our observations reveal that no retinal damage and no apoptosis occurs in light-exposed *c-fos* -/- mice in comparison to wildtype mice. The lack of any cell loss when apoptosis was prevented indicates that apoptosis is one major correlate of light damage. This assumption is supported by observations that growth factors, cytokines and neurotrophins partially rescue photoreceptors from damage by constant light (LaVail *et al.*, 1992; Faktorovich *et al.*, 1992). Those molecules exert antiapoptotic effects in other systems and may have done so in the above experiments. Similarly, other agents that protect against light damage, such as antioxidants and radical scavengers, may act, at least in part, through antiapoptotic mechanisms. They may also, however, reduce light-induced effects that are secondary to the primary event of apoptosis.

4.6.3. *Apoptosis as the final common death pathway in RP*

Several questions emerge in view of apoptosis as the final common death pathway in such heterogeneous conditions as the animal models of RP. The naturally occurring dystrophies as well as those bearing transgenes or gene deletions show great variety on a cellular and molecular level, such as numerous alterations in the rhodopsin molecule, cytoskeletal disturbances in photoreceptors, inhibited or modified phototransduction or defects in ROS phagocytosis (e.g. Lem and Makino, 1996; Wong, 1997). How can those diverse lesions lead to a final common death pathway? The induction of apoptosis may arise within the diseased cell or may be triggered by extracellular signals and both conditions may be enhanced by exogenous factors such as light exposure, nutrition or toxic agents. Light exposure was found to enhance photoreceptor loss in mice bearing a rhodopsin point mutation (Pro23His) indicating that light-induced apoptosis was added to that induced by the genetic defect (Naash *et al.*, 1996). This observation revives the question whether patients suffering from RP should be protected against bright light exposure (Berson, 1980). According to animal studies, however, light deprivation does not retard the cell loss in all forms of RP. Thus, there are attributes of the Pro23His mouse and some other animal models (e.g. the albino RCS rat) that render them especially sensitive to light exposure. Diverse signals may thus converge to a common execution point in the functionally disturbed cell. It remains to be seen at which level of dysfunction the cell is submitted to death.

In contrast to RP animal models, our light-exposure model permits close inspection of the early events following or even accompanying initial cellular lesions. Due to the control of the onset time, our model produces a synchronized burst of apoptosis as well as controlling potentially confounding effects produced by secondary responses. These may include stress reactions, inflammation and proliferation, lipid peroxidation as a result of cellular decay, altered Ca^{++} levels and others. The study of early stages in the apoptotic process permits us to investigate induction pathways and their target effector systems in photoreceptors.

5. TELEOLOGICAL VANTAGE POINTS

One of the most obvious advantages of apoptotic cell death appears to be its confinement to single cells in contrast to necrotic cell loss which spreads indiscriminately beyond the single cell level. The strictly regulated apoptotic program provides the possibility to fine tune cell death according to the requirements of a given situation in development and in mature tissues.

5.1. Adaptation

For the retina, adaptation to different environmental illuminance conditions may be an essential survival mechanism and, as an apparent paradox, may include cell death. Beyond this self-protective adaptation of the retina, the organism as a whole may need to catch a specific number of photons within a 24 h cycle. For this purpose, the retina can adapt to lighting conditions where this set point is achieved. This principle has been discovered and elucidated in detail by Williams and his co-workers and is termed "photostasis" (Penn and Williams, 1986; Williams and Thistle, 1998). Light dependent apoptotic cell death—even though being a point of no return—may be a means to achieve the goal of photostasis in the developing as well as the mature retina.

In contrast to apoptosis, other photoreceptor degradative processes permit to adapt in a reversible manner to varying illuminance levels: disk-shedding from outer segments (LaVail, 1976) and autophagic degradation in inner segments (Remé, 1977; Remé and Young, 1977; Remé *et al.*, 1986; Remé *et al.*, 1991; Remé *et al.*, 1998a). Increased phagosome numbers will reduce the light sensitivity of rods by removing rhodopsin (Schremser and Williams, 1995), autophagic degradation may remove surplus proteins—including those involved in phototransduction—when adaptation requires reduced light sensitivity, and vice versa,

when an increase is necessary. In this context, autophagy may be considered as pre-apoptotic in degrading only parts of a given cell as an "organelle death" without leading to the extinction of the entire cell. Indeed, in our model early apoptotic stages often reveal numerous autophagic vacuoles within the cytoplasm indicating that both processes are initiated by light exposure and may be considered as a continuum, perhaps even sharing some signaling- and degradative-pathways.

5.2. Apoptosis and Immune Privilege

A protective mechanism where apoptosis plays a significant role is the immune privilege of the eye, which is known to consist of a number of important factors. One of these factors is FasL-induced apoptosis of invading inflammatory cells in viral infection. FasL is expressed in ocular structures including the retinal pigment epithelium and photoreceptors, whereas the Fas receptor is not. Invading inflammatory cells presenting the Fas receptor can thus be destroyed (Griffith and Ferguson, 1997; Griffith *et al.*, 1996; Griffith *et al.*, 1995). The origin of and signaling for those macrophages observed after light-induced apoptosis or in other retinal degenerations remains to be elucidated. Macrophage activity apparently is not inhibited, or, alternatively, macrophages are of local origin and may thus not bear the Fas receptor. In human macrophages, FasL failed to induce apoptosis and a protective mechanism downstream of the FasL/receptor interaction has been proposed to exist in those cells (Kiener *et al.*, 1997). Such a mechanism may also be effective in the retina under certain conditions.

The transplantation of mature retinal or pigment epithelial cells in retinal degenerations may profit significantly from the immune privilege due to the FasL system.

6. FUTURE DIRECTIONS

Research on retinal apoptosis will have to include signaling pathways and genes involved in it's regulation in animal models of RP and light-induced degeneration. Of special interest is the role of *c-fos* and its target genes. Is c-Fos directly implicated in apoptosis or does it participate in the regulation of phototransduction and is, therefore, only indirectly linked to the apoptotic machinery by e.g., a modification of light sensitivity? Because apoptosis is a regulated process, there may be a rhythmic expression of the basic death machinery that needs to be triggered in specific death requiring situations. An endogenous rhythm would further contribute to the possibility of regulation and fine tuning. The documented rhythmic expression of *c-fos* may underscore its regulative role in apoptosis.

Of particular interest for applied reserearch might be the prevention of photoreceptor apoptosis in animal models of RP. Although such prevention would not cure the gene defect, it might delay cell death. Impairment of apoptosis might be followed by an alternative, non-apoptotic mode of photoreceptor death, since the deficiency in cellular function would still be present. The reduction of apoptotic cell death may help to prolong vision even if the primary disease is not cured. Further, it will be important to clarify the molecular mechanisms of the enhanced sensitivity to light exposure in some of the RP animal models.

Even though cones in many respects are more important than rods for human visual functions, they are, in general, much less investigated than rods in cell biology. Apoptotic mechanisms in cones are practically unknown and thus need to be studied. For example, it would be important to see why cones are dying in the course of RP and whether they do so by apoptosis. Not only the signaling between rods and cones, but also between the PE and photoreceptors needs to be clarified in the context of apoptosis. Pilot studies in our laboratory have demonstrated cone apoptosis in the thirteen lined ground squirrel (Remé *et al.*, 1998b). In view of degenerative changes and dystrophies of the central retina in humans, such studies should be extended and the mechanisms of cone apoptosis should be sought.

The rodent retina model of apoptosis does not lend itself readily to study apoptosis in cones. Thus, other model systems in duplex retinae of larger animals with longer life spans should be

developed. Not only might the extended time course of cell death observed in human retinae find it's correlate in the retinae of such animals, but such models would elucidate pathways leading to cone death in retinae where the primary disease is in rods.

Both the induction and the effector phases of retinal apoptosis are poorly understood and need to be clarified. With respect to induction, for example, what is the role of mitochondrial damage and what other early mechanisms might there be? And for the late apoptotic periods it remains to be seen, e.g., which signals attract macrophages and why they are not destroyed by the ocular FasL system.

On a subcellular level it will be important to correlate the biochemical events of apoptosis with the light- and electron-microscopic appearance of cellular features. For example, we consistently observe that chromatin condensation precedes immuno-labeling of DNA fragments (TUNEL) in our model. Thus, could the chromatin condensation be reversible, representing a "point of return" which might be considered pre-apoptotic? A basic question concerning light-induced apoptosis but also physiological regulation of light sensitivity is whether light can modulate the expression of specific genes and, if so, how.

In many tissues the signalling between cells and their extracellular matrix constitutes an important survival system and apoptosis is induced when the normal interaction is disturbed. Such signalling may also exist in the retina and PE and apoptosis is triggered when the signalling is impaired. For example, alterations in Bruch's membrane may induce PE apoptosis with subsequent loss of photoreceptors thus mimicking human retinal degenerations.

Light-induced apoptosis in the mouse is confined to photoreceptors in our model, whereas in the rat, both photoreceptors and the pigment epithelium respond with apoptotic cell death. The mouse pigment epithelium may thus contain protective factors that can prevent apoptosis and it will be significant to study those potential factors, especially in view of the potential therapeutic usefulness of such information for human therapies.

Detailed studies of light-induced apoptosis could help to answer the general question "Where does apoptosis end and necrosis begin?". What is the range of illuminance for apoptotic responses? Does necrosis occur at higher illuminance levels? And further, is there a spectral dependence of apoptosis induction, i.e. can we derive an action spectrum?

In conclusion, investigating details of retinal apoptosis will help to clarify basic questions of retinal function as well as lead to therapeutic strategies for conserving and hopefully extending periods of useful vision in various retinal pathologies including degenerative diseases.

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