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Early neural cell death: numbers and cues from the developing neuroretina

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ABSTRACT Programmed cell death is a well established key process required for proper development of the nervous system. The regulatory and executor mechanisms controlling survival/death of projection neurons, as well as of other types of differentiated neurons and glial cells, have been studied intensely during neural development. Much less attention has been paid to earlier cell death events affecting neuroepithelial cells and recently born neurons and glial cells. We review here the reports on cell death during vertebrate retina development, our model system for many years, which has provided clear evidence of the importance of early neural cell death. We tentatively categorize the available observations in three death phases, namely morphogenetic cell death, early neural cell death and neurotrophic cell death. The magnitude and the precise regulation of the early phases of cell death are fully comparable to the much better characterized neurotrophic cell death. Therefore, early neural cell death deserves a profound dedicated study; this will help to obtain an integrated understanding of the development of the retina and other parts of the vertebrate nervous system.

KEY WORDS: *apoptosis, neural development, proliferation, differentiation, neurogenesis*

The nervous system contains an amazingly complex array of cell types and stereotypic connections that form the basis of its sophisticated functionality. The studies of Santiago Ramón y Cajal revealed many aspects of the intricate neuronal layout of the nervous system in detailed microscopic preparations and beautiful drawings (Fig. 1). However, one century later neuroscientists still fail to fully understand just how such diversity and complexity is achieved.

Most of the nervous system arises from apparently undifferentiated neuroepithelial cells that are in a state of proliferation. These neuroepithelial cells undergo a developmental process that takes them through the sequentially stages of neural stem cells, neural progenitors, neuroblasts or glioblasts, until they finally become differentiated neurons or glial cells. Thus, proliferation and differentiation, as well as cell migration, axonal and dendritic growth and navigation, have been clearly recognized as fundamental processes in generating neural complexity and, consequently, these processes have been studied intensely during neural development. As elaborated in the neurotrophic theory, another particularly relevant process in refining neural morphology and physiology is that of programmed cell death (PCD), which affects projecting neurons as well as other types of differentiated neurons and glia (Kuan *et al.*, 2000; Roth and D'Sa, 2001; Davies, 2003; Buss *et al.*, 2006).

However, this is neither the only nor the earliest cell death that occurs in the developing nervous system. Neural stem cells, proliferating progenitors, and recently born neurons and glia are all also affected by cell death (de la Rosa and de Pablo, 2000; Yeo and Gautier, 2004; Boya and de la Rosa, 2005). Although this early phase of neural cell death is increasingly being studied, the cell populations affected, the regulatory mechanisms involved, its magnitude and the functional implications of this process are still

Abbreviations used in this paper: AC, amacrine cell; BC, bipolar cell; E, day of embryonic development; GCL, ganglion cell layer; INL, inner nuclear layer; MC, Müller glial cell; NBL, neuroblastic layer; ONL, outer nuclear layer; P, postnatal day; PCD, programmed cell death; PhR, photoreceptor; RGC, retinal ganglion cell; TUNEL, TdT-mediated dUTP nick end labelling.

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In memoriam Prof. Dr. Uli Schwarz, Max-Planck Institute of Developmental Biology († Dec 21, 2006) and Prof. Ruben Adler, Johns Hopkins University School of Medicine († Dec 31, 2007).

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far from being appropriately characterized. This limited understanding of early neural cell death may be impeding an integrated understanding of neural development.

The vertebrate neuroretina is one of the classic model systems used in developmental neurobiology (Cepko *et al.*, 1996; Harris, 1997; Marquardt and Gruss, 2002; Adler, 2005). The retina is an accessible part of the central nervous system since it is located outside of the brain and it can be subjected to a variety of experimental approaches, including *in vivo* manipulation using classic experimental embryological techniques, particularly in non-mammalian vertebrates. Several reviews have addressed early neural cell death as a global process in the developing nervous system, including those referred to above. Here we will focus on the retina, our model system for many years, which has provided clear evidence of the importance of early neural cell death.

Retinal development is affected by several phases of cell death

Retinal development commences with the induction of the retinal anlage in the neural plate/neural tube, the morphogenesis of the optic vesicle and optic cup, and it proceeds with the proliferation of the neuroepithelial cells, the generation of differentiated neurons and glia, the migration to their proper retinal layer, axon and dendrite growth and navigation, synaptogenesis and functional refinement (Cepko *et al.*, 1996; Chow and Lang, 2001; Levine and Green, 2004; Harada *et al.*, 2007; Lamb *et al.,* 2007). PCD occurs simultaneously to these processes and it can be considered a genuine developmental process. Numerous events of cell death have been independently characterized in several vertebrate species (Tables 1-6, and reviewed in Cellerino *et al.*, 2000; Vecino *et al.*, 2004; Farah, 2006; Guerin *et al.*, 2006), although such studies have more commonly focused on later periods of retinal development. However, by simply considering their timing, many of these studies highlight that most if not all of the aforementioned developmental processes in the retina are affected by PCD, a fact not well recognized in the literature. Here, we review the observations made on PCD in the vertebrate retina, which we have tentatively organized according to the developmental phases in which they occur, denominated: morphogenic cell death (programmed cell death related with optic vesicle evagination, optic cup invagination and closure of the optic fissure); early neural cell death (programmed cell death affecting proliferating neuroepithelial cells and recently born neurons and glia); and neurotrophic cell death (programmed cell death affecting differentiated neurons mostly due to lack of neurotrophic support or inappropriate synaptogenesis).

Before addressing these issues, we should first recall several facts that hinder precise comparison and temporal compartmentalization of cell death during retinal development. The development of the retina occurs along prominent spatio-temporal gradients

TABLE 1

VERTEBRATE SPECIES REFERRED TO IN THIS REVIEW

Common name	Scientific name
Zebrafish	*Danio rerio*
Medaka	*Oryzias latipes*
African cichlid fish	*Haplochromis burtoni*
Brown trout	*Salmo trutta*
Grass frog	*Rana temporaria*
Clawed frog	*Xenopus laevis*
Turtle	*Mauremys leprosa*
Chick	*Gallus domesticus*
Quail	*Coturnix coturnix*
Wallaby	*Setonix brachyurus*
Cat	*Felix catus*
Ferret	*Mustela putorius furo*
Rabbit	*Oryctolagus cuniculus*
Mouse	*Mus musculus*
Rat	*Rattus norvergicus*
Hamster	*Mesocricetus auratus*
Guinea pig	*Cavia porcellus*
Rhesus monkey	*Macaca mulatta*
Human	*Homo sapiens*

Common and scientific names are referred to for identification.

TABLE 2

MORPHOGENIC CELL DEATH IN RETINAL DEVELOPMENT

Species	Developmental stage range & (peak)	Process, distribution & *magnitude*	References
Zebrafish	Hpf 12-18 (Hpf 16)	Invagination of the optic vesicle. *20 apoptotic cells per retina. 0.9% of neuroepithelial cells dying at Hpf 16.*	Li *et al.*, 2000 Cole and Ross, 2001
Medaka	Stg 20-26 (Stg 24-26)	PCD in the neuroepithelium.	Iijima and Yokohama, 2007
Brown trout	5–8 mm (5 mm)	Morphogenesis of the optic vesicle and cup. Proliferating cells dying in the neuroepithelium. *500 apoptotic cells/mm^2 at 5 mm. Cell death decreases just after the formation of the optic cup.*	Candal *et al.*, 2005
Grass frog	Hpf 25-48	Invagination of the optic vesicle. Proliferating cells dying in the central retina.	Glücksmann, 1940
Clawed frog	Stg 26-32	Invagination of the optic vesicle and closure of the optic fissure.	Hensey and Gautier, 1998
Chick	HH 12-17 (HH15)	Formation of the optic cup. PCD in the optic fissure. *1515 pyknotic fragments at HH15.*	Martín-Partido *et al.*, 1988 Trousse *et al.,* 2001 Valenciano *et al.*, 2002
Ferret	E20	Cell death in the optic fissure before its closing.	Strongin and Guillery, 1981
Mouse	E10.5-14.5 (E10.5)	PCD in the neuroepithelium. Closure of the optic fissure. *2% of neuroepithelial cells dying in central retina at E10.5.*	Strongin and Guillery, 1981 Laemle *et* al., 1999 Van Kefflens *et al.*, 1999 Ozeki *et al.*, 2000 Trousse *et al.*, 2001 Péquignot *et al.*, 2003 Rodríguez-Gallardo *et al.* 2005
Rat	E13	Cell death in the optic fissure.	Kuwabara and Weidman, 1974
Hamster	E10	Cell death in the optic fissure before its closing.	Strongin and Guillery, 1981
Human	ND	Cell death in the optic fissure before its closing.	Strongin and Guillery, 1981

Developmental age referred to as: E, day of embryonic development; Hpf, hours post-fertilization; HH and Stg, developmental stages (Medaka, Iwamatsu, 1994; Clawed frog, Nieuwkoop and Faber, 1967; Chick, Hamburger and Hamilton, 1951); mm, length of the embryo; ND, not determined. Abbreviations: PCD, programmed cell death.

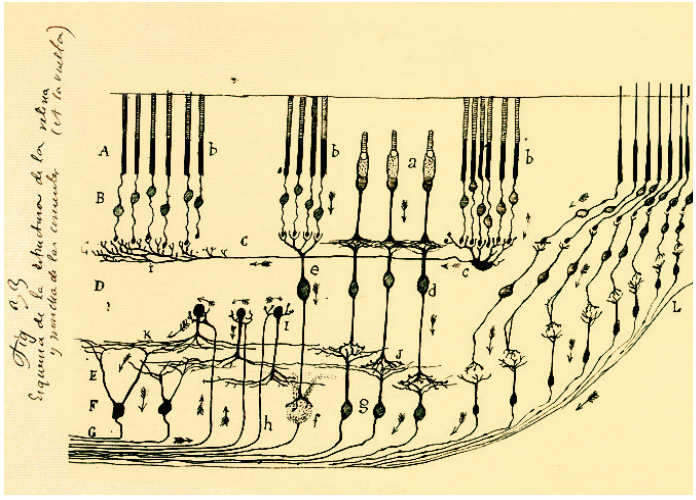


Fig. 1 (Left). Schematic representation of the structure of the mammalian retina. Artistic grouping of cells and the direction of "current flow" as depicted by Santiago Ramón y Cajal (1901). (A) *Layer of rods and cones;* **(B)** *visual cell body layer;* **(C)** *outer plexiform layer;* **(D)** *bipolar cell layer;* **(E)** *inner plexiform layer;* **(F)** *layer of ganglion cells;* **(G)** *optic nerve fiber layer;* **(L)** *central fossa. Reproduced from the original drawing deposited at the Cajal Institute, CSIC, Madrid, Spain with permission.*

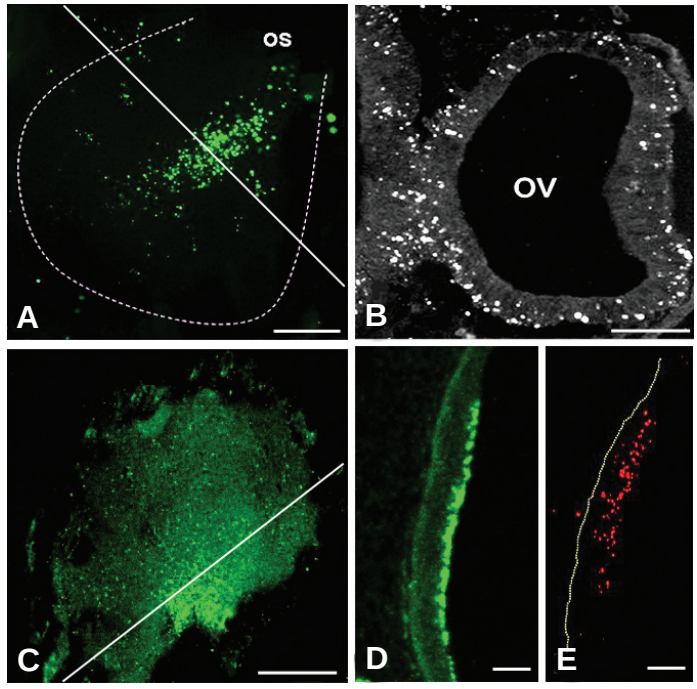


Fig. 2 (Right). Spatiotemporal correlation of proliferation, retinal ganglion cell neurogenesis and cell death. (A) *Distribution of dying cells in the HH16-17 chick retina, as visualized by TUNEL staining of a whole-mount retina. The profile of the neuroretina is marked by a dashed line. The continuous line indicates the approximate plane of the section showed in (B) (reproduced from Valenciano* et al.*, 2002).* **(B)** *Distribution of proliferating cells in the HH16 chick retina, as visualized by BrdU incorporation and immunostaining of a retinal section (reproduced from Mayordomo* et al.*, 2003). Note that TUNEL-positive nuclei ((A), green) are concentrated in the presumptive optic fissure whereas BrdU-positive nuclei (B, white) appear to be scattered throughout the whole retina.* **(C)** *Distribution of dying cells in the E4 chick retina, as visualized by TUNEL staining of a whole-mount retina. The continuous line indicates the approximate plane of the sections showed in (D,E) (reproduced from Díaz* et al.*, 1999).* **(D)** *Distribution of retinal ganglion cells in the E4 chick retina, as visualized by Islet 1/2 immunostaining in a retinal section (reproduced from Díaz* et al.*, 2000).* **(E)** *Distribution of dying cells in the E4 chick retina, as visualized by TUNEL of a retinal section adjacent to that shown in (D) (reproduced from Díaz* et al.*, 2000). Note that TUNEL-positive nuclei ((C) green; (E) red) and RGC, Islet 1/2-positive nuclei ((D) green) are located in overlapping areas of the central retina. The dotted line indicates the outer margin of the retina. Scale bar: 200 μm in (A), 75 μm in (B), (D) and (E), 1 mm in (C). Images reproduced with permission from (A) Lippincott, Williams & Wilkins, (B,C) European Neuroscience Association and (D,E) The Company of Biologists.*

implying that cells at different locations of the same retina may be involved in different developmental processes. Moreover, in lower vertebrates there is indefinite growth of the retina and hence, its initial development is significantly accelerated which results in an overlap of the phases of cell death defined above. In the various vertebrate groups, differences are evident in the cellular composition and developmental timing of the retina often making direct comparisons difficult. Furthermore, in the studies carried out to date, comparisons of cell death have been hindered by the many different methods employed: from electron microscopy in tissue sections to TdT-mediated dUTP nick end labelling (TUNEL) analysis of whole-mount retinas. Similarly, both absolute (total and cumulative numbers) and relative (cell densities and actual numbers) quantification may or may not have been performed. Besides that, cumulative numbers of dead cells are not directly extrapolable from actual numbers at a defined time because apoptotic cells are efficiently removed from the tissue. Thus, we present here a tentative analysis of the descriptive data available from different vertebrate groups, as well as the quantitative information where available, attempting to organize them in relation to the distinct phases of PCD that occur throughout retinal development.

Morphogenic cell death during eye development

Several studies have focused on PCD during the initial stages of eye and retinal development, including during eye morphogenesis and in the early stages of proliferation within the retinal neuroepithelium (Table 2 and references herein). At these stages, the dying cells are selectively associated to the developing optic fissure (Fig. 2A), probably in correlation with the ongoing morphogenesis. It has been suggested that this death is involved in sculpting the retina and facilitating the exit of optic fibers (Cuadros and Ríos, 1988, Ozeki *et al.*, 2000, Morcillo *et al.*, 2006). However, more detailed studies in interference models (i.e. KO mice, *in vivo* manipulation in chick, frogs and fishes) will be necessary to more precisely define the function of this phase of PCD.

At these stages, scattered dying cells can also be found and they are normally most abundant in the central retina. Some studies have characterized these dying cells as proliferating/mitotic cells (Table 2; Glücksmann, 1940; Díaz *et al.*, 1999; Li *et al.*, 2000; Candal *et al.,* 2005). This observation may just be a consequence of their nature as proliferating neuroepithelial cells, or it may indicate a functional coupling between proliferation and cell

TABLE 3

DEVELOPMENTAL TIMING OF RETINAL GANGLION CELLS

Species	First RGCs born	First optic fibers reach extraretinal targets	References
Zebrafish	Hpf 28	Hpf 44	Burrill and Easter, 1994 Schmitt and Dowling, 1994, 1996 and 1999
Medaka	Stg 26	Stg 32	Iwamatsu, 1994 Yoda *et al*., 2004
African cichlidae fish	Before Dpf 4	Dpf 5	Presson and Fernald, 1986 Hagedorn and Fernald, 1992
Brown trout	11 mm	11 mm	Mansour-Robaey and Pinganaud, 1996 Candal *et al*., 2005
Grass frog	Hpf 24		Glücksmann, 1940
Clawed frog	Stg 32	Stg 35	Nieuwkoop and Faber, 1967 Jenkins and Straznicky, 1986
Turtle	E16	E29	Hidalgo-Sánchez *et al*., 2006 Francisco-Morcillo et al., 2006
Chick	E2	E9	Dreher and Robinson, 1988 Prada *et al*., 1991
Quail		E7	Senut and Alvarado-Mallart, 1986
Wallaby		P85	Braekevelt *et al*., 1986 Beazley *et al*, 1988
Cat	E19	E48	Walsh *et al*., 1983 Williams *et al.,* 1986
Ferret	E22	E28	Linden *et al*., 1981 Cucchiaro and Guillery, 1984 Reese *et al*., 1994 and 1996
Rabbit	E16	E21	Dreher and Robinson, 1988 Crabtree, 1989 and 1990
Mouse	E10	E16	Godement *et al*., 1984 Cepko *et al*., 1996
Rat	E14	E16	Horsburgh and Sefton, 1987 Dreher and Robinson, 1988 Galli-Resta *et al*., 1996
Hamster	E10		Sengelaub *et al*., 1986
Guinea pig	E24		Langford and Sefton, 1992
Human	Before week 8		Provis *et al.,* 1985a Sturrock, 1987

Developmental age referred to as: Dpf, days postfertilization; E, day of embryonic development; Hpf, hours postfertilization; mm, length of the embryo; Stg, developmental stages (Medaka, Iwamatsu, 1994; Clawed frog, Nieuwkoop and Faber, 1967); week, gestational week.

TABLE 4

EARLY NEURAL DEATH DURING GENERATION OF RETINAL GANGLION CELLS

Species	Developmental stage range & (peak)	Process, distribution & *magnitude*	References
Zebrafish	Hpf 20-48 (Hpf 36)	No proliferating cells dying. *68 apoptotic cells per retina. 0.2% of dying cells in the GCL.*	Li *et al*., 2000 Cole and Ross, 2001
Medaka	Stg 26-28 (Stg 26)	*50 apoptotic cells per retinal section.*	Iijima and Yokohama, 2007
Brown trout	12-13 mm	*50 apoptotic cells/mm^2.*	Candal *et al.,* 2005
Clawed frog	Stg 32-36	PCD in all layers of neuroepithelium.	Hensey and Gautier, 1998
Chick	E2-6 (E3.5-4)	Proliferating cells dying. *12.000 apoptotic cells/mm^2. 1680 pyknotic fragments.*	Martín-Partido *et al*, 1988 Frade *et al*., 1996 Díaz *et al*., 2000; Mayordomo *et al*., 2003; Chavarría *et al*., 2007
Cat	E19-33 (E28)	PCD surrounding the optic fissure between E19 to E23. *0.23% of RGCs dying at E28.*	Willians *et al.,* 1986
Ferret	E25	PCD surrounding the optic fissure after its closing.	Strongin and Guillery, 1981
Mouse	E15.5-17.5 (E16.5)	*0.5% of cells dying in the NBL.*	Péquignot *et al.,* 2003
Hamster	E12	PCD surrounding optic fissure after its closing.	Strongin and Guillery, 1981

Developmental age referred to as: E, day of embryonic development; Hpf, hours postfertilization; mm, length of the embryo; Stg, developmental stages (Medaka, Iwamatsu, 1994; Clawed frog, Nieuwkoop and Faber, 1967). Abbreviations: GCL, Ganglion Cell Layer; NBL. Neuroblastic Layer; PCD, Programmed Cell Death; RGC: Retinal Ganglion Cell.

death, for example as a mechanism to correct DNA replication or mitotic errors. However, while proliferating cells are evenly disseminated in the retina at these stages (Fig. 2B), the concentration of cell death in the central retina is maintained during early retinal development and later, it is clearly better correlated with cells leaving the cell cycle rather than with cycling cells (see below; Fig. 2, C-E; Díaz *et al*., 2000). Thus, the abundance of dying cells in the central retina may better reflect the onset of the next phase of PCD (see below).

While cell death in the initial stages of eye morphogenesis and retinal development is unequivocally documented (Table 2), quantitative studies of these events are very scarce. Thus, the relative magnitude of morphogenic cell death can only be extrapolated from comparative studies that are performed by the same laboratory using the same method. In the trout (Candal *et al*., 2005), the density of apoptotic cells in the selective locations where morphogenic cell death is taken place is 10-fold higher than that at any other later phase

affecting retinal ganglion cells (RGCs). In mouse (Péquignot *et al.*, 2003), a similar comparison renders 2% of dying cells at E10.5 whereas at P2, 3.5% of cells in the ganglion cell layer (GCL) undergo PCD during the neurotrophic cell death phase. The available data cannot be extrapolated to determine the cumulative numbers of cells eliminated during morphogenic cell death, which characteristically seems to involve a high density of dying cells in defined, narrow spatiotemporal coordinates (Fig. 2A).

Early neural cell death affecting retinal ganglion cell generation

RGCs are the first differentiated cell type generated during retinal development and RGC neurogenesis seems to start in the late period of eye morphogenesis or immediately afterwards (Table 3). RGC progenitors leave the cell cycle following a central-to-peripheral gradient, at least in the best characterized species (Prada *et al.*, 1991). A number of studies have described a phase of PCD that follows the closure of the optic fissure, concomitant with the initiation of differentiation in the retina (Table 4). However, this phase is not always recognized as an independent death event and adequately characterized as such.

A few studies over an extended period of retinal developmental have identified this early phase as an independent developmental PCD event and enabling the relative magnitude to be compared. In various fishes, it is not always easy to isolate the peaks of morphogenetic and early neural cell death, probably due to the accelerated initial development when compared to higher vertebrates (Candal *et al.*, 2005; Iijima and Yokohama, 2007). In chick (Martín-Partido *et al.*, 1988), the numbers reported are very similar for the morphogenetic and early neural phases (1515 vs. 1680 pyknotic fragments, respectively). In the mouse, it is again impossible to isolate these peaks of cell death (Péquignot *et al.*, 2003).

The situation is more clearly defined for later neurotrophic cell death. In the chick (Chavarría *et al.*, 2007), apoptotic cells are tightly present in the central retina at E3-5 (Figs. 3, A-C, and 4A). After a period of little PCD (E6-8, Figs. 3, D and E, and 4A), cell death resurges again and sequentially affects the ganglion cell layer (GCL, peak at E9, Figs. 3, E-H, and 4B), the inner nuclear layer (INL; peak at E11, Figs. 3, F-K, and 4B), and to a small extent the outer nuclear layer (ONL). The density of apoptotic cells during the early peak of PCD, concomitant with RGC generation, is more than 20-fold higher than in the peak of neurotrophic cell death affecting the GCL. In the mouse, the early phase of neural cell death (difficult to identify as mentioned above) appears to be moderate, with 0.5% of the cells in the neuroblast layer undergoing apoptosis at E12.5 (Péquignot *et al.*, 2003). Later, and also in the central retina, the peak of PCD at P2 reaches 3.5% of cells in the GCL. In the cat (Williams *et al.,* 1986), 0.23% of the cells are dying at E28 in the forming GCL in comparison with 0.4% of RGCs with axons that undergo apoptosis at E44-48. Although the peak of early neural cell death is difficult to define in the brown trout (Candal *et al.,* 2005), the incidence of cell death in the corresponding stages (12-13 mm) is identical to the later PCD in the GCL (17 mm; 50 apoptotic cells /mm^2). These numbers essentially indicate that the relative magnitude of early neural cell death is comparable with that of the subsequent neurotrophic cell death. The differences observed between species may correlate with the difference in the timing of neurogenesis and the cell composition of the respective retinas.

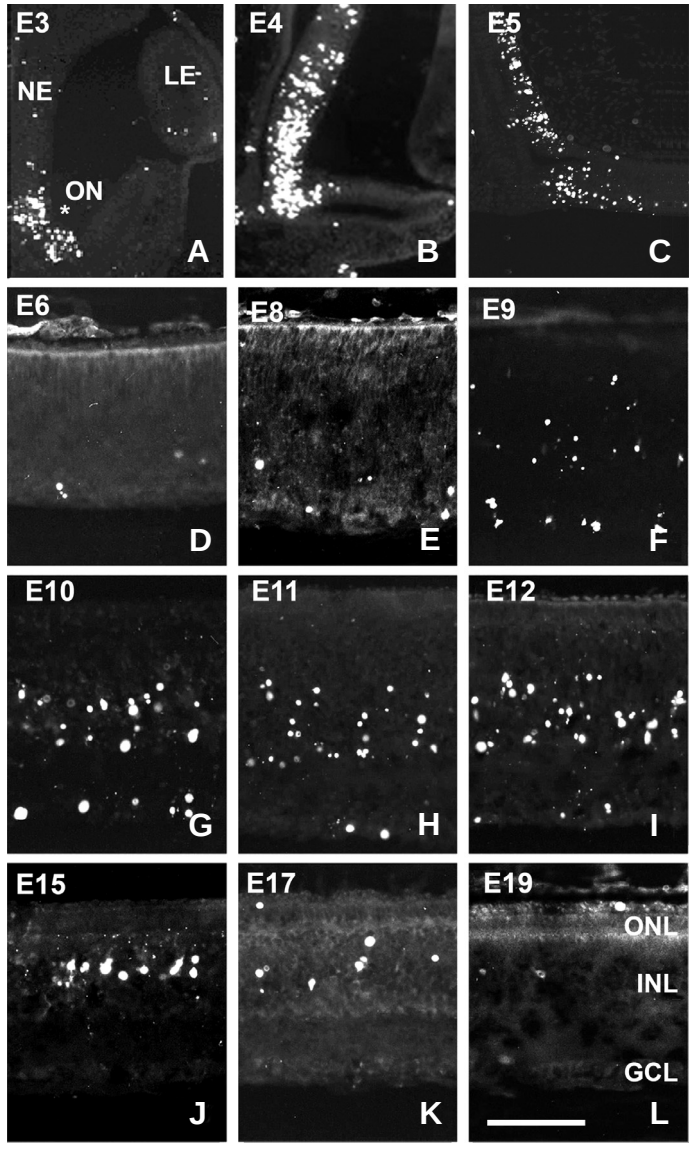


Fig. 3. Developmental course of cell death in the embryonic chick retina. *Dying cells were identified by TUNEL (white spots) in retinal sections of the indicated embryonic age (E). The retinal layers (GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer) are formed sequentially as development proceeds. The asterisk indicates the exit point of the optic nerve. Additional retinal features labeled include: NE, neuroepithelium; LE, lens; ON, optic nerve). Bar represents 400 μm in (A–C) and 200 μm in (D–L). Reproduced from Chavarría* et al., *2007 with permission. © 2007 Wiley Periodicals Inc.*

Interestingly, the phase of early neural cell death is clearly simultaneous with the generation of RGCs, but not with the generation of later neuronal phenotypes (Prada *et al.*, 1991; Cepko *et al.*, 1996; Péquignot *et al.*, 2003; Chavarría *et al.*, 2007). This may indicate that RGCs are more selectively prone to die during their generation. Alternatively, PCD associated with the generation of other neuronal cell types may be masked by later, overlapping phases of neurotrophic cell death. While the latter alternative deserves further study using combined labeling (*ie:* with markers to determine the time of cell cycle exit together with specific neuronal

markers), the former has been demonstrated by *in vivo* interference studies. *In ovo* exposition of chick embryos to caspase inhibitors at HH17 decreases apoptosis and doubles the number of RGC (Figs. 5 and 6; Mayordomo *et al.*, 2003). Conversely, treatment between E2 and E6 with antibodies that block the insulin receptor increases apoptosis and halves the number of RGCs (Figs. 7 and 8), reflecting the survival signals mediated by this receptor in early development (Hernández-Sánchez *et al.,* 2006). Furthermore, overexpression of the proapototic molecule Bax in neurulating chick embryos results in a moderate reduction in the number of RGCs at E9 (10-20%, Sato *et al.*, 2006). Similarly, genetic deletion of Dlx1/Dlx2 in the mouse increases apoptosis at E13.5 and E16.5, as well as decreases RGC number by 34% (De Melo *et al.*, 2005). Thus, the generation of RGCs is a process that is undoubtedly subject to PCD.

These different approaches also provide cumulative quantification and thus, at least, 50% of the ganglion cells generated are selected by PCD shortly alter birth, a magnitude equivalent to that of neurotrophic cell death of RGCs. Certainly, early neural cell death deserves further studies to assess its selectivity for RGCs and to determine how widespread this process is, especially in those species where peaks of PCD are not easily identified, and more importantly, as well as to characterize its function.

Neurotrophic cell death of retinal ganglion cells

RGCs are a paradigm of neurotrophic support/death. A com-

TABLE 5

NEUROTROPHIC CELL DEATH IN THE GANGLION CELL LAYER

Species	Developmental stage range & (peak)	Process, distribution & magnitude	References
Zebrafish	Dpf 2-5 (Dpf 3)	Extraretinal synaptogenesis. *1.09% of dying cells in the GCL.*	Biehlmaier *et al*., 2001
	Dpf 5-9 (Dpf 7)	Intraretinal synaptogenesis and initial visual activity. *0.22% of dying cells in the GCL.*	
African cichlid fish	Dpf 4-6 (Dpf 5)	Extraretinal synaptogenesis. *20 apoptotic cells/mm^2.*	Hoke and Fernald, 1998
Brown trout	15-24 mm (17 mm)	Intraretinal synaptogenesis and initial visual activity. *50 apoptotic cells/mm^2.*	Candal *et al*., 2005
Clawed frog	Stg 53 and metamorphosis	Extraretinal synaptogenesis. *Loss of 20% of generated RGC* Metamorphosis. *40-70% of RGCs born at Stg 32-49.*	Gaze and Grant, 1992
Turtle	E23-64 (E31-34)	Extraretinal synaptogenesis. *120 apoptotic cells/mm^2 at E31. Loss of 40% of RGCs generated.*	Francisco-Morcillo *et al*., 2004 Hidalgo-Sánchez *et al.,* 2006
Chick	E8-11 (E9) E7-18 (E13)	Extraretinal synaptogenesis. *539 apoptotic/mm^2 in the GCL at E9. Loss of 40% of RGCs generated.*	Rager and Rager, 1979 Cook *et al.,* 1998 Chavarría *et al.,* 2007
Quail	E8-13 (E9-11)	Extraretinal synaptogenesis. *500 apototic cells/mm^2 in the GCL at E10.*	Marín-Teva *et al.,* 1999
Wallaby	P30-100 (P50)	Extraretinal synaptogenesis. *1% of dying cells in the GCL at P50. Loss of 30% of RGCs generated.*	Dunlop and Beazley, 1987
Cat	E39-53 (E44)	Extraretinal synaptogenesis. *3-10% of dying cells in the centro-temporal retina at E40.* 0.44% of dying cells in the GCL at E44-E48. Intraretinal synaptogenesis and initial visual activity. *0.22% dying cells in the GCL.*	Williams *et al.,* 1986 Wong and Hughes, 1987
	P12-84 (P12-36)	*All processes account for the loss of 76 % of the RGCs generated.*	
Ferret	P0 to P8	Extraretinal synaptogenesis. *5 TUNEL$^+$ cells/mm^{2}in the GCL.*	Henderson *et al*., 1988 Cusato *et al.,* 2001
Rabbit	E23-P42	*Loss of 62% of the RGCs generated.*	Robinson *et al.,* 1987
Mouse	E18.5 to P12 (P2)	Extraretinal synaptogenesis. *3.5-8.6% of dying cells in the GCL. 1% of dying RGCs.*	Young, 1984 Williams et al., 1990
	P13-21 (P15)	Intraretinal synaptogenesis and initial visual activity. *2.3 % of cells dying in the GCL.* *All processes account for the loss of 60 % of the RGCs generated.*	Bonfanti et al., 1996 Péquignot et al., 2003 Pollock et al., 2003
Rat	P0-14 (P4)	Extraretinal synaptogenesis. *Loss of* 35-90% *of the RGCs generated.*	Potts *et al.,* 1982 Perry, 1983
	P7-14	Intraretinal synaptogenesis and initial visual activity. 50% of the RGCs generated die from P0 to P14.	Horsburgh and Sefton, 1987 Galli-Resta and Ensini 1996
Hamster	P0-10	Extraretinal synaptogenesis. *Loss of 25-50% of the RGCs generated.*	Sengelaub and Finaly, 1982 Sengelaub *et al*., 1986
Guinea Pig	E30-45 (E36)	Extraretinal synaptogenesis. *2.2% dying cells in the GCL at E36. Loss of 37% of the RGCs generated.*	Langford and Sefton, 1992
Rhesus monkey		Extraretinal synaptogenesis. *Loss of 50% of the RGCs generated.*	Radick and Riley, 1983
Human	Week12-30 (week 16-21)	Extraretinal synaptogenesis. *Loss of 70% of the RGCs generated.*	Provis *et al*., 1985b Provis and Van Driel, 1985 Provis, 1987 Sturrock, 1987

Developmental age referred to as: Dpf, days postfertilization; E, day of embryonic development; mm, length of the embryo; P, postnatal day; Stg and HH, developmental stages (Clawed frog, Nieuwkoop and Faber, 1967; Chick, Hamburger and Hamilton, 1951); week, gestational week. Abbreviations: GCL, Ganglion Cell Layer; RGC, Retinal Ganglion Cell.

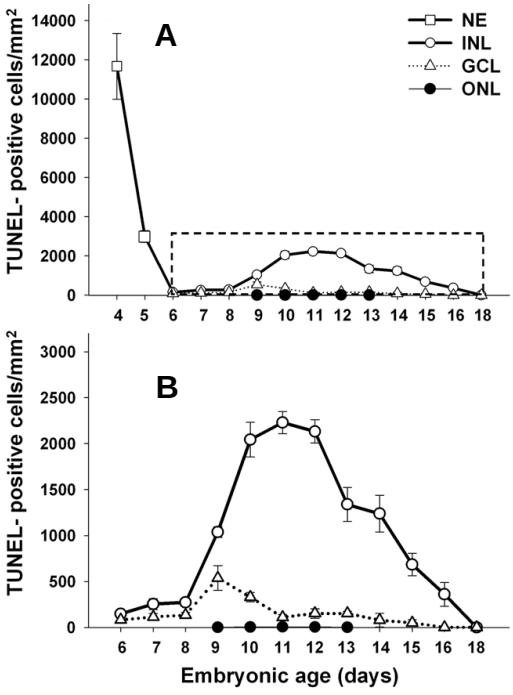


Fig. 4. Magnitude of cell death throughout embryonic chick retinal development. *Density of cell death, expressed as TUNEL-positive nuclei per mm², was quantified in the four most central fields of four sections per retina, such as those shown in Fig. 3. At early stages (E4–E6), the whole neuroepithelium (NE) was scored. The relative densities in the developing retinal layers were considered independently as early as the different layers were identifiable (GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer).* **(B)** *corresponds to the inset in–* **(A)** *with an expanded scale for better visualization of layer specific cell death. Values represent the mean ± SD of four independent retinas at each age. Reproduced from Chavarría* et al.*, 2007 with permission. © 2007 Wiley Periodicals Inc.*

parative analysis of several species (Table 5) shows that RGCs are affected by two consecutive, partially overlapping events during the second half of retinal development. The first follows the postulate of the neurotrophic theory: the axons of the RGCs form the optic nerve and, at their main target tissue (the optic tectum for non-mammalian vertebrates and the lateral geniculate nucleus for mammals), they compete for limiting amounts of BDNF, their best defined neurotrophic factor (Cohen-Cory and Lom, 2004). In addition, later RGC death has been reported in several species that seems to coincide with both, eye opening and a peak of cell death in the ONL (Table 5). Both events occur after RGC generation is completed and thus, the cumulative number of RGCs affected can be easily calculated by comparing the number of axons in the optic nerve before and after this phase of PCD. The loss of RGCs during this period is species-dependent and ranges from 30 to 75% (Table 5), emphasizing the importance of this physiological process for establishing the connectivity of the nervous system. Since early neural cell death affecting recently born RGCs seems to be of a similar magnitude, the functional relevance in retinal development may be similar and it certainly deserves more attention.

Late neuronal cell death in other retinal layers

Most neuronal cell types in the retina are affected by PCD after the neurotrophic peak in the GCL (Figs. 3 and 4). Although an exhaustive discussion of this PCD is beyond the scope of this review, we have referred to these events in Table 6 to provide a comprehensive overview of cell death during retinal development.

Regulation of retinal cell death

Genetically modified mice and *in vivo* manipulation of non-mammalian vertebrates have confirmed the importance of neuronal cell death in the postulate of the neurotrophic theory, including that of the RGCs. This kind of approach usually provides

TABLE 6

LATE NEURONAL CELL DEATH IN OTHER RETINAL LAYERS

Species	Developmental stage range & (peak)	Process, distribution & *magnitude*	References
Zebrafish	Dpf 5-9 (Dpf 6) Dpf 6-27 (Dpf 7)	*0.2 of dying cells in the INL.* *1.1% of dying cells in ONL.*	Biehlmaier *et al.,* 2001
Chick	E8-20 (E10-12 and E17-18)	PCD affecting to postmitotic cells. *2229 apoptotic cells/mm² in the INL. 35 apoptotic TrkA⁺-AC cells/mm². Less than 1% of cells dying in the ONL.*	Cook *et al.,* 1998 Karlsson *et al.,* 2001 Turner *et al.,* 2006 Chavarria *et al.*, 2007
Quail	E8 to P3 (E12)	*3000 apoptotic cells/mm² in the INL.*	Marin-Teva *et al.*, 1999
Cat	E50 to P17 (E58 and P2) P20-30 (P25)	AC. *72,000 apoptotic cells.* Ectopic PhRs in the outer part of the INL. *6 TUNEL⁺ cells/mm². 65,000 apoptotic cells.* *25 TUNEL⁺ PhR /mm² in the ONL.*	Williams *et al.*, 1986 Wong and Hughes, 1987 Robinson, 1988 Maslin *et al.*, 1997
Ferret	P3-42 (P22)	*20 TUNEL⁺ cells/mm² in the INL.*	Cusato *et al.*, 2001
Mouse	P3-21 (P4, P7, P8-10 and P15)	*2.8% of dying cells in INL (60 TUNEL⁺ cells/mm²), 6.4% of dying BC or MC, 7.8% of dying AC, 3.3 % of dying rods, 0.6% dying cells in the ONL (15 TUNEL⁺ cells/mm²).*	Young, 1984 Portera-Cailliau *et al.,* 1994 Mervin and Stone, 2002 Péquignot *et al.,* 2003
Rat	P1-30 (P4, P6-7, P10 and P15-22)	*3-8% of dying cells in the INL die (30 TUNEL⁺ cells/mm²). 25 TUNEL⁺ cells/mm² in the ONL.*	Vogel and Möller, 1980 Spira *et al.,* 1984 Horsburg and Sefton, 1987 Maslin *et al.*, 1997
Guinea pig	E39-E45	*1% of dying AC, 1.2 % of dying BC.*	Langford and Sefton, 1992

Developmental age referred to as: Dpf, days postfertilization; E, day of embryonic development; P, postnatal day. Abbreviations: AC, Amacrine Cells; BC, Bipolar Cells; INL: Inner Nuclear Layer; MC, Müller glial cells; ONL, outer nuclear layer; PhR, photoreceptor.

information regarding both the regulatory pathways involved and the possible function. As discussed previously (de la Rosa and de Pablo, 2000; Boya and de la Rosa, 2005), the models available may also provide additional information beyond that considered at present. However, most of the genetic approaches employed to define the molecules that regulate PCD have analyzed the structure of the retina when development is finished. For instance, 3-week-old caspase-3 null mutant mice show hyperplasia in all retinal layers (Kuida *et al.*, 1996). Although this approach provides information on the final consequences of such genetic modifications, it does not reveal in which phase/s of cell death the molecule is involved.

A few studies have clearly identified modifications in the number of dying cells during the morphogenic cell phase as a result of experimental interference (Table 7). Antisense RNA interference with the serotonin receptor in the clawed frog (De Lucchini *et al.*, 2005) and KO mice for BMP7 (Morcillo *et al.*, 2006) show disturbances in the closure of the optic fissure, establishing a functional correlation between the molecules altered, PCD and this specific morphogenetic process. Other studies have characterized later consequences of the manipulation which deserve to be studied in more detail to determine whether they are secondary consequences of the modification of morphogenic cell death, or independent processes in which the altered molecule is involved.

The manipulations that increase or decrease in the numbers of dying cells during the early phase of neural cell death show reciprocal effects on RGC number (Table 8). Some studies have indicated that the normal phenotype is recuperated at later stages (Pimentel *et al.,* 2000) suggesting a coupling of the different cell death phases to obtain the precise final numbers of RGCs. These observations offer clear evidence that the process of early neural cell death tightly controls the generation of RGCs in a precisely regulated fashion. Conversely, in terms of functional relevance, no clear suggestions on the role of this phase of cell death can be extrapolated from the observations available.

Finally, the survival/death of most retinal neurons at later developmental stages is regulated by multiple families of molecules that provide extrinsic and intrinsic signals (Tables 9 and 10), an extensively studied field.

Concluding remarks

The data presented in this review intends to facilitate the evaluation of several independent phases of cell death during vertebrate retinal development, some of which are little characterized. These distinct phases have been tentatively called morphogenic cell death, early neural cell death and neurotrophic cell death. Neither the magnitude nor the precise regulation of the earlier phases when compared to the much better characterized neurotrophic cell death justifies neglecting their study when trying to obtain an integrated view of retinal development.

The most compelling issue is to understand the physiological meaning of selecting recently born RGCs through PCD. As postulated by the neurotrophic theory, the number of projecting neurons is adjusted to the target field and their connections are refined by a process of PCD. As suggested by some studies, number adjustment may be a multi-step process, a mechanism that may provide both greater flexibility and more precise regulation. Furthermore, we speculate that projecting neurons such as RGCs may have additional fitness and positional information requirements that are achieved by processes related to early neural cell death (extensively discussed in de la Rosa and de Pablo, 2000; Boya and de la

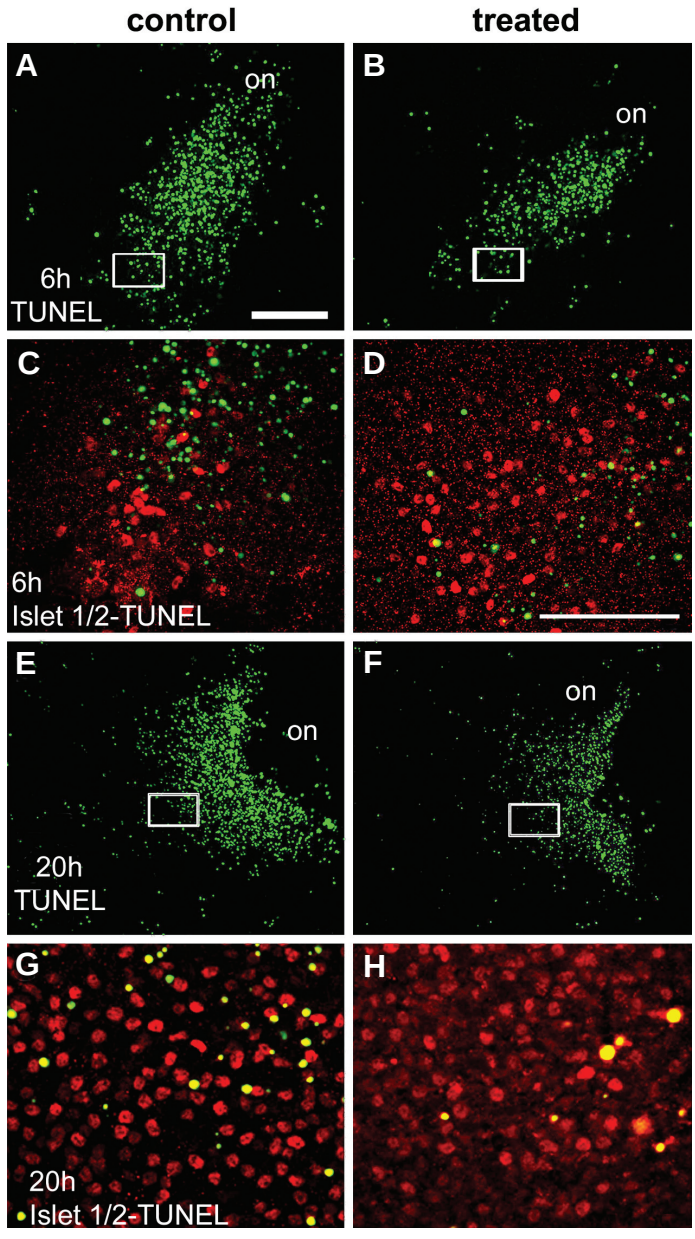
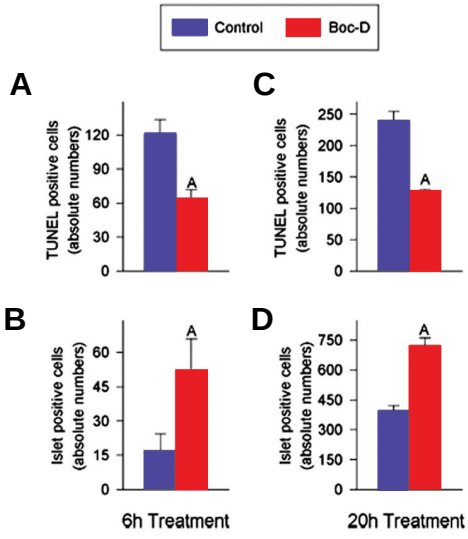


Fig. 5. Modulation of cell death and retinal ganglion cell generation by the pancaspase inhibitor Boc-D. *HH17 chick embryos were treated* in ovo *for 6* **(A–D)** *or 20 h* **(E–H)** *with the caspase inhibitor Boc-D-fmk* **(B,D,F,H)**, *or the corresponding control (0.1% DMSO;* **(A,C,E,G)**). *Whole-mount retinas were double-stained for TUNEL to visualize dying cells (green spots in all panels) and with anti-Islet 1/2 antibody to reveal retinal ganglion cells (red nuclei in* **(C,D,G,H)**); *double-labeled cells at higher magnification in the area indicated by the asterisk in* **(A,B,E,F)**). *Note that TUNEL-positive and Islet-1/2-positive apoptotic bodies appear as yellow spots. The final stage of the treated embryos was HH19 (A–D) or HH21 (E–H). Scale bar, 200 μm. Reproduced from Mayordomo* et al., *2003 with permission. © 2003 European Neuroscience Association.*



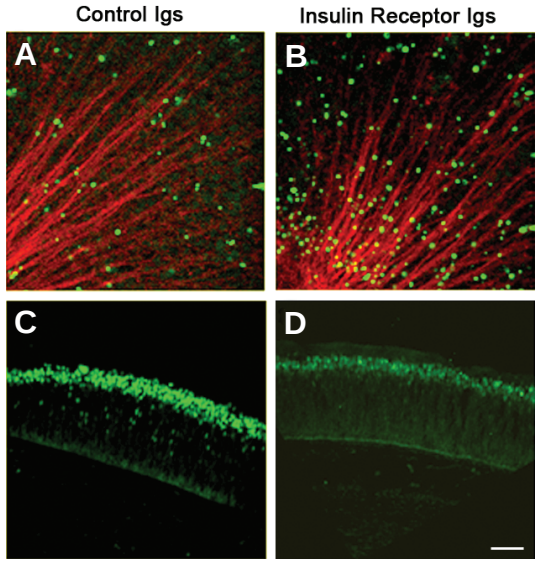


Fig. 6 (Left). Quantitative analysis of the effect of the pancaspase inhibitor Boc-D on cell death and retinal ganglion cell generation. *HH17 chick embryos were treated* in ovo *for 6* **(A,B)** *or 20 h* **(C,D)** *with the caspase inhibitor Boc-D-fmk, or the corresponding control (0.1% DMSO). TUNEL-positive apoptotic bodies* **(A,C)** *and Islet 1/2-positive nuclei* **(B,D)** *were scored in whole-mount retinas such as those shown in Fig. 5. The total number of positive cells are shown as the mean ± SEM (n=8 for all experimental conditions). A, p<0.01, Boc-D vs. control. Reproduced from Mayordomo* et al., *2003 with permission. © 2003 European Neuroscience Association.*

Fig. 7 (Right). Modulation of cell death and retinal ganglion cell generation by blocking insulin signaling. *E2 chick embryos were treated* in ovo *for 2* **(A,B)** *or 4 days* **(C,D)** *with anti-insulin receptor Igs* **(B,D)** *or with the corresponding control Igs* **(A,C)**. *The neuroretinas were processed in whole-mount (A,B) or sections (C,D), and TUNEL (green in (A,B)) was used to characterize dying cells. Immunostaining for G4/Ng-CAM (red in (A,B) or for Islet1/2 (green in (C,D)) was used to identify the retinal ganglion cells axons and nuclei, respectively.* **(A,B)** *Superimposed confocal microscope images illustrate the distribution of the dead cells surrounding the optic nerve head and the retinal ganglion cell axons at E4, after 2-days of treatment. Note the increase of TUNEL-positive nuclei in the retina treated with the blocking antibody against the insulin receptor.* **(C,D)** *Fields adjacent to the optic nerve from retinal sections at E6 after 4-days of treatment. Note the reduced number of Islet1/2-positive cells in the retina treated with the blocking antibody against the insulin receptor. Scale bar, 40 μm. Reproduced from Díaz* et al., *2000 with permission. © 2000 The Company of Biologists.*

TABLE 7

REGULATION OF MORPHOGENIC CELL DEATH IN RETINAL DEVELOPMENT

Molecule / experimental approach	Cell death phenotype	Retinal phenotype	References
GDF6 (BMP13) Treatment of clawed frog embryos (2-cell Stg) with antisense morpholinos.	Apoptotic cells increase at Stg 27.	ND during the morphogenic phase. Strong reduction of several types of mature neurons at Stg 41.	Hanel and Hensey, 2006
Serotonin Treatment of clawed frog embryos (2-cell stage) with antisense morpholinos to 5-HT_2 receptor. Treatment of Stage 15-17 clawed frog embryos with 5-HT_2 receptor antagonist.	Apoptotic cells increase at Stg 40. Apoptotic cells increase at Stgs 15, 28, 33.	Alterations in the closure of optic fissure and reduction of the GCL at Stg 40-42. Microphtalmia.	De Lucchinni *et al.*, 2005
Biotin *In ovo* treatment of HH14-17 chick embryos with biotin.	Apoptotic cells decrease at HH17.	Alterations in the morphogenesis of the eye. Increased thickness of the retina.	Valenciano *et al.*, 2002
BMP4 *In ovo* treatment of HH14 chick embryos with BMP4. *In ovo* treatment of HH14 chick embryos with noggin.	Apoptotic cells increase at HH17. Apoptotic cells decrease at HH17.	ND.	Trousse *et al.*, 2001
BMP7 KO mouse.	Apoptotic cells decrease at E10.5.	Alterations in the closure of optic fissure.	Morcillo *et al.*, 2006
FasL Gld mouse (*fasl* KO mouse).	Apoptotic cells decrease 50% in the NBL at E10.5.	Cell number increases 150% in NBL at E10.5. No altered phenotype in adults.	Péquignot *et al.*, 2003
Msx2 Overexpressing transgenic mouse.	Apoptotic cells increase at E9.5.	Thinner neuroepithelium at E9.5 Lack of RGCs at E14.5.	Wu *et al.*, 2003
$P75^{NTR}$ KO mouse.	42% decrease in apoptotic cells at E12. No reduction observed between E13 and P0.	ND during the morphogenic phase No altered phenotype at P0, P5, P10 and P40.	Harada *et al.*, 2006

Including those treatments that modify apoptotic cell numbers during the morphogenic phase (see Table 2). Developmental age referred to as: E, day of embryonic development; P, postnatal day; Stg and HH, developmental Stgs (Clawed frog, Nieuwkoop and Faber, 1967; Chick, Hamburger and Hamilton, 1951). Abbreviations: GCL, ganglion cell layer; NBL, Neuroblastic Layer; ND, not determined; RGCs, Retinal Ganglion Cells

TABLE 8

REGULATION OF EARLY NEURAL CELL DEATH IN RETINAL DEVELOPMENT

Molecule / experimental approach	Cell death phenotype	Retinal phenotype	References
Cdh4 (cadherin-4) Treatment of zebrafish embryos with antisense morpholinos.	Apoptotic cells increase 3-fold at Hpf 36 and 50.	Reduction of the GCL and optic fibers at Hpf 48.	Babb *et al.*, 2005
Serotonin Treatment of clawed frog embryos (2-cell Stg) with antisense morpholinos to 5-HT_2 receptor. Treatment of Stg 15-17 clawed frog embryos with 5-HT_2 receptor antagonist.	Apoptotic cells increase at Stg 40. Apoptotic cells increase at Stgs 15, 28, 33.	Alterations in the closure of optic fissure and reduction of the GCL at Stg 40-42. Microphtalmia.	De Lucchinni *et al.*, 2005
Bax Overexpression in chick embryos by retroviral infection at HH9.	Apoptotic cells increase in the GCL at E3 and E6.	RGC number decreases 10-20% at E9.	Sato *et al.*, 2006
Caspases *In ovo* treatment of HH17 chick embryos with caspase inhibitors.	Apoptotic cells decrease 50% at HH19.	RGC number increases 50% at HH20.	Mayordomo *et al.,* 2003
Growth hormone *In ovo* treatment of E2 chick embryos with blocking antibodies against GH.	Apoptotic cells increase at E6.	ND.	Sanders *et al.*, 2005
Insulin *In ovo* treatment of E2 chick embryos with blocking antibodies against insulin and insulin receptor.	Apoptotic cells increase 60% at E4. RGC apoptotic cells increase 250% at E4.	RGC number decreases 50% at E6.	Díaz *et al.*, 2000
Integrins Injection of dominant-negative β1 integrin into the anterior neural tube of HH11 chick embryos. *In ovo* treatment from E2 to E5 with α4 β1 intregrin antagonist.	Apoptotic cells increase 5-fold at E6.5. Apoptotic cells increase 3-fold at E6.	RGC number decreases 50% at E6. Reduced thickness of the retina.	Leu *et al.,*2004
Raf Overexpression in chick embryos by retroviral infection of C-Raf at E4.5 of dominant-negative Raf C4.	Apoptotic cells reduced at E6.5 and E7.5.	RGC number decreases at E7.5.	Pimentel *et al.*, 2000
Apaf-1 KO mouse.	Not determined in the retina. Reduced apoptosis in the nervous system.	Increased thickness of the retina at E12.5 and E14.5.	Cecconi *et al.,* 1998
Dlx1/Dlx2 KO mouse.	Apoptotic cells increase 3-fold at E13.5, 66% at E16.5. No modification at E18.5. Dying cells are RGC.	No retinal phenotype at E13.5 and E16.5. RGC number decreases 34% at E18.5. Optic fibres decrease 23% at P0.	De Melo *et al.*, 2005
Phosphatidylserine Receptor KO mouse.	Apoptotic cells increase 6-fold at E17.5.	ND.	Li *et al.*, 2003
Sortilin1 KO mouse.	Apoptotic cells decrease 63% at E15.5.	ND.	Jansen *et al.*, 2007
Tlx KO mouse.	Apoptotic cells increase 4 fold from E16.5 to E18.5.	No retinal phenotype during embryonic period. GCL increases thickness 1.3-fold at P0.	Miyawaki *et al.,* 2004

Including those treatments which modify apoptotic cell number during the early neural phase (see Table 4). Developmental age referred to as: Hpf, hours postfertilization; E, day of embryonic development; Stg and HH, developmental Stgs (Clawed frog, Nieuwkoop and Faber, 1967; Chick, Hamburger and Hamilton, 1951). Abbreviations: GCL, Ganglion Cell Layer; ND, not determined; RGC, Retinal Ganglion Cell.

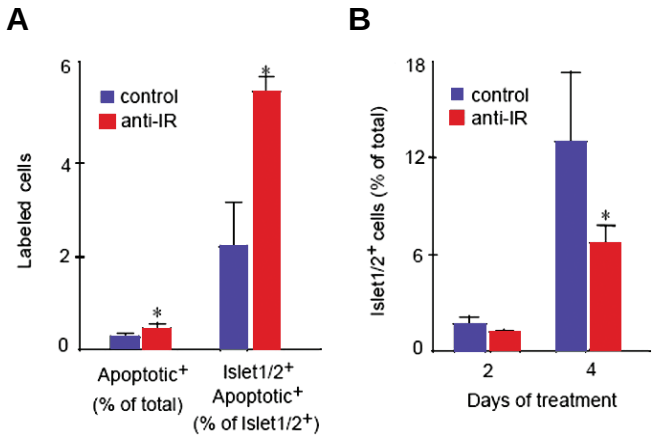


Fig. 8. Quantitative analysis of the effect of blocking insulin signaling on cell death and retinal ganglion cell generation. *E2 chick embryos were treated* in ovo *for 2 or 4 days with anti-insulin receptor Igs* **(A,B)** *or the corresponding control Igs. The neuroretinas were dissociated to a single-cell suspension and stained with DAPI and with anti-Islet1/2.* **(A)** *Relative incidence of apoptosis, after a 2-day exposure, in either the total populations or the Islet1/2-positive subpopulation.* **(B)** *Progressive reduction of the Islet1/2-positive subpopulation with the duration of treatment. A minimum of 1000 DAPI-stained cells were counted in at least three different retinal preparations per treatment. The percentage of apoptotic cells among the Islet1/2-positive cells was determined in the same preparations but scoring all the individual Islet1/2-positive cells. Values in (A,B) represent the mean ± s.d. *, P<0.005 for each experimental pair, calculated by Student's* t*-test. Reproduced from Díaz* et al.*, 2000 with permission. © 2000 The Company of Biologists.*

TABLE 9

REGULATION OF NEUROTROPHIC CELL DEATH IN THE GANGLION CELL LAYER

Molecule / experimental approach	Cell death phenotype	Retinal phenotype	References
Bax			
KO mouse.	Apoptotic cells decrease 98% at P2 and P7 and 94% at P15.	GCL thicker from P2. Cells in the GCL and optic fibers increase 50%. RGC axons increase 226 % in adults.	Mosinger Ogilvie *et al.*, 1998 Péquignot *et al.*, 2003
Bcl-2			
Overexpressing transgenic mouse (NSE promoter)	Apoptotic cells decrease 90% at P2.	No modification in the number of RGCs between P2 and adulthood. RGCs increase 50% in adults.	Bonfanti *et al.,* 1996 Strettoi and Volpini, 2002
BDNF			
$BDNF^{-/-}$ $NT4^{-/-}$ KO mouse.	Apoptotic RGCs increase 1.7 fold at P2.	Delayed retinal development.	Pollock *et al.*, 2003
$BDNF^{-/+}$ $NT4^{+/+}$ KO mouse.	Apoptotic RGCs increase 1.4 fold at P2.		Harada *et al.*, 2006
FasL			
Gld (*fasl* KO mouse).	Apoptotic cells decrease 72% in the GCL at P2, and 81% at P15.	No altered phenotype in adult.	Péquignot *et al.*, 2003
Rb			
KO mouse.	Caspase-3 activity increase 90% in the GCL at E18.5.	Ectopic proliferating cells in the GCL at E18.5.	McPherson *et al.*, 2004
KO mouse (lox-nestin promoter X cre-pax6 promoter; deletion starts at E10).	Caspase-3 activity increase 90% in the GCL at E18.5.	RGCs decrease 90% in 3 week- old mice.	
Tlx			
KO mouse.	Apoptotic cells increase 4-fold from E18.5 to P3, and 2-fold at P7. No modification in the number of apoptotic cells from P9 to P14.	1.3-fold increase in the thickness of the GCL at P0. Decrease in thickness of the GCL at P14 and in thickness of all retinal layer at P28.	Miyawaki *et al.,* 2004
TrkB			
KO mouse.	Apoptotic RGCs increase 2-fold at P2 and P6.	No modification in the number of RGC axons in adult mice.	Pollock *et al.,* 2003

Included those treatments which modify apoptotic cell number during the neurotrophic phase (see Table 5). Developmental age referred to as: E, day of embryonic development; P, postnatal day. Abbreviations: GCL, ganglion cell layer; RGC, retinal ganglion cell; ND, not determined.

TABLE 10

REGULATION OF NEUROTROPHIC CELL DEATH IN THE INNER AND OUTER NUCLEAR LAYERS

Molecule / experimental approach	Cell death phenotype	Cellular phenotype	References
TrkB			
Overexpression in chick embryos by retroviral infection of trunckated TrkB at E2.5.	Increased apoptosis at E10-14.	Cells in the INL increase 23% (likely AC). PhR number decreases 41% al E15.	Rickman and Rickman, 1996 Rohrer *et al.*, 1999 Turner *et al.*, 2006
KO mouse.		Slight reduction of AC and lack of OFF-cone BC. Delayed PhR morphological differentiation.	
Treatment of newborn rats (P0-7) with antisense oligonucleotides.		Parvalvumin-expressing AC at P10-15 decrease 56%.	
Bax			
KO mouse.	Apoptotic cells decrease 83% in the INL at P7 and P9, and 72% in the ONL at P15.	Cells in INL increase 50%. Cells in the ONL increase 75% in adults.	Mosinger- Ogilvie *et al.,* 1998 Eversole-Cire *et al.,* 2002 Péquignot *et al.*, 2003
Overexpressing transgenic mouse (rod-opsin promoter).	Apoptotic PhR increase in the ONL at P11.	Reduced thickness of the ONL at P11. PhR number decreases 9-fold at P30.	
Bax & Bak			
Double KO mouse.	Apoptotic cells decrease in the INL at P7 and P9.	Cells in the INL increase 50% in adults. Ectopic rods in the INL.	Hahn *et al.*, 2003
Bcl-2			
Overexpressing transgenic mouse (NSE promoter).	Decreased apoptosis.	Cells in the INL increase in adults: BC, 50%; AC, 10-fold; HC, 19%.	Strettoi and Volpini, 2002
Bhlbh4			
KO mouse.	Caspase-3^{+} cells increase from P8 (45%) to P10 (32%).	Thickness of the INL decreases 21% in adults. Rod BC number decreases 64% at P8 and 92% at P12.	Bramblett *et al.*, 2004
BMPr1b			
KO mouse.	Apoptotic cells increase in the INL at P7.	BC number decreases.	Li *et al.*, 2003
FasL			
Gld (*fasl* KO mouse).	Apoptotic cells decrease 60% in the ONL at P15.	No altered phenotype in adult.	Péquignot *et al.*, 2003
IRS-2			
KO mouse.	Apoptotic cells increase 200% at P7, 60% at P14, normal values at P21. Caspase-3 activity increased in 9 week-old mice.	PhR numbers decrease 10% at P7, 50% at P14, 50% in adult mice.	Yi *et al.,* 2005
Rb			
KO mouse.	Apoptotic cells increase in the INL/ONL at P3.	INL disorganization and ectopic cells in OPL in adults. PhR numbers decrease 50% in 3 week-old, 4 month-old and adult mice.	McPherson *et al.*, 2004
KO mouse (lox-nestin promoter X cre-pax6 promoter; deletion starts at E10).	Apoptotic cells increase in the INL and the ONL at P12.	Slight reduction of AC and lack of BC at P12. Loss of lamination in the INL and decrease of PhR number in 3 week-old mice.	
Tlx			
KO mouse.	Apoptotic cells increase 3 fold in the INL at P7 and P9. No modification in the number of apoptotic cells at P14.	1.3-fold increase in the thickness of the INL at P7. Decrease in thickness of the INL at P14 and in thickness of all retinal layer at P28.	Miyawaki *et al.*, 2004

Included those treatments which modify apoptotic cell number during the neurotrophic phase (see Table 6). Developmental age referred to as: E, day of embryonic development; P, postnatal day. Abbreviations: AC, amacrine cells; BC, bipolar cells; INL: inner nuclear layer; ONL, outer nuclear layer; PhR, photoreceptor.

Rosa, 2005). We encourage more detailed studies of retinal development to address the more complex involvement of cell death in retinal development as probably also occurs in the central nervous system as a whole.

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References

ADLER, R. (2005). Challenges in the study of neuronal differentiation: a view from the embryonic eye. *Dev. Dyn*. 234: 454–463.

BABB, S.G., KOTRADI, S.M., SHAH, B., CHIAPPINI-WILLIAMSON, C., BELL, L.N., SCHMEISER, G., CHEN, E., LIU, Q. and MARRS J.A. (2005). Zebrafish R-cadherin (Cdh4) controls visual system development and differentiation. *Dev. Dyn.* 233: 930-945.

BEAZLEY, L.D., DUNLOP, S.A.,–HARMAN, A.M., and COLEMAN, L.A. (1988). Development of cell density gradients in the retinal ganglion cell layer of marsupials. *Aust. Paediatr. J.* 24 Suppl 1: 43-47.

BIEHLMAIER, O., NEUHAUSS, S.C. and KOHLER, K. (2001). Onset and time course of apoptosis in the developing zebrafish retina. *Cell Tissue Res.* 306: 199-207.

BONFANTI, L., STRETTOI, E., CHIERZI, S., CENNI, M.C., LIU, X.H., MARTÍNOU, J.C., MAFFEI, L. and RABACCHI S.A. (1996). Protection of retinal ganglion cells from natural and axotomy-induced cell death in neonatal transgenic mice overexpressing bcl-2. *J. Neurosci*. 16: 4186-4194.

BOYA P. and DE LA ROSA E. J. (2005). Cell death in early neural life. *Birth Defects Res. C Embryo Today* 75: 281–293.

BRAEKEVELT C.R., BEAZLEY L.D., DUNLOP S.A., DARBY J.E. (1986). Numbers of axons in the optic nerve and of retinal ganglion cells during development in the marsupial Setonix brachyurus. *Brain Res*. 390: 117-125.

BRAMBLETT, D.E., PENNESI, M.E., WU, S.M., and TSAI, M.J. (2004). The transcription factor *bhlhb4* is required for rod bipolar cell maturation. *Neuron* 43: 779–793.

BURRILL J.D. and EASTER, S.S. (1994). Development of the retinofugal projections in the embryonic and larval zebrafish (*Brachydanio rerio*). *J. Comp. Neurol.* 346: 583-600.

BUSS, R.R., SUN, W. and OPPENHEIM R.W. (2006). Adaptive roles of programmed cell death during nervous system development. *Annu. Rev. Neurosci*. 29: 1-35.

CANDAL, E., ANADÓN, R., DEGRIP, W.J. and RODRÍGUEZ-MOLDES, I. (2005). Patterns of cell proliferation and cell death in the developing retina and optic tectum of the brown trout. *Brain Res. Dev. Brain Res.* 154: 101-119.

CECCONI, F., ÁLVAREZ-BOLADO, G., MEYER, B.I., ROTH, K.A. and GRUSS P. (1998). Apaf1 (CED-4 Homolog) Regulates Programmed Cell Death in Mammalian *Dev. Cell* 94: 727–737.

CELLERINO, A., BÄHR, M., and ISENMANN, S. (2000). Apoptosis in the developing visual system. *Cell Tissue Res*. 301: 53-69.

CEPKO, C.L., AUSTIN, C.P., YANG, X., ALEXIADES, M., and EZZEDDINE, D. (1996). Cell fate determination in the vertebrate retina. *Proc. Natl. Acad. Sci. U. S. A.* 93: 589-595.

CHAVARRÍA, T., VALENCIANO, A.I., MAYORDOMO, R., EGEA, J., COMELLA, J.X., HALLBÖÖK, F., DE PABLO, F. and DE LA ROSA E.J. (2007). Differential, age-dependent ERK and PI3K activation by insulin acting as a survival factor in early retinal development. *Dev. Neurobiol*. 67: 1777-1788.

CHOW, L.R. and LANG R.A. (2001). Early eye development in vertebrates. *Annu Rev Cell Dev Biol*. 17: 255-296.

COHEN-CORY S, LOM B. (2004). Neurotrophic regulation of retinal ganglion cell synaptic connectivity: from axons and dendrites to synapses. *Int. J. Dev. Biol*. 48: 947-956.

COLE, L.K. and ROSS, L.S. (2001). Apoptosis in the developing zebrafish embryo. *Dev. Biol*. 240: 123-142.

COOK, B., PORTERA-CAILLIAU, C. and ADLER, R. (1998). Developmental neuronal death is not a universal phenomenon among cell types in the chick embryo retina. *J. Comp. Neurol* 396: 12–19.

CRABTREE, J.W. (1989). Prenatal development of retinocollicular projections in the rabbit: an HRP study. *J. Comp. Neurol*. 286.504–513.

CRABTREE JW**.** (1990). Prenatal development of retinogeniculate projections in the rabbit: an HRP study. *J. Comp. Neurol.* 299: 75-88.

CUADROS, M. A. and RÍOS, A. (1988). Spatial and temporal correlation between early nerve fiber growth and neuroepithelial cell death in the chick embryo retina. *Anat. Embryol*. 178: 543-551.

CUCCHIARO J. and GUILLERY R.W. (1984). The development of the retinogeniculate pathways in normal and albino ferrets. *Proc. R. Soc. Lond. B. Biol. Sci*. 223: 141-164.

CUSATO, K., STAGG, S.B. and REESE, B.E. (2001). Two phases of increased cell death in the inner retina following early elimination of the ganglion cell population. *J. Comp. Neurol*. 439: 440-449.

DAVIES, A.M. (2003). Regulation of neuronal survival and death by extracellular signals during development. *EMBO J*. 22: 2537-2545.

DÍAZ, B., PIMENTEL, B., DE PABLO, F. and DE LA ROSA, E. J. (1999). Apoptotic cell death affecting proliferating neuroepithelial cells in the embryonic retina is prevented by insulin. *Eur. J. Neurosci.* 11: 1624-1632.

DÍAZ, B., SERNA, J., DE PABLO, F. and DE LA ROSA E. J. (2000). In vivo regulation of cell death by embryonic (pro)insulin and the insulin receptor during early retinal neurogenesis. *Development* 127**:** 1641-1649.

DREHER, B. and ROBINSON, S.R. (1988). Developmental of the retinofugal pathway in birds and mammals: evidence for a common “timetable”. *Brain Beh. Evol*. 31: 369–390.

DUNLOP, S.A. and BEAZLEY, L.D. (1987). Cell death in the developing retinal ganglion cell layer of the wallaby *Setonix brachyurus*. *J. Comp. Neurol*. 264: 14-23.

EVERSOLE-CIRE, P., CHEN, J. and SIMON, M.I. (2002). Bax is not the heterodimerization partner necessary for sustained anti-photoreceptor-cell-death activity of Bcl-2. *Invest. Ophthalmol. Vis. Sci*. 43: 1636-1644.

FARAH, M.H. (2006). Neurogenesis and cell death in the ganglion cell layer of vertebrate retina. *Brain Res. Rev*. 52: 264-274.

FRADE, JM., RODRÍGUEZ-TEBAR, A. AND BARDE, Y.A. (1996). Induction of cell death by endogenous nerve growth factor through its p75 receptor. *Nature*. 383: 166-168.

FRANCISCO-MORCILLO, J., HIDALGO-SÁNCHEZ, M. and MARTÍN-PARTIDO G. (2004). Spatial and temporal patterns of apoptosis during differentiation of the retina in the turtle. *Anat. Embryol. Berl.* 208: 289-299.

FRANCISCO-MORCILLO, J., HIDALGO-SÁNCHEZ, M. and MARTÍN-PARTIDO G. (2006). Spatial and temporal patterns of proliferation and differentiation in the developing turtle eye. *Brain Res*. 1103: 32-48.

GALLI-RESTA, L. and ENSINI, M. (1996). An intrinsic limit between genesis and death of individual neurons in the developing retinal ganglion cell layer. *J. Neurosci.* 16: 2318–2324.

GAZE, R.M. and GRANT, P. (1992). Spatio-temporal patterns of retinal ganglion cell death during Xenopus development. *J. Comp. Neurol.* 315: 264–274.

GLÜCKSMANN, A. (1940). Development and differentiation of the tadpole eye. *Brithish J. Opthalmol.* 154-178.

GODEMENT, P, SALAÜN, J, and IMBERT, M. (1984). Prenatal and postnatal development of retinogeniculate and retinocollicular projections in the mouse. *J. Comp. Neurol.* 230: 552-575.

GUERIN, M.B., MCKERNAN, D.P., O’BRIEN, C.J. and COTTER, T.G. (2006). Retinal ganglion cells: dying to survive. *Int. J. Dev. Biol*. 50: 665-674.

HAGEDORN, M., and FERNALD, R.D. (1992). Retinal growth and cell addition during embryogenesis in the teleost, *Haplochromis burtoni*. *J. Comp. Neurol*. 321: 193-208.

HAHN P., LINDSTEN, T., YING, G.S. BENNETT, J., MILAM, A.H., THOMPSON, C.B. and DUNAIEF J.L. (2003). Proapoptotic *bcl-2* family members, *bax* and *bak*, are essential for developmental photoreceptor apoptosis. *Invest. Ophthalmol. Vis. Sci.* 44: 3598–3605.

HAMBURGER, V. and HAMILTON, H.L. (1951). A series of normal stages in the

development of the chick embryo. *J. Morph.*, 88: 49–92.

HANEL, M.L. and HENSEY C. (2006). Eye and neural defects associated with loss of GDF6. *BMC Dev. Biol*. 6: 43.

HARADA, C., HARADA, T., NAKAMURA, K., SAKAI, Y., TANAKA, K. and PARADA L.F. (2006). Effect of p75NTR on the regulation of naturally occurring cell death and retinal ganglion cell number in the mouse. *Dev. Biol* 290: 57-65.

HARADA, T., HARADA, C. and PARADA, L.F. (2007). Molecular regulation of visual system development: more than meets eye. *Genes Dev*. 8: 960-976.

HARRIS, W.A. (1997). Cellular diversification in the vertebrate retina. *Curr. Opin. Genet. Dev*. 7: 651-658.

HENDERSON, Z., FINLAY, B.L., and WIKLER, K.C. (1988). Development of ganglion cell topography in ferret retina. *J. Neurosci* 8: 1194–1205.

HENSEY, C. and GAUTIER, J. (1998). Programmed cell death during Xenopus development: a spatio-temporal analysis. *Dev. Biol*. 203: 36-48.

HERNÁNDEZ-SÁNCHEZ, C., MANSILLA, A., DE LA ROSA, E.J. and DE PABLO, F. (2006). Proinsulin in development: New roles for an ancient prohormone. *Diabetologia.* 49: 1142-1150.

HIDALGO-SÁNCHEZ, M., FRANCISCO-MORCILLO, J., NAVASCUÉS, J. and MARTÍN-PARTIDO, G. (2006). Developmental changes in the fibre population of the optic nerve follow an avian/mammalian-like pattern in the turtle *Mauremys leprosa*. *Brain Res*. 1113: 74-85.

HOKE, K.L. and FERNALD, R.D. (1998). Cell death precedes rod neurogenesis in embryonic teleost retinal development. *Brain Res. Dev. Brain Res*. 111: 143-146.

HORSBURGH, G.M. and SEFTON, A.J. (1987). Cellular degeneration and synaptogenesis in the developing retina of the rat. *J. Comp. Neurol*. 263: 553-566.

IJIMA, N. and YOKOYAMA, T. (2007). Apoptosis in the Medaka Embryo in the Early Developmental Stage. *Acta Histochem. Cytochem*. 40: 1–7.

IWAMATSU, T. (1994). Stages of normal development in the medaka. Oryzias latipes. *Zool. Sci*. 11: 825–839.

JENKINS, S. and STRAZNICKY, C. (1986). Naturally occurring and induced ganglion cell death. A retinal whole-mount autoradiographic study in Xenopus. *Anat. Embryol. Berl*. 174: 59-66.

KARLSSON, M., MAYORDOMO, R., REICHARDT, L.F., CATSICAS, S., KARTEN, H.J. and HALLBÖÖK, F. (2001). Nerve growth factor is expressed by postmitotic avian retinal horizontal cells and supports their survival during development in an autocrine mode of action. *Development* 128: 471-479.

KUAN, C.Y., ROTH, K.A., FLAVELL, R.A. and RAKIC, P. (2000). Mechanisms of programmed cell death in the developing brain. *Trends Neurosci*. 23: 291-297.

KUIDA, K., ZHENG, T. S., N.A, S., KUAN, C., YANG, D., KARASUYAMA, H., RAKIC, P., and FLAVELL, R.A. (1996). Decreased apoptosis in the brain and premature lethality in CPP32-deficient mice. *Nature* 384**:** 368-372.

KUWABARA T., and WEIDMAN, T.A. (1974). Development of the prenatal rat retina. *Invest. Ophthalmol. Vis. Sci.* 13: 725-739.

LAMB, T.D., COLLIN, S.P., PUGH, E.N. Jr. (2007). Evolution of the vertebrate eye: opsins, photoreceptors, retina and eye cup. *Nat. Rev. Neurosci*. 8: 960-976.

LAEMLE, L. K., PUSZKARCZUK, M., and FEINBERG, R. N. (1999). Apoptosis in early ocular morphogenesis in the mouse. *Brain. Res. Dev. Brain Res.* 112**:** 129-133.

LANGFORD, C. and SEFTON, A.J. (1992). The relative time course of axonal loss from the optic nerve of the developing guinea pig is consistent with that of other mammals. *Vis. Neurosci*. 9: 555-564.

LEU, S.T., JACQUESA, S.A.L., WINGERDA, K.L., HIKITAA, S.T., TOLHURSTA, E.C., PRINGA, J.L., WISWELLA, D., KINNEYA, L., GOODMANA, N.L., JACKSONB, D.Y, CLEGGA, D.O. (2004). Integrin alpha4beta1 function is required for cell survival in developing retina. *Dev. Biol.* 276: 416-430.

LEVINE, E.M. and GREEN, E.S. (2004). Cell-intrinsic regulators of proliferation in vertebrate retinal progenitors. *Semin. Cell Dev. Biol.* 151: 63-74.

LI, M.O., SARKISIAN, M.R., MEHAL, W.Z., RAKIC, P. and FLAVELL, R.A. (2003). Phosphatidylserine receptor is required for clearance of apoptotic cells. *Science* 302: 1560-1563.

LI, Z., HU, M., OCHOCINSKA, M.J., JOSEPH, M.N. and EASTER, S.S. (2000). Modulation of cell proliferation in the embryonic retina of zebrafish (*Danio rerio*). *Dev. Dyn*. 219: 391–401.

LINDEN, D.C., GUILLERY, R.W. and CUCCHIARO, J. (1981). The dorsal lateral geniculate nucleus of the normal ferret and its postnatal development. *J. Comp. Neurol*. 203: 189–211.

DE LUCCHINI, S., ORI, M., CREMISI, F., NARDINI, M. and NARDI, I. (2005). 5-HT2B-mediated serotonin signaling is required for eye morphogenesis in Xenopus. *Mol. Cell. Neurosci*. 29: 299–312.

MACPHERSON, D., SAGE, J., KIM, T., HO, D., MCLAUGHLIN, M.E. and JACKS T. (2004). Cell type-specific effects of Rb deletion in the murine retina. *Genes Dev*. 18: 1681-1694.

MANSOUR-ROBAEY, S. and PINGANAUD, G. (1996). Development of the retino-tectal arborisation of the trout. *Anat. Embryol*. 194: 279–287.

MARÍN-TEVA, J.L., CUADROS, M.A, CALVENTE, R., ALMENDROS, A. and NAVASCUES, J. (1999). Naturally occurring cell death and migration of microglial precursors in the quail retina during normal development. *J. Comp. Neurol.* 412: 255–275.

MARQUARDT, T. and GRUSS, P. (2002). Generating neuronal diversity in the retina: one for nearly all. *Trends Neurosci.* 25: 32–38.

MARTÍN-PARTIDO, G., RODRÍGUEZ-GALLARDO L., ÁLVAREZ, I.S and NAVASCUES, J. (1988). Cell death in the ventral region of the neural retina during the early development of the chick embryo eye. *Anat. Rec*. 222: 272-281.

MASLIM, J., VALTER, K., EGENSPERGER, R., HOLLANDER, H., and STONE, J. (1997). Tissue oxygen during a critical developmental period controls the death and survival of photoreceptors. *Invest. Ophthalmol. Vis. Sci.* 38**:** 1667-1677.

MAYORDOMO, R., VALENCIANO, A.I., DE LA ROSA, E.J. and HALBÖÖK, F. (2003). Generation of retinal ganglion cells is modulated by caspase-dependent programmed cell death. *Eur. J. Neurosci.* 18: 1744-1750.

DE MELO, J., DU, G., FONSECA, M., GILLESPIE L.A., TURK, W.J., RUBENSTEIN, J.L.R. and EISENSTAT, D.D. (2005). Dlx1 and Dlx2 function is necessary for terminal differentiation and survival of late-born retinal ganglion cells in the developing mouse retina. *Development* 132: 311-322.

MERVIN, K. and STONE, J. (2002). Developmental death of photoreceptors in the C57BL/6J mouse: Association with retinal function and self-protection. *Exp. Eye Res*. 75: 703-713.

MIYAWAKI, T., UEMURA, A., DEZAWA, M., YU, R.T., IDE, C, NISHIKAWA, S., HONDA, Y., TANABE, Y., and TANABE, T. (2004). Tlx, an orphan nuclear receptor, regulates cell numbers and astrocyte development in the developing retina. *J. Neurosci*. 24: 8124–8134.

MORCILLO, J., MARTÍNEZ-MORALES, J.R., TROUSSE, F., FERMIN, Y., SOWDEN, J.C. and BOVOLENTA P. (2006). Proper patterning of the optic fissure requires the sequential activity of BMP7 and SHH. *Development*. 133: 3179-3190.

MOSINGER OGILVIE, J., DECKWERTH, T.L., KNUDSON, C.M. and KORSMEYER S.J. (1998). Suppression of developmental retinal cell death but not of photoreceptor degeneration in Bax-deficient mice. *Invest. Ophthalmol. Vis. Sci.* 39: 1713–1720.

NIEUWKOOP, P.D. and FABER, J. (1967). Normal Table of *Xenopus laevis* (Daudin). North Holland, Amsterdam.

OZEKI, H., OGURA, Y., HIRABAYASHI, Y., and SHIMADA, S. (2000). Apoptosis is associated with formation and persistence of the embryonic fissure. *Current Eye Res.* 20: 367-372.

PÉQUIGNOT, M. O., PROVOST, A. C., SALLE, S., TAUPIN, P., SAINTON, K. M., MARCHANT, D., MARTINOU, J. C., AMEISEN, J. C., JAIS, J. P., and ABITBOL, M. (2003). Major role of BAX in apoptosis during retinal development and in establishment of a functional postnatal retina. *Dev. Dyn.* 228: 231-238.

PERRY, V.H., HENDERSON, Z. and LINDEN, R. (1983) Postnatal changes in retinal ganglion and optic axon populations in the pigmented rat. *J. Comp. Neurol.* 219: 356–368.

PIMENTEL, B., SANZ, C., VARELA-NIETO, I., RAPP, U.R., DE PABLO, F. and DE LA ROSA, E.J.(2000). c-Raf regulates cell survival and retinal ganglion cell morphogenesis during neurogenesis. *J. Neurosci*. 20: 3254-3262.

POLLOCK, G.S., ROBICHON, R., BOYD, K.A., KERKEL, KA, KRAMER, M., LYLES, J., AMBALAVANAR, R., KHAN, A., KAPLAN, D.R., WILLIAMS, R.W. and FROST D.O. (2003). TrkB receptor signaling regulates developmental death dynamics, but not final number, of retinal ganglion cells. *J. Neurosci*. 23: 10137-10145.

PORTERA-CAILLIAU, C., SUNG, C.H., NATHANS, J. and ADLER, R. (1994). Apoptotic photoreceptor cell death in mouse models of retinitis pigmentosa.–*Proc. Natl. Acad. Sci. U.S.A.* 91: 974-978.

POTTS, R.A., DREHER, B. and BENNETT, M.R. (1982). The loss of ganglion cells in the developing retina of the rat. *Brain Res*. 255: 481-486.

PRADA, C., PUGA, J., PÉREZ-MÉNDEZ, L., LÓPEZ, R. and RAMÍREZ, G. (1991). Spatial and temporal patterns of neurogenesis in the chick retina. *Eur. J. Neurosci.* 3: 559–569.

PRESSON, J.C. and FERNALD, R.D. (1986). Development of the optic tract in the cichlid fish *Haplochromis burtoni*. *Brain Res.* 391: 179-186.

PROVIS, J.M. and VAN DRIEL, D. (1985). Retinal development in humans: the roles of differential growth rates, cell migration and naturally occurring cell death. *Aust. N. Z. J. Ophthalmol.* 13: 125-133.

PROVIS, J.M., VAN DRIEL, D., BILLSON, F.A., and RUSSELL, P. (1985a). Development of the human retina, patterns of cell distribution and redistribution in the ganglion cell layer. *J. Comp. Neurol.* 233: 429-451.

PROVIS, J.M., VAN DRIEL, D., BILLSON, F.A., and RUSSELL, P. (1985b). Human fetal optic nerve: overproduction and elimination of retinal axons during development *J. Comp. Neurol.* 238: 92-100.

PROVIS, J.M. (1987). Patterns of cell death in the ganglion cell layer of the human fetal retina. *J. Comp. Neurol.* 259: 237-246.

RADICK, P. and RILEY, K.P. (1983).Overproduction and elimination of retinal axons in the fetal rhesus monkey. *Science* 219: 1441-1444.

RAGER, G. and RAGER, U. (1979). Systems-matching by degeneration in the developing retino-tectal projection of the chicken. *Prog Brain Res* 51: 439-443.

REESE, B.E., THOMPSON, W.F. and PEDUZZI, J.D. (1994) Birthdates of neurons in the retinal ganglion cell layer of the ferret. *J. Comp. Neurol* 341: 464-475.

REESE B.E., JOHNSON PT, and BAKER GE. (1996). Maturational gradients in the retina of the ferret. *J. Comp. Neurol.*375: 252-73.

RICKMAN, D.W. and RICKMAN, B.C. (1996). Suppression of trkB expression by antisense oligonucleotides alters a neuronal phenotype in the rod pathway of the developing rat retina. *Proc. Natl. Acad. Sci. U. S. A.* 93: 12564-12569.

ROBINSON, S.R., HORSBURGH, G.M., DREHER, B. and MCCALL, M.J. (1987). Changes in the numbers of retinal ganglion cells and optic nerve axons in the developing albino rabbit. *Brain Res.* 432(2): 161-74.

ROBINSON, S.R. (1988). Cell death in the inner and outer nuclear layers of the developing cat retina. *J. Comp. Neurol*. 267: 507-515.

RODRÍGUEZ-GALLARDO, L., LINEROS-DOMÍNGUEZ, M.C., FRANCISCO-MORCILLO, J. and MARTÍN-PARTIDO, G. (2005). Macrophages during retina and optic nerve development in the mouse embryo: relationship to cell death and optic fibres. *Anat. Embryol.* 210: 303-316.

ROHRER, B., KORENBROT, J.I., LAVAIL, K.R. and REICHARDT, L.F. (1999). Role of neurotrophin receptor TrkB in the maturation of rod photoreceptors and establishment of synaptic transmission to the inner retina. *J. Neurosci.* 19: 8919-8930.

DE LA ROSA, E. J. and DE PABLO, F. (2000). Cell death in early neural development: beyond the neurotrophic theory. *Trends Neurosci*. 23: 454-458.

ROTH, K.A. andÄD'SA, C. (2001) Apoptosis and brain development. *Ment. Retard Dev. Disabil. Res. Rev.* 7: 261-266.

SANDERS, E.J., PARKER, E., ARÁMBURO, C. and HARVEY, S. (2006). Retinal growth hormone is an anti-apoptotic factor in embryonic retinal ganglion cell differentiation. *Exp. Eye Res*. 81: 551-560.

SATO, N., SAKUMA, C., SATO, Y., GOULD, T.W., OPPENHEIM, R.W., and YAGINUMA, H. (2006). Distinct susceptibility of developing neurons to death following Bax overexpression in the chicken embryo. *Cell Death Diff.* 13: 435-445.

SCHMITT, E.A. and DOWLING, J.E. (1994). Early eye morphogenesis in the zebrafish, *Brachydanio rerio. J. Comp. Neurol.* 344: 532-542.

SCHMITT, E.A. and DOWLING, J.E. (1996). Comparison of topographical patterns of ganglion and photoreceptor cell differentiation in the retina of the zebrafish, Danio rerio. *J. Comp. Neurol*. 371: 222-234.

SCHMITT, E.A. and DOWLING, J.E. (1999). Early retinal development in the zebrafish, *Danio rerio*: light and electron microscopic analyses. *J. Comp. Neurol.* 404: 515-536.

SENGELAUB, D.R. and FINLAY, B.L. (1982). Cell death in the mammalian visual system during normal development : I. Retinal ganglion cells. *J. Comp. Neurol.* 204: 311-317.

SENGELAUB, D.R., DOLAN, R.P. and FINLAY, B.L. (1986). Cell generation, death, and retinal growth in the development of the hamster retinal ganglion cell layer. *J Comp Neurol.* 246: 527-543.

SENUT, M.C. and ALVARADO-MALLART, R.M. (1986). Development of the retinotectal system in normal quail embryos: cytoarchitectonic development and optic fiber innervation. *Dev. Brain Res* 29: 123-140.

SPIRA, A., HUDY, S. and HANNA, R. (1984) Ectopic photoreceptor cells and cell death in the developing rat retina. *Anat. Embryol. Berl.* 169: 293-301.

STRETTOI, E. and VOLPINI, M. (2002). Retinal organization in the bcl-2-overexpressing transgenic mouse.'*J. Comp. Neurol*. 446: 1-10.

STRONGIN, A.C. and GUILLERY, W. (1981). The distribution of melanin in the developing optic cup and stalk and its relation to cellular degeneration. *J. Neurosci.* 1: 1193-1204.

STURROCK, RR. (1987). Changes in the number of axons in the human embryonic optic nerve from 8 to 18 weeks gestation *J. Hirnforsch.* 28: 649-652.

TROUSSE, F., ESTEVE, P. and BOVOLENTA, P. (2001). BMP4 mediates apoptotic cell death in the developing chick eye. *J. Neurosci* 21: 1292-1301.

TURNER, B.A., SPARROW, J., CAI, B., MONROE, J., MIKAWA, T. and HEMPSTEAD, B.L. (2006). TrkB/BDNF signaling regulates photoreceptor progenitor cell fate decisions. *Dev. Biol.* 299: 455-465.

VALENCIANO, A.I., MAYORDOMO, R., DE LA ROSA, E.J. and HALLBÖÖK, F. (2002). Biotin decreases retinal apoptosis and induces eye malformations in the early chick embryo. *Neuroreport*. 13: 297-299.

VAN KLEFFENS, M., GROFFEN, C., NECK J.W., VERMEIJ-KEERS, C. and DROP, S.L. (1999). mRNA and protein localization of the IGF system during mouse embryonic development in areas with apoptosis. *Growth Horm. IGF Res*. 9: 195-204.

VECINO, E., HERNÁNDEZ, M. and GARCÍA, M. (2004). Cell death in the developing vertebrate retina. *Int. J. Dev. Biol.* 48: 965-974.

VOGEL, M. and MÖLLER, K. (1980). Cellular decay in the rat retina during normal post-natal development: a preliminary quantitative analysis of the basic endogenous rhythm. *Albrecht Von Graefes Arch. Klin. Exp. Ophthalmol*. 212: 243-260.

WALSH, C, POLLEY, E.H, HICKEY, T.L. and GUILLERY, R.W. (1983). Generation of cat retinal ganglion cells in relation to central pathways. *Nature.* 302: 611-614.

WILLIAMS, M.A., PIÑON, L.G.P., LINDEN, R. and PINTO, L.H. (1990). The pearl mutation accelerates the schedule of natural cell death in the early post-natal retina. *Exp. Brain Res* 82: 393-400.

WILLIAMS, R.W, BASTIANI, M.J., LIA, B. and CHALUPA, L.M. (1986). Growth cones, dying axons, and developmental fluctuations in the fiber population of the cat's optic nerve. *J. Comp. Neurol.* 246: 32-69.

WONG, R.O. and HUGHES, A. (1987). Role of cell death in the topogenesis of neuronal distributions in the developing cat retinal ganglion cell layer. *J. Comp. Neurol*. 262: 496-511.

WU L.Y., LI, M., HINTON, D.R., GUO, L., JIANG, S., WANG, J.T., ZENG, A., XIE, J.B., SNEAD, M., SHULER, C., MAXSON R.E., AND LIU, Y.H. (2003). Microphthalmia resulting from MSX2-induced apoptosis in the optic vesicle. *Invest. Ophthalmol. Vis. Sci.* 44: 2404-2412.

YEO, W. and GAUTIER, J. (2004) Early neural cell death: dying to become neurons. *Dev. Biol*. 274, 233-244.

YI, X., SCHUBERT, M., PEACHEY, N.S., SUZUMA, K. BURKS, D.J., KUSHNER, J.A., SUZUMA, I., CAHILL, C., FLINT, C.L., DOW, M.A., LESHAN, R.L., KING, J.L. and WHITE, M.F. (2005). Insulin receptor substrate 2 is essential for maturation and survival of photoreceptor cells. *J. Neurosci.* 25: 1240-1248.

YODA, H., HIROSE, Y.,nYASUOKA, A., SASADO, T., MORINAGA, C., DEGUCHI, T., HENRICH, T., IWANAMI, N., WATANABE, T., OSAKADA, M., KUNIMATSU, S., WITTBRODT, J., SUWA, H., NIWA, K., OKAMOTO, Y., YAMANAKA, T., KONDO, H.H. and FURUTANI-SEIKI, M. (2004). Mutations affecting retinotectal axonal pathfinding in Medaka, *Oryzias latipes. Mech. Dev*. 121: 715-728.

YOUNG, R.W. (1984) Cell death during differentiation of the retina in the mouse. *J Comp. Neurol.* 229: 362-373.

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Nicolas Dos-Santos, Thomas Rubin, Fabienne Chalvet, Pierre Gandille, Frederic Cremazy, Jacqueline Leroy, E. Boissonneau and Laurent Théodore
Int. J. Dev. Biol. (2008) 52: 21-31

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Jacob Souopgui, Tiemo J. Klisch, Tomas Pieler and Kristine A. Henningfeld
Int. J. Dev. Biol. (2007) 51: 339-343

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Reyna I. Martinez-De Luna and Heithem M. El-Hodiri
Int. J. Dev. Biol. (2007) 51: 235-240

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Malcolm Maden, Aida Blentic, Susan Reijntjes, Sophie Seguin, Emily Gale and Anthony Graham
Int. J. Dev. Biol. (2007) 51: 191-200

Expression of Bmp ligands and receptors in the developing Xenopus retina
Jennifer C. Hocking and Sarah McFarlane
Int. J. Dev. Biol. (2007) 51: 161-165

NMDA-receptor blockade enhances cell apoptosis in the developing retina of the postnatal rat
María Hernández, Inmaculada Guerrikagoitia, Luis Martínez-Millan and Elena Vecino
Int. J. Dev. Biol. (2007) 51: 117-122

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Shane D. Madden, Maryanne Donovan and Thomas G. Cotter
Int. J. Dev. Biol. (2007) 51: 415-424

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Jana Setkova, Herve Lesot, Eva Matalova, Kirsti Witter, Petra Matulova and Ivan Misek
Int. J. Dev. Biol. (2006) 50: 481-489

Retinal ganglion cells: dying to survive
Marc B. Guerin, Declan P. McKernan, Colm J. O'Brien and Thomas G. Cotter
Int. J. Dev. Biol. (2006) 50: 665-674

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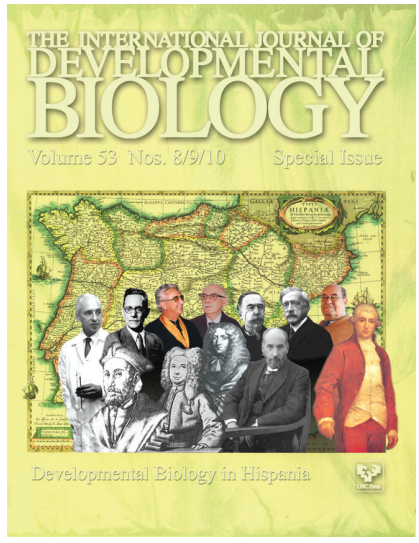
Elena Vecino, María Hernández and Mónica García
Int. J. Dev. Biol. (2004) 48: 965-974



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