Limited Role of Developmental Programmed Cell Death Pathways in *Drosophila norpA* Retinal Degeneration

Cheng-Da Hsu,* Michelle A. Whaley,* Kristin Frazer, Douglas A. Miller, Kathleen A. Mitchell, Sheila M. Adams, and Joseph E. O'Tousa

Department of Biological Sciences, University of Notre Dame, Notre Dame, Indiana 46556-0369

We examined the role of programmed cell death (PCD) pathways in retinal degeneration caused by a mutation in the *norpA* gene. *norpA* degeneration shows morphological hallmarks of programmed cell death, specifically cytoplasmic condensation and engulfment of the dying photoreceptor cells by neighboring retinal pigment cells. However, genetic mosaic analysis of adult photoreceptors lacking *rpr, hid,* and *grim* show that these PCD inducers are not required for *norpA* degeneration. We showed previously that ectopic expression of either *rpr* or *hid* triggers rapid PCD in adult photoreceptors, and this is completely suppressed by the coexpression of the baculoviral P35 caspase inhibitor. In contrast, expression of P35 does not suppress *norpA* retinal degeneration, although a small delay in the rate of degeneration is observed in low light–low temperature conditions. P35 does not alter the morphological characteristics of *norpA* cell death. Overexpression of the *Drosophila* inhibitor of apoptosis Diap1 or a dominant-negative form of the Dronc caspase, even when coexpressed with P35, does not dramatically alter the time course of *norpA* degeneration. These results establish that the pathways responsible for PCD in development do not play a major role in adult retinal degeneration caused by *norpA*.

*Key words: retinal degeneration; rpr; hid; diap1; Dronc; baculovirus P35; norpA; programmed cell death; Drosophila vision*

**Introduction**

Programmed cell death (PCD) occurs as a normal process in the development and homeostasis in both vertebrates and invertebrates (Song and Steller, 1999). All cells appear capable of executing PCD, and the decision is controlled by the interplay of PCD activator and suppressor genes. In *Drosophila melanogaster*, PCD activator genes include *reaper (rpr)*, *head involution defective (hid)*, and *grim*. These genes play a role in developmental PCD decisions and also PCD triggered by chromosomal damage (White et al., 1996). Rpr- and Hid-induced PCD is blocked by caspase inhibitors, such as the baculoviral P35 gene product (Grether et al., 1995; White et al., 1996), and Diap1, a *Drosophila* inhibitor of apoptosis (Hay et al., 1995). The dominant-negative mutant of the Dronc caspase, DroncDN, also known as Dronc*<sup>G318S</sup>*, also suppresses Rpr- and Hid-induced cell death (Meier et al., 2000).

In addition to its role during development, PCD is implicated in the death process of differentiated cells. In the case of retinal diseases, mutations in a large number of different photoreceptor genes trigger retinal degeneration in both vertebrates (Clarke et al., 2000) and *Drosophila* (Ranganathan et al., 1995). Experiments show that these PCD inducers are not required for *norpA* degeneration. Under certain conditions, P35 and DroncDN delay the retinal degeneration process in *norpA* mutants but cannot prevent cell death. These results show that norpA triggers retinal degeneration through cell death processes that are significantly different than those responsible for developmental PCD.
Materials and Methods

Drosophila strains. All transgenic strains used in the study were described previously: P[ninaEhid] (Hsu et al., 2002), P[GMRP35] and P[GMRhid] (Hay et al., 1994), P[GMRiapl] (Hay et al., 1995), and P[GMRDroncDN] (Hawkins et al., 2000). We verified that the dupl and DroncDN transgenes were active by showing they partially protect from GMRhid-driven cell death using the rescue of the small eye phenotype as described previously (Hay et al., 1995; Hawkins et al., 2000). However, unlike P35 (Hsu et al., 2002), they were not effective in the rescue of ninaEhid (data not shown).

Creation of Df(H99) mosaic. Genetic mosaics were created to analyze the effect of homozygous Df(H99) on norpA+/- photoreceptor cells. The mosaics were generated using the flipase recombinase/flipase recombinase target (FLP/FRT) system using the w w FRT 80B chromosome designated 80-w (Xu and Rubin, 1993) and eyFLP (New Dome et al., 2000). Standard genetic crosses were used to generate the Df(3L)H99 FRT 80B chromosome. To facilitate construction of this chromosome, the P[lacW]/Mi-23D4 w P element present within the 76C salivary chromosome region was recombined onto the FRT 80B chromosome. Because Df(H99) is located in the 75C salivary chromosome region, loss of this w P element marker identified likely Df(3L)H99 FRT 80B recombinants chromosomes from Df(3L)H99 w P 80B females. A chromosome identified in this manner was checked for lethality in the Df(3L)H99 FRT 80B/df(3L)H99 genotype. The presence of Df(3L)H99 on this chromosome was confirmed by our observation that 25% of the embryos from Df(3L)H99 FRT 80B/TM2 parents lack acridine orange staining, because homozygous Df(3L)H99 FRT embryos lack nearly all embryonic cell death and hence do not stain (White et al., 1994). To generate norpA/Y; df(99) FRT 80B/w+ FRT 80B (mosaic) males, subsequently referred to as norpA; ΔH99 mosaics, w norpA eyFLP; w w FRT 80B/TM3 females were crossed to w/Y; df(3L)H99 FRT 80B/TM2 males. The norpA norpAΔH99 mosaic female progeny from this cross provided the norpA norpAΔH99 mosaic controls.

Analysis of Drosophila mutant retina. Retinal degeneration was triggered by the amorphic mutant allele norpA+/- (Pearn et al., 1996) in most experiments. ninaE+/+ (Kurada and O'Tousa, 1995) and rdgB+ (Vithellic et al., 1991) were used in experiments shown in Figure 5. Relevant genotypes were generated by standard genetic crosses; typically, a cross scheme was designed such that the progeny contained both experimental and control animals to minimize genetic background and environmental rearing differences. Unless specified in the text, flies were raised at room temperature (22°C) in a 12 hr light/dark cycle with a light intensity of 2900 lux. The deep pseudopupil (DPP) was visualized in red-eyed flies (Fig. 1) in a 1 hr light/dark cycle with a light intensity of 2900 lux. The deep pseudopupil (DPP) was visualized in red-eyed flies (Fig. 1) when a bright white light illuminates the retina from the back of the head (Franceschini, 1975). During degeneration, the DPP becomes increasingly diffused before being completely lost. The DPP was scored as negative as soon as it was judged to be more diffused than would ever be observed in a wild-type retina. In all DPP experiments, the displayed data were compiled from three to five independent experimental trials that contained 30–50 flies of each genotype.

Microscopic analysis. Eyes were prepared for light and electron microscopy using procedures described by Washburn and O'Tousa (1992). Light microscopy sections 1 μm thick were stained with equal parts 1% methylene blue in 1% borax and 1% azure II. Electron microscopy sections were 80–100 nm thick, stained first in 5% uranyl acetate in 50% EtOH and then in Reynolds' lead citrate. The micrographs shown in all figures are taken from ommatidia cross-sectioned at the depth of the rhabdomere. The pigment cells (black arrows) possess a homogeneous granular cytoplasm and are located on the basal side of the photoreceptor cell opposite the rhabdomere. B. The 1-d-old norpA photoreceptors, at initial stages of degeneration, possess smaller, less organized, rhabdomeres and condensed cytoplasm. The pigment cell (black arrow) has expanded and begins to surround the photoreceptor as it loses contact with neighboring photoreceptors. C, D. At 2 d, many photoreceptors (white arrows) are completely engulfed by an enlarged pigment cell. The photoreceptors become filled with vesicles and vacuoles, and the rhabdomeres are further disintegrated. Scale bars, ~5 μm.

Results

norpA photoreceptors show morphological features of programmed cell death

In cross-sectioned wild-type cells (Fig. 1 A), the photoreceptor rhabdomere (R) appears as an organized microvillar structure extending into the intraommatidial space. Pigment cells located on the basal side of the photoreceptor opposite the rhabdomere surround and insulate the photoreceptors of a single ommatidium. The pigment cells, even in white-eyed flies lacking the large pigment granules, are distinguished from photoreceptors by the homogeneous and granular appearance of their cytoplasm. The rhabdomeres of 1-d-old norpA photoreceptors are at early stages of degeneration (Fig. 1 B), appearing smaller and less organized. The photoreceptor cell bodies are reduced in size, and pigment cells swell to occupy this space. At later stages of degeneration (Fig. 1 C, D), rhabdomeres are further deteriorated, and cell bodies are shrunken with many aberrant vesicles and electron-dense material. Similar morphological changes are seen in adult photoreceptors destined to die because of either the Hid or Rpr proteins (Hsu et al., 2002).
Figure 1, C and D, documents the engulfment of the dying norpA photoreceptors. The photoreceptor in the middle of each micrograph has lost contact with neighboring cells. The pigment cell is the phagocytic cell responsible for engulfing the photoreceptor. Additional evidence that the pigment cell is the engulfing cell is gained from similar temporal studies using $w^+$ norpA (red-eyed) flies. In these studies, the engulfing cell always contains numerous large pigment granules found only in pigment cells (data not shown).

norpA cells degenerate in absence of Rpr, Hid, and Grim activity
The rpr, hid, and grim genes are clustered in a small chromosome region referred to as the H99 region (White et al., 1994). A homozygous deletion of the H99 region, referred to here as $\Delta H99$, prevents PCD that normally occurs during embryonic development (White et al., 1994). To examine the requirement of this region in adult photoreceptor PCD, mitotic recombination was used to make homozygous Df(H99) mutant cells, hereafter called $\Delta H99$ cells, in the retina. In our experimental design, these cells lacked the $w^+$ gene and therefore could be identified by the absence of pigment granules. Light and electron microscopic analysis showed that most $\Delta H99$ cells looked similar to wild-type cells at 4 d of age in norpA $^+$ flies (Fig. 2A, C). A few $\Delta H99$ cells showed a different morphology (arrow) but were easily discernable from degenerating norpA photoreceptors.

The viability of homozygous H99 photoreceptor cells allowed us to test for a role of genes within the H99 region in norpA degeneration. Figure 2, B and D, shows that $\Delta H99$ cells (no pigment) undergo a similar time course of degeneration as the neighboring pigmented H99 $^+$ cells. We observed no difference in the timing and morphological correlates of norpA degeneration in H99 $^+$ and $\Delta H99$ photoreceptors. Thus, unlike developmental PCD processes, the genes of the H99 region do not play a role in the cellular processes responsible for norpA degeneration.

Limited P35 suppression of norpA degeneration
We examined the ability of the baculoviral P35 protein to alter the degeneration process in norpA mutants by introducing GMRp35 into norpA flies (Fig. 3). To accentuate differences in rates of degeneration, flies were reared on a 12 hr light/12 hr dark cycle. As a result, the time course of degeneration in the control norpA flies is notably slower than in the experiment described in Figure 1. Figure 3A shows data collected from observation of the DPP structure in this experiment. We confirmed that GMRp35 was capable of suppressing photoreceptor degeneration triggered by hid expression under control of the ninaE rhodopsin promoter (Hsu et al., 2002). In contrast, the DPP was retained for only ~1 d longer in norpA with P35 than seen in norpA flies lacking P35.

These results show that P35, although active in suppressing Hid-induced degeneration, allows only limited protection from the loss of the DPP attributable to the norpA mutation.

Electron microscopy was used to examine the photoreceptor morphology of these flies at ages for which the DPP study suggested differences. Figure 3B–D shows electron micrographs from 6-d-old retina of GMRp35 alone (Fig. 3B), norpA alone (Fig. 3C), and norpA; GMRp35 (Fig. 3D). Expression of GMRp35 has no effect on retinal structure in norpA $^+$ flies. norpA flies show an improved retinal structure attributable to GMRp35 expression (Fig. 3, compare C,D). These cells lack the frayed rhabdomere structure seen in the norpA flies, and the cytoplasm shows a normal appearance, lacking electron-dense material and accumulation of vesicles, at these time points. On the other hand, the norpA; GMRp35 cells are clearly in the process of degeneration, notably showing significant loss of rhabdromeric material. Thus, the images are in agreement with DPP results showing that P35 expression has a modest effect on slowing, but not preventing, degeneration in the norpA mutant.
In this experiment, P35 extended the DPP
flies were raised at 18
generation. Figure 4
small but reproducible capability of P35 to slow the rate of de-
expression of DroncDN
norpA
gated the ability of P35 to suppress

The influence of P35 on photoreceptor cell death in the norpA
mutants. Flies were reared in a 12 hr light/dark cycle
at 22°C. A, Rate of retinal degeneration assessed by DPP analysis of norpA and norpC; GMRp35. GMRp35 delayed the loss of the DPP
in norpA by a 1 d. The same GMRp35 transgene completely suppressed niniaElid degeneration. B, Electron micrograph of 6-d-old
GMRp35 retina without norpA from this experiment. Photoreceptors retain wild-type morphology. C, Electron micrograph of 6-d-old norpA retina without GMRp35 from this experiment. Photoreceptors show frayed rhodromeres and are full of vesicles and electron-dense material. D, Electron micrograph of 6-d-old norpA; GMRp35 retina from this experiment. The photoreceptors show signs of retinal degeneration, notably the loss of rhabdomeric volume. There is less degeneration than in norpA lacking p35 (C), consistent with the DPP results. Scale bars, 5 μm.

To confirm that P35 provided limited protection, we investigat-
gating the ability of P35 to suppress norpA under a variety of reduced light and temperature conditions expected to attenuate the rate of norpA degeneration. In all cases, we documented the small but reproducible capability of P35 to slow the rate of degeneration. Figure 4A shows the result of an experiment in which flies were raised at 18°C and under constant illumination of 2700 lux. In this experiment, P35 extended the DPP + period in norpA by a 5 d. Figure 4C–E shows the photoreceptor morphology of flies from this experiment. At the 13 d time point, the norpA photoreceptors are more degenerated than the norpA; P35 photoreceptors. However, by 20 d (Fig. 4E), the norpA; P35 flies show the same morphological changes, including cytoplasmic condensation and the engulfment of dying photoreceptor cells, seen in norpA flies at 13 d. We repeated this experiment using lower light conditions with similar results. These studies show that experimental conditions that better distinguish the protective effects of P35 are mild conditions that also dramatically slow the norpA degeneration rate. In no case, however, was P35 able to completely protect against norpA degeneration.

Because of the limited protection of norpA by P35, two genes that have been implicated in P35-insensitive cell death pathways, diap1 and DroncDN (Meier et al., 2000; Igaki et al., 2002), were analyzed for effects on the rates of norpA degeneration. We first showed that, like P35, expression of diap1 or DroncDN does not cause retinal degeneration (Fig. 4B). GMR-driven expression of diap1 did not alter the norpA degeneration process. GMR-driven expression of DroncDN provided weak protection similar to that observed for P35. The pseudopupil analysis was confirmed by electron microscopic observations of these genotypes (Fig. 4F–H) in which 13-d-old photoreceptors show reduced rhodromeres that further deteriorate by 20 d of age. Given the possibility that Dronc represents a P35-insensitive caspase (Meier et al., 2000), we also examined whether the combination of P35 and DroncDN, or alternatively P35 and Diap1, could suppress degeneration. These results, shown in Figure 4B, provided no evidence of synergistic suppression. The electron microscopic images of photoreceptors from these genotypes are consistent with this conclusion (Fig. 4I–K).

Limited P35 suppression of other retinal degeneration mutants
Other researchers have reported significant suppression of the rdgC and niniaE mutants by expression of P35 (Davidson and Steller, 1998; Alloway et al., 2000). The failure to document suppression of norpA by P35 caused us to reevaluate the effect of P35 on other retinal degeneration mutants. Figure 5A–C shows the effects of P35 expression on rdgB' reared under constant light at 22°C. As with norpA, although we observed a subtle delay in the rate of degeneration, P35 was not able to prevent the degenerative process. Electron microscopic examination at an intermediate time point suggests that P35 may influence the rate of cellular condensation and other morphological attributes of the degeneration process (Fig. 5B, C). We performed similar experiments with niniaE', a dominant rhodopsin mutant causing late onset of retinal degeneration (Kurada and O’Tousa, 1995). Again, P35 had a modest effect on the rate of degeneration but could not prevent degeneration (Fig. 5D–F). Similar results were also observed for the niniaE91227 and for rdgC' (data not shown).

The GMR promoter is active in the adult eye and
mutant photoreceptors
One explanation for the inability of GMRp35, GMRdiap1, and GMRDroncDN to suppress degeneration is that genes controlled by the GMR promoter are not expressed in adult photoreceptors or in norpA, rdgB, and niniaE211 mutants. To study GMR promoter activity, the GFP content of wild-type and mutant norpA, rdgB, or niniaE211 flies expressing GFP under GMR control were analyzed by protein blot. Figure 6 shows that the GMR promoter is active in adult wild-type flies (lanes 1–3). In addition, before degeneration, mutant norpA, rdgB, and niniaE211 retinas have high GMR promoter activity (lanes 4–6). Therefore, lack of expression in adult and mutant photoreceptors is not the reason for ineffective suppression by P35, Diap1, and DroncDN.

Discussion
norpA encodes the phospholipase C responsible for phototransduction and is representative of a large number of photoreceptor-specific genes active in phototransduction that, when mutated, trigger retinal degeneration (O’Tousa, 1997). Because of the lack of a receptor potential in norpA mutants, there is no rise in intra-
cellular Ca\(^{2+}\) levels (Peretz et al., 1994). The failure to activate Ca\(^{2+}\)-dependent enzymes required for rhodopsin recycling causes excessive endocytosis of rhodopsin (Orem and Dolph, 2002), which is thought to initiate the cell death process (Alloway et al., 2000; Kiselev et al., 2000). We show here, in confirmation of a previous report (Alloway et al., 2000), that norpA degeneration is accompanied by morphological changes consistent with PCD processes, such as loss of cell contact, cytoplasmic condensation, and engulfment of the dying cells (Arends and Wyllie, 1991). The neighboring retinal pigment cells perform the phagocytosis of the dying photoreceptor cells. The primary goal of this study was to determine the role of known PCD pathways in the retinal degeneration processes triggered by the norpA mutant. We present a series of results establishing that characterized developmental PCD components are not required for norpA retinal degeneration. Our results suggest that novel cell death processes, not those characterized from developmental PCD studies, play the major role in retinal degeneration syndromes.

We first showed that rpr, hid, and grim gene activity are not required for retinal degeneration triggered by norpA mutants. Our experimental design created a homozygous deficiency for the H99 region containing the rpr, hid, and grim genes in the norpA adult eye. This homozygous deficiency in the embryo eliminates nearly all developmental cell death (White et al., 1994). Thus, the underlying events responsible for norpA cell death cannot be identical to those responsible for developmental PCD. sickle, a fourth gene in the 75C region, maps just outside the H99 region (Christich et al., 2002; Srinivasula et al., 2002; Wing et al., 2002). In the adult Drosophila eye, sickle expression is not able to induce cell death on its own, but it can enhance the cell death triggered by an rpr or an rpr/grim chimera (Wing et al., 2002), and its action is antagonized by P35 and DIAP1. It is proposed to act by antagonizing the activity of IAPs, inhibitors of apoptosis proteins (Christich et al., 2002; Srinivasula et al., 2002). Because sickle is not removed by the H99 deletion, its role in the norpA cell death process was not addressed in the mosaic experiment. However, sickle is postulated to signal through IAPs to caspases. The P35 and DIAP1 studies described below suggest that this pathway does not play a major role in the norpA cell death process.

We then examined the ability of the anti-apoptotic baculoviral protein P35 to

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**Figure 4.** P35, Drunc, and Diap suppression of norpA in a low temperature environment. All flies were reared at 18°C under constant light of 2700 lux. A, Rate of retinal degeneration assessed by DPP analysis of norpA and norpA; GMRp35. GMRp35 delayed the loss of the DPP in norpA by 5 d. Under these conditions, and all others tested, the same GMRp35 transgene provided complete suppression of ninein-hid-triggered degeneration. B, Rate of norpA retinal degeneration assessed by DPP analysis in the presence of DruncDN, DruncDN, and P35, Diap1, and Diap1 and P35 cell death suppressors. Neither DruncDN nor Diap1 enhanced P35 suppression. C–E, Electron micrographs of photoreceptors from norpA and norpA; GMRp35 flies at 13 d (C, D) and norpA; GMRp35 flies at 20 d (E) from the same experiment shown in A. Consistent with the DPP analysis and previous experiments, GMRp35 provides some suppression at the earlier time point, with more extensive degeneration evident by 20 d. F–H, Electron micrographs of photoreceptors from norpA; GMRDruncDN and norpA; GMRDruncDN flies at 13 d (F, G) and norpA; GMRDruncDN flies at 20 d (H) from the same experiment shown in B. Consistent with the DPP analysis, initial signs of degeneration are seen at 13 d, with more extensive degeneration evident by 20 d (data not shown for GMRDiap1). I–K, Electron micrographs of norpA photoreceptors expressing P35 with either DruncDN or Diap1 at 13 d (I, J) and P35; DruncDN at 20 d. Consistent with the DPP analysis, no improvement in retinal structure is provided by simultaneous expression of two different caspase inhibitors. Scale bars, −5 μm.
suppress norpA retinal degeneration. P35 is a broad-spectrum caspase inhibitor and hence blocks PCD subsequent to the action of Rpr, Hid, and Grim. P35 has a modest impact on the rate of degeneration in norpA. Electron microscopy showed that, at intermediate stages of degeneration, there are two notable effects of P35 on the norpA PCD process. First, although the rhabdomere volume still decreases dramatically in the presence of P35, most rhabdomeres do not show the severe unraveled structure seen in norpA in the absence of P35. Second, progression of the cell bodies to an electron-dense and particulate appearance takes longer in the presence of P35. The P35 cells become electron dense at later time points and are engulfed by the neighboring pigment cells. These results support the view that P35-sensitive caspase pathways are active in some processes of dying photoreceptors but are not pivotal in the cell death decision.

Our conclusion that the major cell death determinants in retinal degeneration mutants are not sensitive to P35 contrasts with two previous reports. Davidson and Steller (1998) reported that P35 is a strong suppressor of rdgC and ninaERH27 degeneration. In another report, Alloway et al. (2000) showed evidence of P35 suppression of the norpAEE5 mutant. The differences in our results cannot be explained by the use of different alleles or mutations, because we also observed no or limited rescue of rdgC1, ninaED1, ninaERH27, or rdgB5. In an effort to reconcile these results, we showed that reduced light and temperature conditions can accentuate morphological differences between the mutant with and without P35, giving the appearance of strong suppression at certain time points. However, we found no experimental situations for which P35 shows complete protection from degeneration.

In this and all previous reports examining the effect of P35 on retinal degeneration mutants, P35 was expressed under control of the GMR promoter. We showed that GMR does maintain high

**Figure 5.** The influence of P35 in the control of photoreceptor cell death in other retinal degeneration mutants. Flies were reared under constant light at 22°C. A, Retinal degeneration assessed by deep pseudopupil analysis in rdgB and rdgB; P35. B, C, Electron micrographs of 2-d-old rdgB (B) and rdgB; P35 (C) photoreceptors. The degenerated photoreceptor (marked by * in B) appears in the process of being phagocytosed. D, Retinal degeneration assessed by deep pseudopupil analysis in ninaED1 and ninaED1; P35. E, F, Electron micrographs of 2-d-old ninaED1 retina (E) and ninaED1; P35 (F). The degenerated photoreceptors (marked by * in E) are being phagocytosed. Scale bars, 5 μm.

**Figure 6.** Western blot analysis of GFP driven by the GMR promoter in adult and mutant photoreceptors. Flies were raised in 12 hr light/dark cycle at 22°C. Flies carrying both GMR-Gal4 and UAS-GFP were crossed into norpA, rdgB, and ninaED1/H11001 mutant backgrounds. The protein equivalent of one fly head is loaded into each lane for GFP detection (top row); the same blot was also probed for actin (bottom row) to examine loading differences. The GFP protein is detected in wild-type 1-, 3-, and 5-d-old heads (lanes 1–3). Similar levels of GFP protein are detected in 1-d-old norpA (lane 4), rdgB (lane 5), and ninaED1 (lane 6) heads.
levels of gene expression throughout adult life in wild-type and mutant eyes through Western blot analysis using a GFP reporter construct. In addition, P35 expressed from the GMR and the major rhodopsin promoter, which is active throughout adult life, show similar suppression of Rpr- and Hid-triggered photoreceptor degeneration (Hsu et al., 2002). In these experiments, the rhodopsin promoter drove Rpr or Hid expression exclusively in photoreceptors after retinal cell type specification and continued to be expressed throughout the adult life.

We then sought evidence that P35-insensitive PCD processes are involved in norpA degeneration. The Drunc caspase is identified as a P35-insensitive caspase in one study (Meier et al., 2000) but as P35 sensitive from other results (Dorstyn et al., 1999; Colussi et al., 2000). Drunc is sensitive to the inhibitory effects of Diap1 (Meier et al., 2000). Diap1 is reported to inhibit a Drunc complex responsible for both caspase-dependent and caspase-independent cell death processes (Igaki et al., 2002). So, given the possibility that Drunc or Diap1 would affect P35-insensitive PCD processes, we examined their effect on norpA degeneration. We found that neither overexpression of a dominant-negative Drunc gene nor the diap1 gene in the adult eye prevented norpA degeneration. Furthermore, simultaneous expression of P35 and DruncDN, or P35 and Diap1, did not further suppress the rate of degeneration.

The failure to prevent norpA degeneration by known components of developmental PCD implies that other cellular events play the major role in the norpA degeneration process. It is not known whether these events constitute a separate cell death signaling pathway and whether similar events occur in other mutant-triggered retinal degenerations. Genetic approaches were successful in characterizing the components of developmental PCD, so we anticipate that identification and analysis of retinal degeneration suppressors will help answer these questions.

References


