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# Activation of Caspase-3 in the Retina of Transgenic Rats with the Rhodopsin Mutation S334ter during Photoreceptor Degeneration

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The role of caspase-3 in photoreceptor degeneration was examined in a line of transgenic rats that carry a rhodopsin mutation S334ter. Photoreceptor degeneration in these animals is rapid. It is detected as early as postnatal day (PD) 8, and by PD 20, only one of the original 12 rows of nuclei remain in the outer nuclear layer. At PD 11 and 12, the number of photoreceptors dying per day reaches a peak of ~30% of the total photoreceptors in the retina. Coincident with this rapid degeneration is an increase in caspase-3-like activity as assessed by the cleavage of a fluorescent substrate *N*-acetyl-Asp-Glu-Val-Asp-aminomethylcoumarin and an increase in activated caspase-3 as determined by Western blot analysis for its 12 kDa subunit. Intraocular injection of an irreversible caspase-3 inhibitor *N*-benzyloxycarbonal-Asp(OMe)-Glu(OMe)-Val-Asp(Ome)-fluoromethyketone partially protected photoreceptors from degeneration. These findings indicate that a caspase-3-dependent mechanism is operative in photoreceptor death in the transgenic rats under investigation.

*Key words: caspase-3; photoreceptors; z-DEVD-fmk; rhodopsin mutation; degeneration; retina; transgenic rat*

Inherited retinal degenerations, collectively known as retinitis pigmentosa, are characterized by progressive death of photoreceptors. Mutations in any of a number of photoreceptor-specific proteins, including rhodopsin (Dryja et al., 1990a,b; Farrar et al., 1990), peripherin (Travis et al., 1989; Farrar et al., 1991; Kajiwara et al., 1991), the $\beta$ subunit of the cGMP phosphodiesterase (Bowes et al., 1990; McLaughlin et al., 1993, 1995), and a rod outer segment protein, ROM1 (Kajiwara et al., 1994), can lead to photoreceptor degeneration. Moreover, introduction of mutant rhodopsin genes into the genomes of mouse (Olsson et al., 1992), pig (Petters et al., 1997), or rat (Steinberg et al., 1996, 1997) has successfully mimicked the phenotypes of retinitis pigmentosa as observed in humans.

Photoreceptor cell death in animal models is considered an apoptotic process, because DNA fragmentation has been demonstrated in degenerating photoreceptors (Chang et al., 1993; Portera-Cailliau et al., 1994). Since apoptosis, or programmed cell death, was originally shown to be a cell suicide process with distinct morphological characteristics (Wyllie, 1987), enormous progress has been made in unraveling the components of the death mechanism. Pioneering studies performed on the nematode *Caenorhabditis elegans* identified a complement of genes related to cell death, including *ced-3, ced-4*, and *ced-9*. The first identified mammalian homolog of CED-3 was the interleukin-1 $\beta$-converting enzyme (ICE) (Yuan et al., 1993). In turn, a search for ICE-related proteins revealed an entire family of proteases in mammals. These comprise at least 12 members termed caspases (for cysteine-containing, aspartate-specific proteases) (Thornberry and Lazebnik, 1998). Among them, activation of caspase-3 has been shown to participate in the initiation of apoptosis (Thornberry and Lazebnik, 1998), especially in neurons (Kuida et al., 1996; Armstrong et al., 1997; Yakovlev et al., 1997; Cheng et al., 1998; Namura et al., 1998).

In the present work, we examine the activation of caspase-3 in a line of transgenic rats that carry the rhodopsin mutation S334ter. Photoreceptors in these animals undergo rapid degeneration: >50% of photoreceptors die in just 2 d, postnatal days (PD) 11 and 12. There is a significant increase in caspase-3 activation during the rapid phase of photoreceptor degeneration. Furthermore, intraocular administration of specific peptide active-site inhibitor of caspase-3 inhibits photoreceptor degeneration. These results indicate that a caspase-3-dependent mechanism plays an important role in the demise of photoreceptors.

## MATERIALS AND METHODS

*Transgenic animals and intraocular injections.* Homozygous breeders of line 3 of transgenic rats that carry a murine rhodopsin mutant S334ter (S334ter-3) were kindly provided by Dr. M. M. LaVail (University of California, San Francisco, CA). Heterozygous S334ter-3 rats were produced by mating homozygous breeders with wild-type Sprague Dawley rats. All experiments were performed using heterozygous S334ter-3 rats. Controls were age-matched Sprague Dawley rats. Animals were kept in a 12 hr light/dark cycle at an in-cage illuminance of <10 foot-candles (1 foot-candle = 10.76 lux). The in-cage temperature was kept at 20–22°C. Intraocular injections were given directly into the vitreous by 32 gauge needles. The caspase-3 inhibitor *N*-benzyloxycarbonal-Asp(OMe)-Glu(OMe)-Val-Asp(Ome)-fluoromethyketone (z-DEVD-fmk) was purchased from Enzyme Systems Products (Dublin, CA).

*Histology and Terminal dUTP Nick End Labeling.* Animals were killed by $CO_2$ overdose, immediately followed by vascular perfusion with mixed aldehydes (LaVail and Battelle, 1975). Eyes were embedded in an Epon-Araldite mixture and sectioned at 1 $\mu$m thickness to display the entire retina along the vertical meridian (LaVail and Battelle, 1975). Retinal sections were examined by light microscopy. Twenty-seven transgenic animals from three litters were used for experiments described in Figure 1. Twelve wild-type Sprague Dawley rats were used for experiments described in Figure 2. The terminal dUTP nick end-labeling (TUNEL) method (Gavrieli et al., 1992) was used to detect DNA fragmentation.

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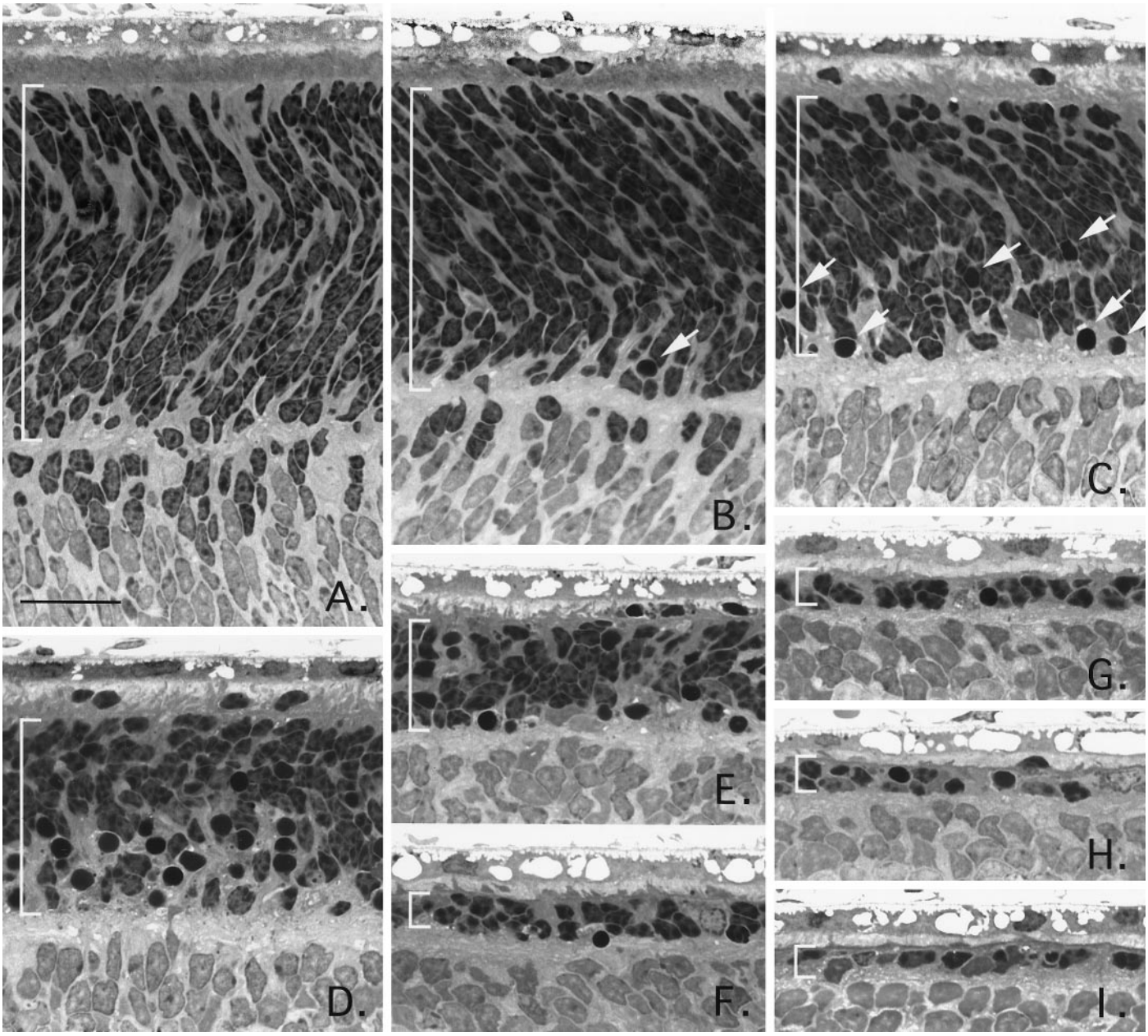


*Figure 1.* Photoreceptor degeneration in the S334ter-3 rats. Plastic-embedded sections of retinas from S334ter-3 rats of PD 6 (*A*), 8 (*B*), 10 (*C*), 11 (*D*), 12 (*E*), 14 (*F*), 16 (*G*), 18 (*H*), and 20 (*I*) were examined by light microscopy (superior region). The ONL is indicated in each panel by a *white bar*. Pyknotic nuclei in *B* and *C* are indicated by *white arrowheads*. Loss of photoreceptors becomes evident at PD 8. Peak of photoreceptor death occurs at PD 11 and 12. Pyknotic nuclei are found mainly in the proximal half of the ONL at PD 8 (*B*), 10 (*C*), and 11 (*D*). Some photoreceptor nuclei are displaced to the subretinal space (*B–E*). Sections were stained with toluidine blue. Scale bar, 20 μm.

Eyes were removed from 4% paraformaldehyde-perfused animals (three transgenic and three control rats), cryoprotected with 20% sucrose, frozen in Tissue-Tek OCT compound (Miles, Elkhart, IN) in powdered dry ice, and stored at −80°C. Cryosections of 10 μm were cut through the entire retina along the vertical meridian and thaw-mounted onto Super Frost Plus glass slides (Fisher Scientific, Pittsburgh, PA). TUNEL was performed using an apoptosis detection system (Promega, Madison, WI) according to the manufacturer's instructions.

*Measurement of caspase activities.* Retinas were dissected, snap-frozen in powdered dry ice, and stored at −80°C. Total protein was obtained by homogenizing retinas in a lysis buffer that contained 100 mM HEPES, pH 7.5, 10% sucrose, 1 mM EDTA, 20 mM EGTA, 0.2% 3[3-cholamidopropyl)dimethylammonio]-1-propane-sulfonate, 10 mM dithiothreitol, 10 μg of leupeptin, 2 μg/ml pepstatin, 2 μg/ml aprotinin, 1 mM PMSF, and 0.03% digitonin. The amount of total protein of each sample was determined by the BCA protein assay (Pierce, Rockford, IL), and the samples were stored at −80°C. Caspase-3 or caspase-1 activity was assessed by measuring the cleavage of fluorogenic substrate Ac-DEVD-AMC or Ac-YVAD-AMC, respectively, using a luminescence spectrometer (LS-50B; Perkin-Elmer, Norwalk, CT). Cleavage of *N*-acetyl-Asp-Glu-Val-Asp-aminomethylcoumarin (Ac-DEVD-AMC) or *N*-acetyl-Tyr-Val-Ala-Asp-aminomethylcoumarin (Ac-YVAD-AMC) was measured for 900 sec for each sample of 100 μg of total protein (accumulation of fluorescence was linear for at least 2 hr). The rate of fluorescence accumulation was calculated as the activity of a given enzyme. Experiments were repeated three times with samples from three litters of transgenic or control animals.

*Protein preparation and immunoblotting analysis.* Retinas were dissected, snap-frozen in powdered dry ice, and stored at −80°C. Pooled retinas were homogenized, and the concentration of total protein in each

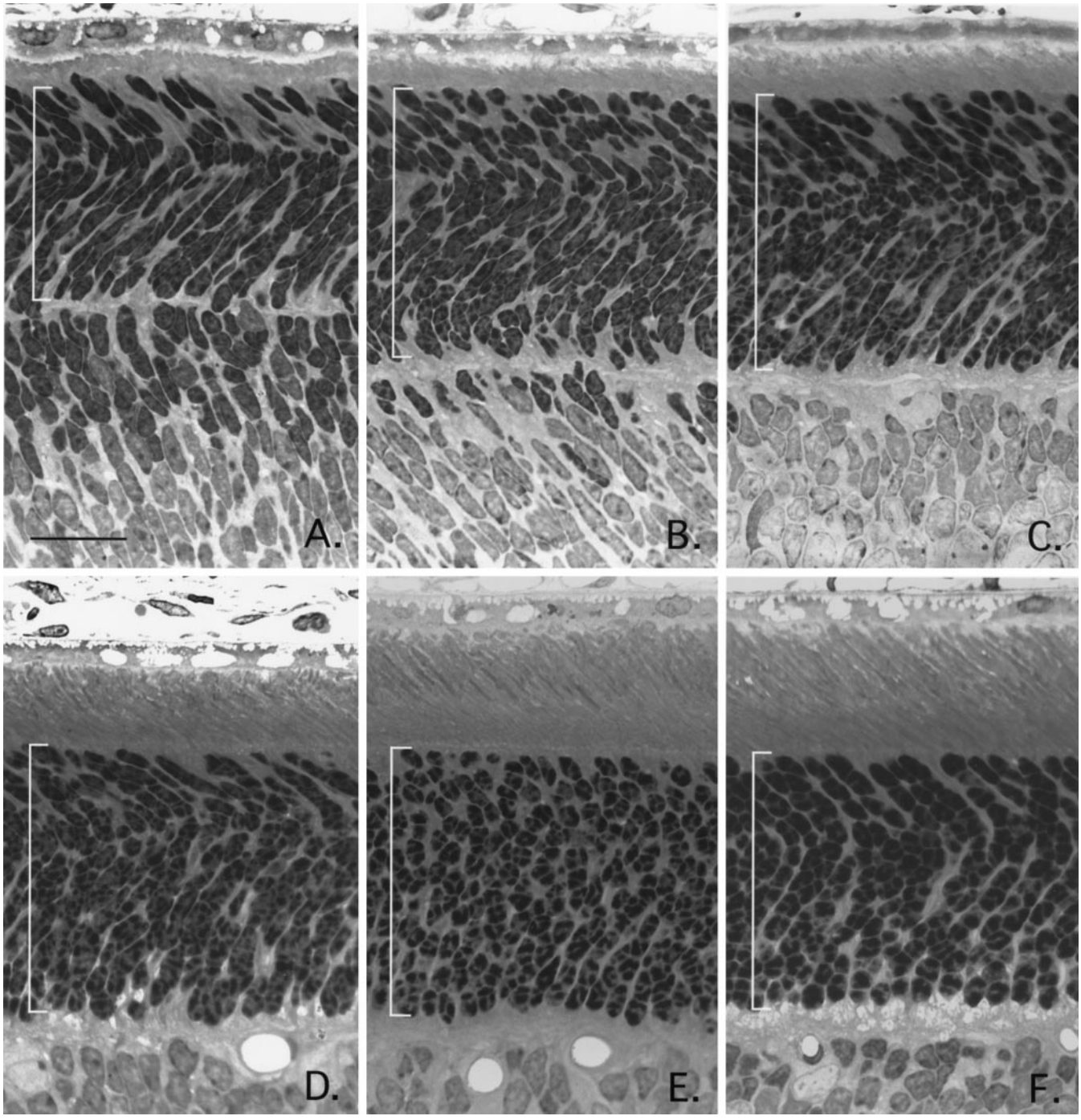


*Figure 2.* Photoreceptors in normal Sprague Dawley rats during postnatal development. Plastic-embedded sections of retinas from Sprague Dawley rats of PD 6 (*A*), 8 (*B*), 10 (*C*), 12 (*D*), 16 (*E*), and 20 (*F*) were examined by light microscopy (superior region). The ONL is indicated in each panel by a *white bar*. Development of rod outer segments is evident at PD 10 (*C*). Rod outer segments are approximately half of the length of those in the mature retina by PD 12 (*D*). Photoreceptors in PD 16 (*E*) and 20 (*F*) animals resemble closely those found in mature animals. Sections were stained with toluidine blue. Scale bar, 20 μm.

sample was determined by the BCA protein assay (Pierce). Total protein of 100 μg from each sample was electrophoresed on polyacrylamide gels and transferred to nitrocellular membranes (Bio-Rad, Hercules, CA). Blots were stained briefly with Ponceau S for visual inspection of transfer efficiency. Immunoblotting analysis was performed using polyclonal antibodies against the 12 kDa subunit of the active form of caspase-3 (Santa Cruz Biotechnology, Santa Cruz, CA). Signals were visualized using an ECL kit (Amersham, Arlington Heights, IL) and recorded on Hyperfilm (Amersham). All experiments were repeated three times to verify the consistency of the results.

## RESULTS

### Photoreceptor degeneration in heterozygous transgenic rats

The progressive photoreceptor degeneration in the retinas of heterozygous S334ter-3 rats follows a pattern described by M. M. LaVail (personal communication). Figure 1 shows representative light micrographs of heterozygous S334ter-3 rat retinas from PD 6 to 20. The overall appearance of the retina of a transgenic

animal at PD 6 (Fig. 1*A*) is similar to an age-matched control (Fig. 2*A*), differing slightly in stage of postnatal development. However, in the retina of a PD 8 S334ter-3 rat, 30–50 pyknotic nuclei in the outer nuclear layer (ONL) are found in an entire retinal section (Fig. 1*B*), in contrast to only one or two in the control ONL (data not shown). By PD 10, the retina of the transgenic rats differs greatly from that of control: not only are many pyknotic nuclei found in the ONL in the transgenic rats, but the inner segments of photoreceptors are now disorganized (Fig. 1*C*). Substantial loss of photoreceptors occurs at PD 11 (Fig. 1*D*). By PD 12, the ONL contains only six or seven rows of nuclei, in contrast to 12–13 rows in normal controls (Fig. 2*C*). The inner segments are even more disorganized (Fig. 1*E*). By PD 14, the rows of nuclei in the ONL decreased to three or four in the S334ter-3 rats, and the remaining inner segments are now short stumps (Fig. 1*F*). The nuclei in the ONL further decline to two or three rows at PD 16. Only one row remains at PD 20 with residual inner segments (Fig. 1*G–I*). No outer segments ever develop in the heterozygous S334ter-3 rats.

In the early stages of photoreceptor degeneration in the S334ter-3 rats (PD 8–11), pyknotic nuclei are distributed mainly in the proximal half of the ONL (Fig. 1*B–D*). As the degeneration progresses, they are seen in the distal ONL as well (Fig. 1*D,E*).

During degeneration, some photoreceptor nuclei become dislocated to the subretinal space next to the retinal pigment epithelial (RPE) cells (Fig. 1*B–E*). No such dislocation is observed in control animals (Fig. 2).

For comparison, Figure 2 shows representative light micrographs of normal Sprague Dawley rat retinas from PD 6 to 20. At PD 6, the overall morphological appearance of Sprague Dawley retina (Fig. 2*A*) is similar to that of the transgenic rats (Fig. 1*A*). At PD 8, the ONL (Fig. 2*B*) thickens, and the inner segments are well organized (Fig. 2*B*). By PD 10, the outer segments of rod photoreceptors begin to develop (Fig. 2*C*). At PD 10, the rod outer segments are approximately half of the length of those in mature retina (Fig. 2*D*). Photoreceptors in PD 16 (Fig. 2*E*) and 20 (Fig. 2*F*) animals are similar to those found in mature normal animals. Only one or two pyknotic nuclei are observed in the ONL from PD 10 through 20 (data not shown).

To determine whether photoreceptor death in the S334ter-3 rats was apoptotic, we used the TUNEL method (Gavrieli et al., 1992) to detect DNA fragmentation. As shown in Figure 3, many cells in the ONL of a PD 11 transgenic rat are TUNEL-positive. The distribution of TUNEL-labeled cells is denser in the proximal than in the distal ONL (Fig. 3*A*). This is consistent with the distribution of pyknotic nuclei (Fig. 1*D*). In age-matched control animals (Fig. 3*B*), no TUNEL-labeled cells are found in the ONL. In the inner nuclear layer (INL), a few cells are TUNEL-positive in both control (Fig. 3*B*) and transgenic (Fig. 3*A*) animals.

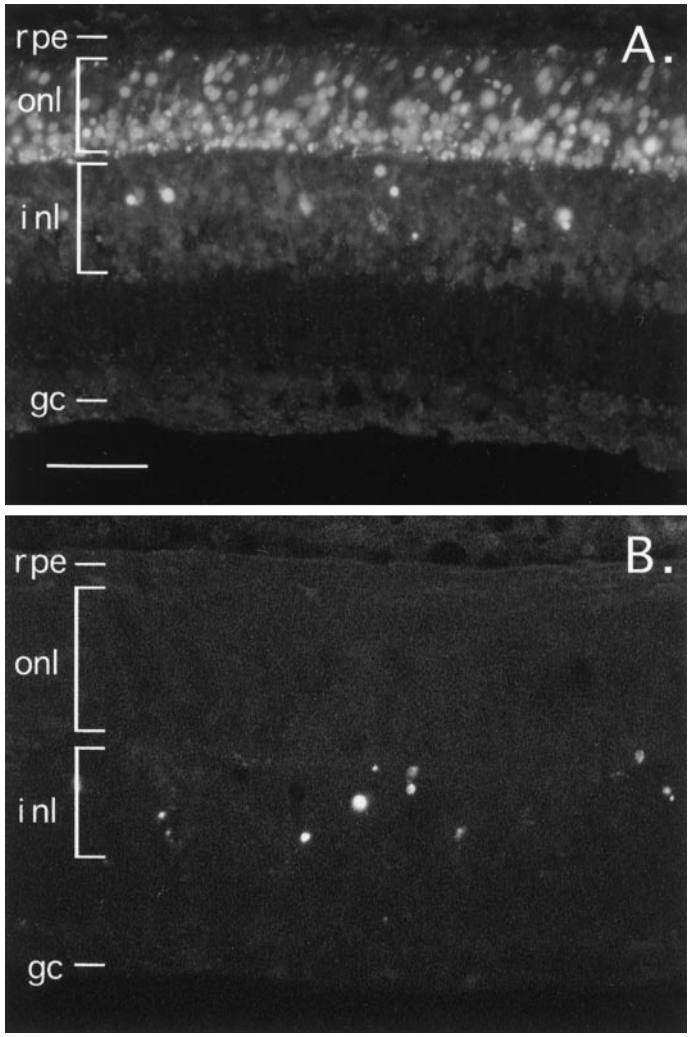


*Figure 3.* DNA fragmentation in degenerating photoreceptors of the S334ter-3 rats. Cryosections (10 $\mu$m) from the retina of a PD 11 S334ter-3 (*A*) and a wild-type Sprague Dawley rat (*B*) were TUNEL-labeled with fluorescein to visualize the DNA fragmentation. In the S334ter-3 rat (*A*), numerous cells in the ONL are labeled. The distribution of labeled cells is denser in the proximal than in the distal ONL. No labeled cells are found in the ONL of the control retina (*B*). A few cells in the INL are also labeled in the control (*B*) and transgenic (*A*) rats. *rpe*, Retinal pigment epithelium; *onl*, outer nuclear layer; *inl*, inner nuclear layer; *gc*, ganglion cell layer. Scale bar, 50 $\mu$m.

## Activation of caspase-3 in the S334ter-3 rat retina during degeneration

The activity of caspase-3-like proteases was assessed by measuring the cleavage of a fluorogenic substrate Ac-DEVD-AMC. Retinal samples were collected from transgenic and age-matched Sprague Dawley control animals at PD 8–14 and 16. A large increase in Ac-DEVD-AMC cleavage was observed in transgenic animals at PD 11–14. It was close to sixfold of control level at PD 11 and 12 and declined to approximately twofold at PD 13 and 14 (Fig. 4*A*). Because Ac-DEVD-AMC is also cleaved by caspase-1, we ruled out this possibility by using a caspase-1-specific fluorogenic substrate, Ac-YVAD-AMC. Substrates with a sequence of YVAD, based on the recognition sequence for caspase-1 YVHD, have high selectivity for caspase-1 over caspase-3. In fact, the tetrapeptide aldehyde Ac-YVAD-CHO is highly selective, possessing an affinity for caspase-1 six orders of magnitude higher than for caspase-3 (Fernandes-Alnemri et al., 1995; Margolin et al., 1997). As shown in Figure 4*B*, no significant alteration in Ac-YVAD-AMC cleavage was observed from PD 8 to 16 in the S334ter-3 rats, compared with the controls, indicating that caspase-1 is not significantly activated during photoreceptor degeneration.

Caspases-3 is synthesized as a 32 kDa inactive proenzyme and is cleaved at Asp-28-Ser-29 and Asp-175-Ser-176 to generate a subunit of 17 kDa and a smaller one of 12 kDa after activation (Nicholson et al., 1995). To assess the amount of active caspase-3 during photoreceptor degeneration, we measured the amount of

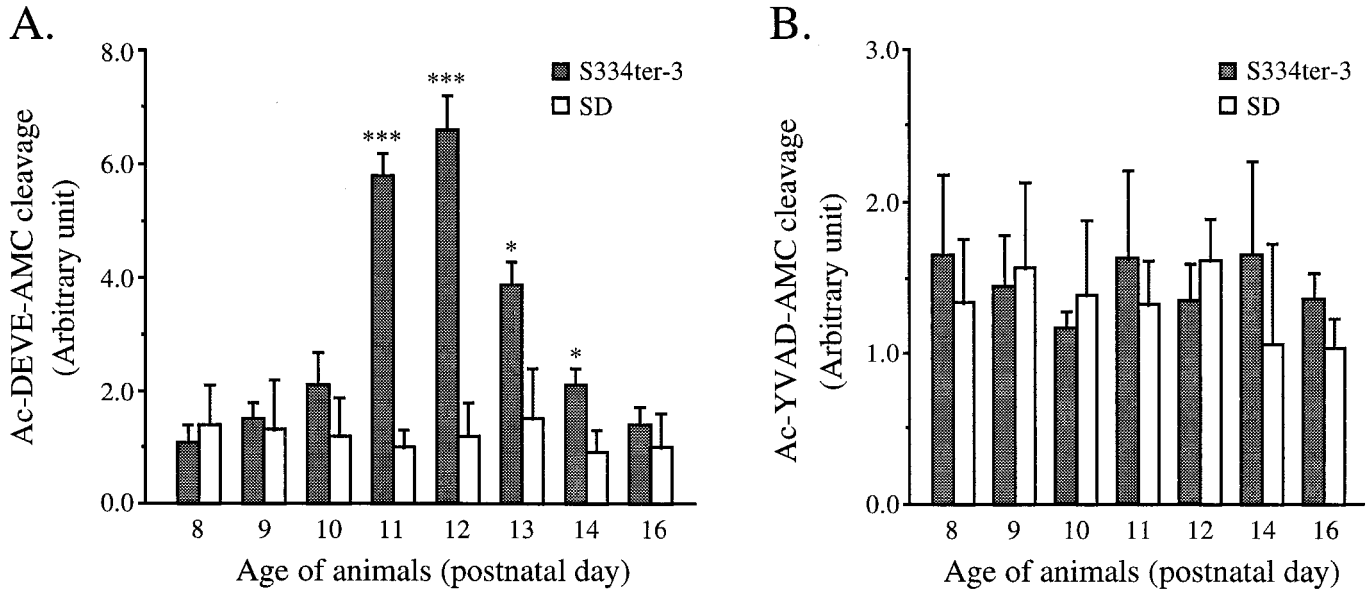


*Figure 4.* Activities of caspase-3-like and caspase-1-like proteases in the retina. Activities of caspase-3-like or caspase-1-like proteases were determined by measuring the cleavage of Ac-DEVD-AMC or Ac-YVAD-AMC, respectively. Retinas were collected at PD 8–14 and 16 from S334ter-3 or normal Sprague Dawley rats. Intensity of fluorescence was measured on a Perkin-Elmer LS-50B luminescence spectrometer at 30 sec intervals for 900 sec. The rate of accumulation of fluorescence was calculated as the activity of a given enzyme. Data are presented as mean ± SD ($n = 3$). $^{***}p < 0.001$; $^{*}p < 0.01$ (Student's $t$ test).

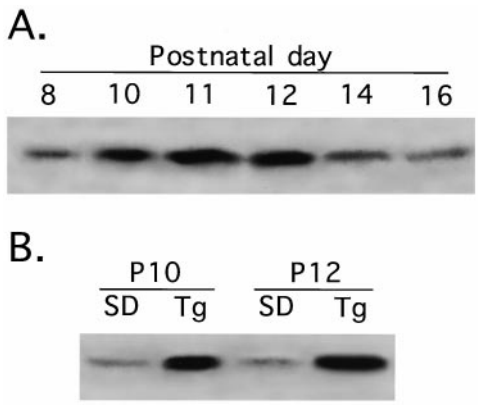


*Figure 5.* p12 subunit of activated caspase-3 during photoreceptor degeneration in the S334ter-3 rats. Immunoblotting analyses were performed to detect the p12 subunit of the active form of caspase-3, using polyclonal antibodies against the p12 subunit of caspase-3. *A*, In the retinas of S334ter-3 rats, significant increases in the p12 subunit are found at PD 10–12, which coincides with the rapid degeneration phase. *B*, The amounts of the 12 kDa subunit in the retina of PD 10 (*P10*) and 12 (*P12*) S334ter-3 animals (*Tg*) are significantly higher than age-matched Sprague Dawley rats (*SD*).

the 12 kDa subunit in retinas of the S334ter-3 rats by immunoblotting analysis. Figure 5*A* displays the relative amounts of the 12 kDa subunit during photoreceptor degeneration. Significant increases were found at PD 10–12 in the transgenic rats, coincident with the rapid phase of photoreceptor loss in the retina. The amounts of the 12 kDa subunit in the transgenic rats during the rapid degeneration phase are much higher than those in the age-matched Sprague Dawley control rats (Fig. 5*B*).

### Protection of photoreceptors by peptide caspase inhibitor z-DEVD-fmk

To test whether caspase-3 is important for photoreceptor death in the S334ter-3 rats, we used an irreversible inhibitor for caspase-3, z-DEVD-fmk. Six S334ter-3 rats (littermates) were injected at PD 9 with 50 μg of z-DEVD-fmk (in 1 μl of DMSO) into the left eyes and 1 μl of DMSO into the right eyes. The eyes were collected at PD 20, and retinas were examined by light microscopy. As shown in Figure 6, the retina of a PD 20 normal animal is fully developed and intact. The ONL contains 12–13 rows of nuclei, and the outer and inner segments are well organized (Fig. 6*A*). The retina of a S334ter-3 rat treated with DMSO (Fig. 6*B*) corresponds to those without any treatment (Fig. 1*I*): only a single row of nuclei remain in the ONL (Fig. 6*B*). In contrast, the ONL of the z-DEVD-fmk-treated retina from the other eye of the same animal contains four or five rows of nuclei (Fig. 6*A*). In all six animals, the ONL in the control eyes has one or two rows of nuclei, whereas the treated eyes have three to five rows.

To verify that z-DEVD-fmk did inhibit caspase-3 activation in the retinas of S334ter-3 rats, we measured the 12 kDa caspase-3 subunit after inhibitor injection. Three transgenic rats were injected with 50 μg of z-DEVD-fmk (in 1 μl of DMSO) into the left eyes and 1 μl of DMSO into the right eyes at PD 9, and retinas were collected at PD 11. As shown in Figure 7, the amount of the 12 kDa subunit in retinas treated with z-DEVD-fmk is significantly lower than in those treated only with DMSO. Nevertheless, it is still higher than in the normal Sprague Dawley rats.

## DISCUSSION

Since the discovery of mutations in the rhodopsin gene in human with retinitis pigmentosa (Dryja et al., 1990a,b; Farrar et al., 1990), various mutations of the visual pigment have been introduced into the genomes of animals as models of these disorders (Olsson et al., 1992; Steinberg et al., 1996, 1997; Petters et al., 1997). These transgenic animals are valuable tools for retinal degeneration research. In the present work, we used a line of transgenic rats that carry a murine rhodopsin mutation, S334ter (Steinberg et al., 1996, 1997). These animals undergo a degenerative course leading to the death of >90% of all photoreceptors

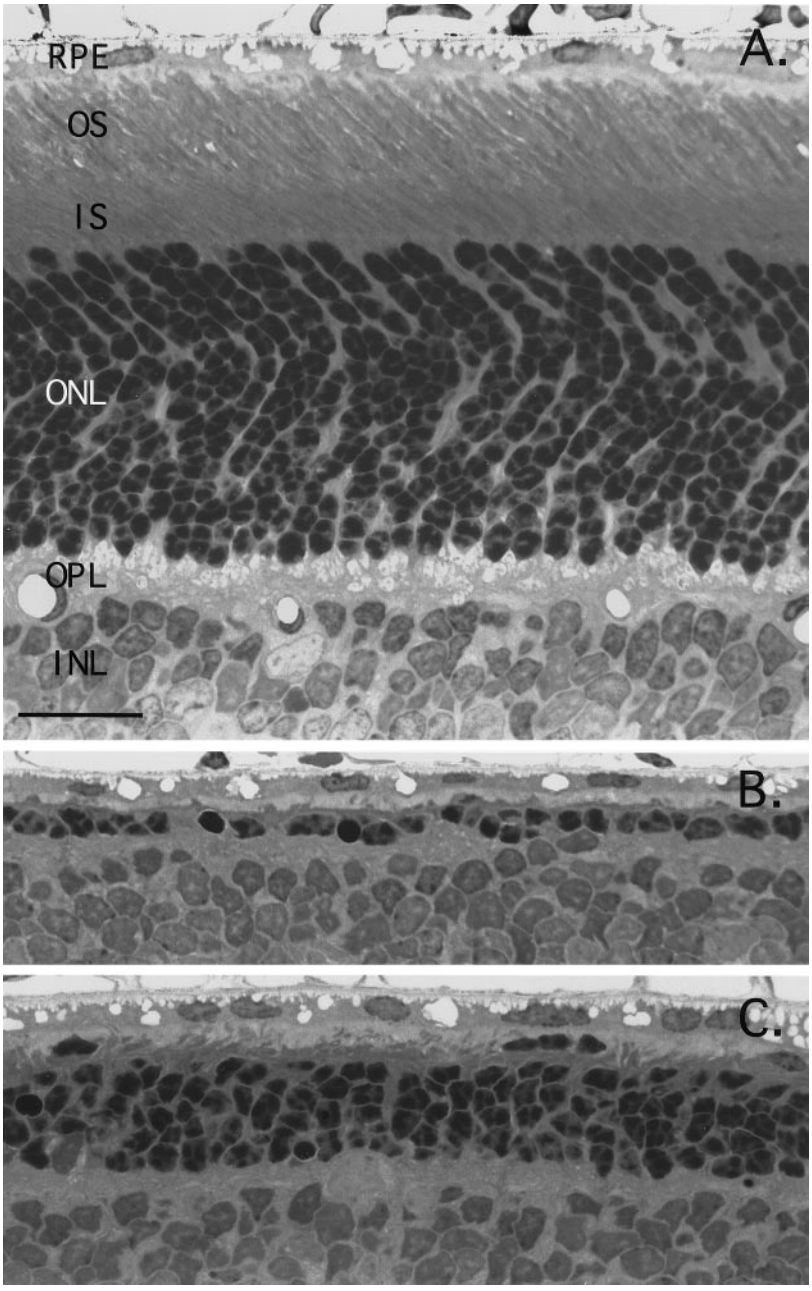


*Figure 6.* Protection of photoreceptors by z-DEVD-fmk. Plastic-embedded sections of retinas from a normal rat (*A*) and a S334ter-3 rat treated with DMSO (*B*) or z-DEVD-fmk (*C*) at PD 20 were examined by light microscopy. The normal retina has well developed outer and inner segments. The ONL has 12 or 13 rows of nuclei (*A*). In the DMSO-treated retina, the ONL has only one row of nuclei, and inner segments become short stumps (*B*). In the z-DEVD-fmk-treated retina from the same animal (*C*), the ONL has four or five rows of nuclei. The inner segments are better preserved, although still shortened and disorganized. Some dislocated cells are found in the subretinal space next to the RPE. Sections were stained with toluidine blue. *OS*, Outer segment; *IS*, inner segment; *OPL*, outer plexiform layer. Scale bar, 20 $\mu$m.

in <2 weeks. At the peak rate of photoreceptor death, the loss of photoreceptors reaches three or four rows of nuclei in the ONL in 1 day, or close to 30% of the photoreceptors in the entire retina (Fig. 1). It is estimated that in the rat retina one row of nuclei in the ONL represents about 1 million photoreceptors. Thus, at the peak of degeneration, the death rate is 3–4 million rods/d. The massive photoreceptor death in these animals provides an opportunity to investigate the biochemistry of photoreceptor degeneration.

Histological analysis shows that photoreceptor degeneration begins at the proximal portion of the ONL, because pyknotic nuclei are found mainly in the proximal half of the ONL from PD 8 through 11 (Fig. 1). In addition, more TUNEL-labeled cells are observed in the proximal than in the distal ONL at PD 11 (Fig. 2). The reason why cells in the proximal ONL are more susceptible to degeneration is not clear. It is possible that these cells are more advanced in development than those in the distal portion of the ONL and therefore die sooner.

The displacement of photoreceptor nuclei to the subretinal space (Fig. 1) is likely to be a phenomenon related to the degenerative process, because it is observed only in the retinas of transgenic animals undergoing photoreceptor degeneration.

The first indication that caspases are involved in photoreceptor death derives from *Drosophila*, in which mutations in the rhodopsin gene also cause photoreceptor degeneration (Steele and O'Tousa, 1990; Colley et al., 1995; Kurada and O'Tousa, 1995). Experiments in two strains of *Drosophila* with rhodopsin mutations, $ninaE^{RH27}$ and $rdgC^{306}$, showed that eye-specific expression of baculovirus protein p35 not only protects photoreceptors but also preserves their normal function (Davidson and Steller, 1998). p35 blocks cell death after viral infection and is regarded as antiapoptotic (Friesen and Miller, 1987; Clem et al., 1991).

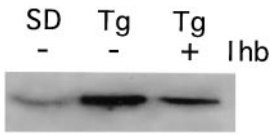


*Figure 7.* The p12 subunit of activated caspase-3 after intraocular injection of caspase-3 inhibitor z-DEVD-fmk. PD 9 S334ter-3 rats were injected with 50 μg of z-DEVD-fmk (in 1 μl of DMSO) to the left eyes and 1 μl of vehicle (DMSO) to the right eyes. Retinas were collected at PD 11. Retinas of age-matched Sprague Dawley rats served as control. Immunoblotting analyses were performed to detect the p12 subunit of the active form of caspase-3, using polyclonal antibodies against the p12 subunit of caspase-3. In the S334ter-3 rats, the amount of the p12 subunit is much less in inhibitor treated retinas (*Tg, Ihb* +) than in vehicle-treated retinas (*Tg, Ihb* −), although it is still higher than normal Sprague Dawley controls (*SD, Ihb* −).

Purified recombinant p35 inhibits a broad range of caspases, including caspase-1, -2, -3, and -4 (Bump et al., 1995; Xue and Horvitz, 1995). The demonstration by Davidson and Steller (1998) that p35 protects photoreceptors indicates that in *Drosophila* photoreceptor cell death likely is mediated by a caspase-dependent mechanism.

Caspase-3 was first cloned using the sequence of an expressed sequence tag in the sequence database that shared similarity with ICE and was named CPP32 (Fernandes-Alnemri et al., 1994). Subsequently, two other groups independently identified it and named it Yama (Tewari et al., 1995) and apopain (Nicholson et al., 1995). Among known caspases, it has the highest homology to *C. elegans* CED-3 in both amino acid sequence and substrate specificity. It is recognized as one of the key executioners of apoptosis. Moreover, activation of caspase-3 is important for the initiation of apoptosis in neurons. For instance, in caspase-3 knock-out mice, there is a selective defect in cell death in the CNS that leads to a doubling of brain size (Kuida et al., 1996). This finding indicates that caspase-3 plays a critical role during morphogenetic cell death in the brain. In cultured mouse cerebellar granule neurons, $K^+$-serum withdrawal also activates caspase-3, inducing apoptosis (Armstrong et al., 1997). In addition, caspase-3 is found to contribute to apoptosis in brain neurons after traumatic brain injury or experimental cerebral ischemia (Yakovlev et al., 1997; Cheng et al., 1998; Namura et al., 1998). Last, in the retina caspase-3 is involved in ganglion cell death after optic nerve transection (Kermer et al., 1998).

The present work establishes a role of caspase-3 in the photoreceptor degeneration in S334ter-3 rats. A clear relationship among the increase in Ac-DEVD-AMC cleavage, the increase in the 12 kDa subunit of caspase-3, and the course of photoreceptor degeneration indicates a correlation between caspase-3 activation and photoreceptor degeneration. That inhibition of caspase-3 by the irreversible caspase-3 inhibitor z-DEVD-fmk protects photoreceptors provides further evidence that caspase-3 activation promotes the death of photoreceptors.

Our present work leaves unanswered the question of whether photoreceptor death by causes other than rhodopsin mutations is also mediated by the same mechanism. However, preliminary experiments (R. Wen, unpublished observations) do indicate that caspase-3 is involved in photoreceptor death in other animal models as well, suggesting a common mechanism for photoreceptor degeneration in these disorders. Further analysis of the involvement of caspases not only will deepen our understanding of photoreceptor cell death but also might provide the basis for a new approach to therapies for retinal degenerative disorders. The present work represents a step toward that goal.

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