Synergistic Effect of Bcl-2 and BAG-1 on the Prevention of Photoreceptor Cell Death

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PURPOSE. Ectopic expression of Bcl-2 in photoreceptors of mice with retinal degenerative disease slows progression of the disease. BAG-1 has previously been shown to augment the inhibitory effect of Bcl-2 on programmed cell death in cultured cell systems. This study was designed to determine whether the coexpression of BAG-1 and Bcl-2 in the photoreceptors of mice with an autosomal dominant form of retinitis pigmentosa (RP) would enhance the protective effect provided by Bcl-2 alone.

METHODS. An expression vector using the 5’ regulatory region of the murine opsin gene was used to target the expression of BAG-1 specifically to photoreceptor cells of mice. The BAG-1 transgenic mice were crossed to Bcl-2 transgenics to obtain animals that coexpress the two transgenes in photoreceptor cells. BAG-1/Bcl-2 animals were then crossed to an RP mouse model (a transgenic line overexpressing the S334ter rhodopsin mutant) to assess the effect of coexpression of BAG-1 and Bcl-2 on retinal degeneration. Morphologic analysis was performed on retinas isolated at various times after birth to monitor disease progression.

RESULTS. High levels of BAG-1 expression resulted in retinal degeneration that was not prevented by Bcl-2 expression. However, coexpression of appropriate levels of BAG-1 and Bcl-2 was found to have a profound inhibitory effect on retinal degeneration caused by overexpression of a mutant rhodopsin transgene. Whereas expression of Bcl-2 alone was previously found to delay degeneration of the retina from 2 weeks to approximately 4 weeks of age, coexpression of BAG-1 and Bcl-2 inhibited photoreceptor cell death for as long as 7 to 9 weeks.

CONCLUSIONS. The synergistic effect against photoreceptor cell death produced by the coexpression of Bcl-2 and BAG-1 indicates that these proteins can function in concert to prevent cell death. At the correct dosage, coexpression of Bcl-2 and BAG-1 may serve as a potential means to treat retinal degenerative diseases. (Invest Ophthalmol Vis Sci. 2000;41:1953–1961)

Retinitis pigmentosa (RP) refers to a group of inherited retinopathies affecting approximately 1 of every 3000 individuals.1,2 The various forms of this progressive disease include autosomal dominant RP (adRP), autosomal recessive RP (arRP), and X-linked RP (xIRP). Degeneration of rod photoreceptors initially results in diminished night vision. However, rod photoreceptor cell death causes a loss of retinal tissue that eventually affects overall vision. The retinal epithelium thins, and deposits of pigment form on the retinal surface, ultimately leading to blindness.

Several components of the visual transduction pathway may be involved in the origin of RP. Many mutations within genes whose products are involved in phototransduction have been linked to the various forms of RP.3–5 Numerous mutations have been identified within the rhodopsin gene that are associated with the autosomal dominant form of RP.5 In addition, defects in the genes encoding the α and β subunits of rod cyclic guanosine monophosphate (cGMP) phosphodiesterase, as well as in the α subunit of cGMP-gated cation channel, have also been associated with the occurrence of RP.6–8 Mutations within the genes encoding peripherin and rom-1, which encode structural components of the outer disc membrane, also lead to degeneration of the retina.9–11 Retinal degeneration may also result from cellular insults such as constant light exposure.12–14 Many of the genetic defects and damaging effects of light act by initiating a programmed cell death pathway in photoreceptor cells.15–17

Programmed cell death (apoptosis) is a physiological process that occurs during normal development and in certain pathologic states.18 Morphologic changes including cell shrinkage, chromatin condensation, and nuclear DNA fragmentation characterize the process. Resultant cellular debris is immediately phagocytosed by adjacent cells to avoid an inflammatory response. Many of the physical manifestations of apoptosis can easily be seen in a degenerating retina. Although, retinal de-
generation results from a variety of mutations in genes involved in photoreception, as well as from certain environmental insults, the cellular response ultimately converges on a programmed cell death pathway. The initiating event that precipitates the decision to activate a programmed cell death pathway in photoreceptor cells remains to be determined.

A programmed cell death pathway that is regulated by several cellular genes such as Bcl-2 and BAG-1 has been described. Overexpression of Bcl-2 in photoreceptors of mice with retinal degeneration temporarily delays disease progression. Bcl-2 is a novel Bcl-2-binding protein that interacts both physically and functionally with Bcl-2 and has been shown to augment the anti-cell death activity of Bcl-2 in vitro. The temporary effect produced by the overexpression of Bcl-2 in slowing the onset of programmed cell death associated with retinal degeneration may result from low levels or the absence of proteins, such as BAG-1, which form a functional complex with Bcl-2. Therefore, Bcl-2 and BAG-1 were ectopically expressed in the photoreceptors of mice with retinal degenerative disease to determine whether their functional complex would enhance the protective effect afforded by Bcl-2 alone.

**MATERIALS AND METHODS**

**Transgene Construct**

The BAG-1 expression vector used to generate transgenic mice was constructed by fusing the 5′ regulatory region of the murine opsin gene to the murine BAG-1 cDNA sequence. Briefly, a SalI–BamHI fragment containing the BAG-1 coding sequence was inserted between a 4.4-kb fragment of the rod opsin 5′ flanking sequence and the mouse protamine 1 polyadenylation sequence that had been cloned into the multiple cloning site of pBluescript KS (+) (Stratagene, La Jolla, CA). The rod opsin promoter sequence contains the standard CCAAT and TATA sequences and a small portion of the opsin 5′ untranslated region preceding the translational start site. The fusion gene was released from the vector by digesting with KpnI and XbaI, gel purified, and used for microinjection. Because the 5′ untranslated region of the murine BAG-1 gene (which contains a noncanonical CTG initiation codon) is not included in the construct, only the short form of the BAG-1 protein should be translated. A similar expression vector was previously used to direct expression of Bcl-2 in photoreceptor cells.

**Generation of Transgenic Mouse Lines**

Transgenic mice were generated by microinjection of the purified expression construct into the pronuclei of fertilized eggs. Injected embryos were allowed to develop to the two-cell stage by incubating overnight at 37°C in 5% CO2 in M16 medium (Cell & Molecular Technologies, Inc., Lavallette, NJ). Two-cell embryos were then implanted into the oviducts of pseudopregnant foster mothers. Genotype analysis of offspring was accomplished by performing polymerase chain reaction (PCR) on genomic DNA isolated from tail biopsy samples of offspring. Founder mice, designated RhBAG-IA through D, were identified and mated to wild-type animals to expand the colonies for further analysis. All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Genotype Analysis**

PCR was performed on genomic DNA isolated from tail biopsy samples to screen for the presence of the various transgenes in the offspring as follows: BAG-1: initial denaturation at 95°C for 3.5 minutes, followed by 30 cycles of 94°C for 1 minute, 63°C for 1.5 minutes, and 72°C for 1.5 minutes. DNA oligos Rh1.1 and BAG OP1 were used to amplify the BAG-1 sequence; Bcl-2: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 94°C for 1 minute, 63°C for 2 minutes, and 72°C for 2 minutes. PCR samples were then cooled to 4°C. DNA oligos Rh1.1 and Bcl2 were used for Bcl-2 amplification; S34ter rhodopsin mutant (RhoΔCT): the same PCR conditions used for amplification of Bcl-2 sequences were used, except the annealing temperature was 54°C instead of 63°C. PCR was performed using DNA oligos Rh2 and Rh3. Oligos were Rh1.1 5′ GTGCCTGGAGTTGCGCTGTGGG 3′, BAG OP1 5′ GTCA-CACTCTGTCAAGAACACCTGA 3′, Bcl2 5′ CCCTGTTCG-CCAGCTGCGGC 3′, Rh2 5′ TGGAAGATGACGAGCCTAA 3′, and Rh3 5′ TGAGGGAGCGGGTACAGATCC 3′.

**Western Blot Analysis**

Protein immunoblot analysis was performed using retinal extract preparations to assess expression levels of the transgenes. Extracts were prepared by homogenizing a single retina in 100 μl of phosphate-buffered saline (PBS). An equal volume of 2× sample-loading dye was added to each sample, and aliquots were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) using 12% Tris-HCl acrylamide gels. Proteins were transferred to nylon membranes, which were blocked overnight at 4°C in a solution containing 5% dry milk in 1× TBST (20 mM Tris [pH 7.5], 137 mM NaCl, and 0.1% Tween-20). Filters were then incubated with the BAG-1 primary antibody at a 1:1000 dilution or with the Bcl-2 antibody at a 1:500 dilution in a solution containing 2% bovine serum albumin (BSA) in 1× TBST at room temperature for 1 hour. BAG-1 antibody solution was supplemented with 0.050 μg/μl ovalbumin. Filters were rinsed several times with 1× TBST over approximately 30 minutes. Anti-rabbit Ig horseradish peroxidase (HRP; Amersham, Arlington Heights, IL) was used as the secondary antibody at a 1:2000 dilution in 1× TBST containing 0.050 μg/μl ovalbumin for BAG-1 and 1× TBST containing 5% dry milk for Bcl-2. Secondary antibodies were incubated with the filters for 1 hour at room temperature. After several rinses with 1× TBST, a chemiluminescence detection system was used to visualize the antibody–protein complex (ECL; Amersham). The rabbit polyclonal antibody (BAG 1735-13) was generated using a synthetic peptide corresponding to the C-terminal 16 amino acid residues of the mouse BAG-1 protein. Bcl-2 antibody was a rabbit polyclonal antibody (product number SC783; Santa Cruz Biotech, Santa Cruz, CA). Purified recombinant His6-huBcl-2 and mBAG-1 protein were used as controls.

**Tissue Processing for Light Microscopy**

Retinal tissues for morphologic analysis were processed as previously described with minor modifications.

**Immunohistochemistry**

Eyes were enucleated and placed in a solution containing 4% paraformaldehyde in PBS. Eyecups, formed by removing the cornea and lens, were kept in 4% paraformaldehyde for ap-
proximately 1 hour on ice. Eyecups were rinsed extensively in cold PBS by changing the solution several times over the course of an hour. Eyecups were then embedded in polymerized acrylamide for sectioning, as previously described. Cryosectioning was performed and 10-μm sections were collected and stored at −80°C. Before immunostaining, sections were thawed at room temperature and placed in a blocking solution containing 0.5% BSA and 0.5% Triton X-100 in PBS. Several drops of normal serum, obtained from the species in which the secondary antibody was produced, were also added. After blocking was performed for approximately 1 hour, the BAG-1 primary antibody was added at a 1:1000 dilution and the Bcl-2 primary antibody at a 1:25 dilution in blocking solution for 1 hour. Sections were washed three times for 5 minutes with blocking solution. Fluorescein anti-rabbit IgG (H + L; Vector, Burlingame, CA) was used as the secondary antibody, at a 1:100 dilution for 1 hour. Sections were washed three times for 5 minutes each in blocking solution followed by three washes in PBS. Sections were mounted under a glass coverslip in mounting medium (Vectashield; Vector).

**TdT-Mediated dUTP Nick End Labeling**

TdT-mediated dUTP nick end labeling (TUNEL) analysis was performed using the in situ cell death detection kit (Boehringer–Mannheim, Mannheim, Germany) according to the manufacturer’s instructions for cryopreserved tissue sections. Sections were mounted under a glass coverslip in mounting medium (Vectashield; Vector) containing 4′, 6-diamidino-2-phenylindole (DAPI) as a counterstain (Vector).

**Morphometric Analysis**

Morphometric analysis was performed by counting photoreceptor cells within a 200-μm span of the central region of wild-type and transgenic retinas that were oriented superior to the rod and cone photoreceptor cells in an appropriate spatial and temporal manner during retinal development. Protein immunoblot analysis performed using retinal extracts prepared from nontransgenic and transgenic littersates at P26. Samples were derived from a nontransgenic littermate (lane 1) and from transgenic animals representing lines RhBAG-1B (lane 2) and RhBAG-1E (lane 3). Amount of extract run on gel was equivalent to 10% of the entire retina. Various amounts of purified BAG-1 protein were run as control samples (lanes 4, 5, and 6).

**Effect of BAG-1 Overexpression on Retinal Morphology**

Morphologic analysis showed that overexpression of BAG-1 in the photoreceptors of mice had an adverse affect on the transgenic retina. As early as postnatal day (P)15 the photoreceptor outer segments appeared disorganized and slightly thinner in an RhBAG transgenic retina than in a nontransgenic retina. Many photoreceptor nuclei were also found to be pyknotic at this time (data not shown). Loss of photoreceptor cells gradually continued over time, resulting in approximately a 30% to 50% decrease in the outer nuclear layer by 5 weeks of age. Morphology of a nontransgenic retina and an RhBAG transgenic retina at 5 weeks of age is shown in Figures 2A and 2B, respectively. The rate of photoreceptor cell death in response to the high levels of BAG-1 expression was slow compared with that observed in a transgenic line overexpressing the S334ter rhodopsin mutant (Rh0ΔCT), in which a nearly complete loss of photoreceptor cells occurs by P15.

Although Bcl-2 has previously been shown to delay retinal degeneration induced by a variety of apoptotic stimuli, including the overexpression of the S334ter mutant rhodopsin, Bcl-2 expression failed to inhibit the death of photoreceptor cells overexpressing BAG-1 in double-transgenic retinas, RhBAG-1B/Bcl-2B (Fig. 2C). The level of Bcl-2 expression in photoreceptors in the Bcl-2 transgenic line has a minimal effect on the retinal structure, because the morphology of Bcl-2 retinas is similar to nontransgenic animals at this age. The effect of BAG-1 cannot be attributed to a nonspecific effect of overexpressing a foreign gene because targeted expression of several different genes in the photoreceptors using the same rhodopsin promoter has not led to deleterious effects on retinal.

**Figure 1.** Expression of BAG-1 transgene in RhBAG-1 transgenic retinas. Western immunoblot analysis using retinal extracts prepared from nontransgenic and transgenic littersates at P26. Samples were derived from a nontransgenic littermate (lane 1) and from transgenic animals representing lines RhBAG-1B (lane 2) and RhBAG-1E (lane 3). Amount of extract run on gel was equivalent to 10% of the entire retina. Various amounts of purified BAG-1 protein were run as control samples (lanes 4, 5, and 6).
morbidity, indicating that photoreceptors can tolerate the expression of foreign genes. In fact, levels of Bcl-2 expression similar to the level of BAG-1 expression in photoreceptor cells has a minimal effect on retinal morphology (see description later). Therefore, the effect produced by BAG-1 is a consequence of its expression and is not related to overexpression of genes that are foreign to the retina. The mechanism by which the ectopic expression of BAG-1 caused photoreceptor degeneration is currently unknown.

**Expression Levels of BAG-1 and Bcl-2 in the Transgenic Retinas**

Quantitation of Bcl-2 and BAG-1 transgene expression by Western immunoblot analysis using retinal extract preparations demonstrated that the Bcl-2 and BAG-1 transgenes were expressed at similar levels in the single transgenic retinas at 1 month of age (Fig. 3A). Estimates of expressed protein levels were made by comparing the intensity of bands obtained using various amounts of recombinant BAG-1 or Bcl-2 protein with the overall levels of transgene expression. The total expressed protein is estimated to be approximately 400 to 800 ng per retina for both the BAG-1 and the Bcl-2 transgenes. The amount of the ectopically expressed proteins is considerably lower than the amount of endogenous rhodopsin expression in a 4-week-old mouse retina (0.31 nanomoles [12.4 µg] rhodopsin per retina). Expression levels of BAG-1 and Bcl-2 in the double-transgenic retinas, RhBAG-1B/Bcl-2B, were also analyzed and were found to be similar (Fig. 3B). Therefore, whether expressed alone or together in the photoreceptors, the overall protein levels of BAG-1 and Bcl-2 were approximately the same.

**Distribution Patterns of BAG-1 and Bcl-2 in RhBAG-1B/Bcl-2B Transgenic Retinas**

The distribution pattern of BAG-1 and Bcl-2, when expressed singly and together in photoreceptor cells, was determined using retinal sections and immunofluorescence microscopy. Retinal sections used for the immunolocalization analysis were obtained from animals at approximately 1 month of age, when the degenerative effect of BAG-1 expression is clear. Immunolocalization demonstrated that BAG-1 and Bcl-2 exhibited similar distribution patterns in the RhBAG-1B/Bcl-2B transgenic retina (Fig. 4), as well as in the RhBAG-1B and Bcl-2B single transgenic retinas (data not shown). Both proteins were expressed throughout the entire photoreceptor cell layer with the highest level of expression for both BAG-1 and Bcl-2 appearing to be in the inner segment where the mitochondria are primarily located. Although, the overall pattern of Bcl-2 expression reported here differed from a previous report in which Bcl-2 was primarily localized to the synaptic layer of the photoreceptor cell in a Bcl-2 transgenic retina, there was some bright punctate staining for Bcl-2 at the synaptic terminal region (see Fig. 4C). The different yet overlapping immunolocalizations for Bcl-2 are thought to result from the use of two

**Figure 2.** Overexpression of BAG-1 in photoreceptor cells affects retinal morphology. Light microscopy was performed on retinal sections derived from a wild-type (A), a RhBAG-1B (B), and a RhBAG-1B/Bcl-2B (C) transgenic sibling at 5 weeks of age. ONL, outer nuclear layer. Bar, 25 µm.
Bcl-2 transgenes. (A) Protein immunoblot analysis using retinal extracts prepared from animals at approximately 1 month of age. Retinal extracts were prepared from wildtype littermates and transgenic animals (lanes 1 and 2, respectively). Amount of extract run on gel was equivalent to 5% of the entire retina. Increasing amounts of purified BAG-1 or Bcl-2 protein were used to compare the overall level of transgene expression (lanes 3 through 7). The total expressed protein is estimated to be approximately 400 to 800 ng per retina for both the BAG-1 and the Bcl-2 transgenes. (B) Western blot analysis using retinal extracts from single (RhBAG-1B and Bcl-2B, lane 1) and double-transgenic animals (RhBAG-1B/Bcl-2B, lane 2) at P31. Amount of extract run on gel was equivalent to 10% of the entire retina.

Effect of BAG-1 and Bcl-2 against Photoreceptor Programmed Cell Death

The BAG-1 and Bcl-2 transgenes were introduced into an adRP mouse model to compare the effects of expression of BAG-1 and Bcl-2, alone and in combination, on the process of retinal degeneration. The RhBAG-1/Bcl-2 double-transgenic animals were crossed to an RP transgenic mouse model that overexpresses a truncated form of rhodopsin created by introducing a stop codon at amino acid position 334 in exon 5 of a rhodopsin transgene. Overexpression of the truncated form of rhodopsin causes nearly complete degeneration of photoreceptors by P15 through an apoptotic mechanism. Morphologic analysis was performed on retinas isolated at various times after birth to compare the effect of expression of BAG-1, Bcl-2, and the combination of BAG-1 and Bcl-2 on retinal development and disease progression. An initial time point at 3 weeks after birth was taken so that the degenerative effect produced by the overexpression of BAG-1 was minimal, and residual rod outer segments were present. The frequency of pyknotic nuclei was assessed by TUNEL analysis. Expression of BAG-1 did not slow photoreceptor cell death in the adRP mouse model at 3 weeks of age (Figs. 5A, 5B), whereas expression of Bcl-2 alone caused some inhibition of photoreceptor cell death during this time (Figs. 5C, 5D, compare thickness of the outer nuclear layer [ONL] between samples). Coexpression of BAG-1 and Bcl-2, however, was found to exert a synergistic protective effect against photoreceptor cell death caused by expression of the mutant rhodopsin transgene (Figs. 5E, 5F). The protective effect produced by the combined expression of BAG-1 and Bcl-2 was clearly greater than that produced when either protein was expressed singly.

The synergistic protective effect produced by BAG-1 and Bcl-2 against photoreceptor cell death is also demonstrated in Figure 6A. Morphometric counts of surviving photoreceptor cells in the various transgenic lines are summarized in Figure 6B. Although morphometric counts are given for only two animals for each genotype, similar results were observed for additional animals (see Fig. 5 for example) indicating that the effect is quite reproducible. Whereas expression of Bcl-2 alone was previously found to delay degeneration of the retina from 2 weeks to approximately 4 weeks of age in mice expressing mutant rhodopsin, coexpression of BAG-1 and Bcl-2 inhibited photoreceptor cell death for as long as 7 to 9 weeks (Fig. 7). The slow yet continued progress of retinal degeneration did not result from absence of transgene expression, because BAG-1 was still found to be expressed at 9.5 weeks, when assessed by Western blot analysis and at 13 weeks by immunocytochemical analysis in single RhBAG-1 transgenic retinas (data not shown). The Bcl-2 transgene should also still be expressed at this age, because both transgenes are under the control of the opsin promoter. Coexpression of BAG-1 and Bcl-2 therefore produces a marked inhibitory effect on retinal degeneration caused by expression of the mutant rhodopsin transgene.

DISCUSSION

Retinal degeneration induced by the overexpression of a truncated form of rhodopsin has previously been shown to be temporarily delayed by the ectopic expression of Bcl-2 in photoreceptors of mice. Expression of the mutant rhodopsin causes nearly a complete loss of photoreceptor cells within the
first 2 weeks of age. Overexpression of Bcl-2 reduces photoreceptor cell death significantly in the first 2 weeks (80%); however, the protective effect is temporary. The temporary effect produced by Bcl-2 theoretically results from the absence of proteins that form functional complexes with Bcl-2, such as BAG-1, that may be necessary for maximum and sustained Bcl-2 anti-cell death activity. The synergistic effect on the prevention of photoreceptor cell death that was found in the coexpression of BAG-1 and Bcl-2 supports this hypothesis.

Because overexpression of BAG-1 was found to adversely affect the normal physiology of the photoreceptor cell and led to a moderate course of retinal degeneration, the photoreceptor environment in which the mutant rhodopsin transgene was expressed was somewhat compromised when coexpressed with BAG-1. Perhaps this compromised state of the photoreceptor cell does not permit the full degenerative effect usually produced by expression of the truncated rhodopsin to be exerted. It can be argued that it is this compromised state that allows the combined expression of BAG-1 and Bcl-2 to be more effective in preventing photoreceptor cell death than when Bcl-2 is expressed alone. However, this argument is not consistent with the observation that expression of BAG-1 alone is incapable of preventing photoreceptor cell death caused by the mutant rhodopsin transgene. The combined protective effect against photoreceptor cell death produced by the coexpression of BAG-1 and Bcl-2 suggests a synergy between these two proteins in the prevention of photoreceptor cell death.

Although the coexpression of BAG-1 and Bcl-2 significantly delays the retinal degenerative process, the rescued photoreceptor cells are probably not functional with respect to visual transduction. The level of BAG-1 expression in the transgenic lines analyzed produced an adverse affect on the overall morphology of the photoreceptor cell, specifically causing disorganization and shortening of the rod outer segments. Nonetheless, the integrity of the retina was maintained, and that could be sufficient to prolong function of the cone cells. Perhaps a lower level of BAG-1 expression that did not alter the physiology of the photoreceptor cell yet still enhanced the protective effect produced by Bcl-2 would result in functional photoreceptors and retention of vision in autosomal dominant forms of RP.

A variety of genes have been ectopically expressed in the retina without causing adverse effects. Although very high levels of Bcl-2 expression can lead to retinal degeneration, expression levels of Bcl-2 comparable to those of BAG-1 in the RhBAG-1B transgenic retinas had a minimal effect on retinal degeneration. Nonetheless, the integrity of the retina was maintained, and that could be sufficient to prolong function of the cone cells. Perhaps a lower level of BAG-1 expression that did not alter the physiology of the photoreceptor cell yet still enhanced the protective effect produced by Bcl-2 would result in functional photoreceptors and retention of vision in autosomal dominant forms of RP.

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This indicates that the retina can tolerate certain levels of ectopic expression. Therefore, it was surprising that relatively low levels of BAG-1 expression caused photoreceptor cell death. This observation was also unexpected because previous reports indicate that high levels of BAG-1 expression in several cell culture systems as well as in certain tissues does not lead to cell death.\textsuperscript{22,23} Expression of BAG-1 caused a loss of photoreceptor cells within the first postnatal month; however, some photoreceptor cells survived for as long as 9 weeks (data not shown). The mechanism by which BAG-1 expression causes photoreceptor cells to die is not yet known. Many of the photoreceptors expressing BAG-1 were found to experience nuclear DNA fragmentation, evidenced by positive TUNEL staining (data not shown), suggesting that perhaps BAG-1 overexpression induces photoreceptors to die by an apoptotic mechanism. Perhaps, similar to Bcl-2, there is a minimal level of BAG-1 expression that would not cause death of photoreceptor cells. In future experiments it may be possible to introduce transgenes with inducible promoters that would permit in vivo manipulation of ectopic gene expression.

It has previously been shown that one of the isoforms of the human homologue of BAG-1 (RAP46) interacts in vitro with the nuclear hormone receptors for glucocorticoid, estrogen, and thyroid.\textsuperscript{3,2} Recently, it has been reported that the murine
BAG-1 protein expressed here also interacts with the retinoic acid receptor (RAR) and interferes with certain biologic effects induced by trans-retinoic acid. Mice that are null for RARβ2 and RARγ2 exhibit dysplasia and degeneration of the retina, indicating that the RAR signaling pathway plays a fundamental role in retinal development and maintenance. The importance of retinoic acid in photoreceptor differentiation has also been demonstrated. It is possible that the overexpression of BAG-1 in photoreceptors causes abnormal retinal morphology and degeneration by interacting with retinoic acid receptors and inhibiting activation of their downstream effectors, which are normally required for retinal development and maintenance.

The introduction of both the BAG-1 and Bcl-2 genes into photoreceptors of mice with an autosomal dominant form of RP was more effective in delaying the advance of retinal degeneration than the introduction of either gene alone. The additive inhibitory effect on the programmed cell death process produced by the combined expression of BAG-1 and Bcl-2 has also been demonstrated in cultured neuronal PC12 cells deprived of nerve growth factor. Coexpression of BAG-1 and Bcl-2 prevented the death of PC12 cells by interfering with caspase activation and with the generation of reactive oxygen species. It is not yet clear whether this is also the mechanism by which the combined expression of BAG-1 and Bcl-2 inhibits programmed cell death in the neural retina.

The programmed cell death process results in the formation of cell remnants or apoptotic bodies that are immediately phagocytosed by neighboring cells to avoid an inflammatory response. This rapid process is evident in photoreceptor cells that express the truncated rhodopsin transgene. A complete loss of photoreceptor cells occurs approximately between P11 and P15 in the RhoΔCT animals. The mechanism by which expression of the truncated rhodopsin protein leads to this rapid cell death is currently unknown. Expression of BAG-1 and Bcl-2 in photoreceptor cells expressing a defective rhodopsin transgene blocks the photoreceptor cells from completing the cell death process. Many photoreceptor cells undergo DNA fragmentation within the first month, as assessed by TUNEL analysis (data not shown). This fragmentation is thought to be a late stage in the apoptotic pathway, occurring just before the disintegration of the cell and subsequent phagocytosis. However, many photoreceptor nuclei remain relatively intact for more than 2 months. Although, the coexpression of BAG-1 and Bcl-2 may interfere with a late event in the cell death pathway initiated by the mutant rhodopsin transgene, it probably is not with the removal process of defective cells, because the surviving photoreceptor cells were not all positive for the TUNEL assay.

The enhanced protective effect against photoreceptor cell death produced by the coexpression of Bcl-2 and BAG-1 indicates that these proteins act in concert to prevent cell death and play an important role in determining cell survival. The similar distribution patterns of BAG-1 and Bcl-2 proteins in the photoreceptor cell are consistent with BAG-1 and Bcl-2 interacting and cooperating to prevent cell death. Future experiments may elucidate the mechanism by which the combined expression of BAG-1 and Bcl-2 proteins in the photoreceptor cell are consistent with BAG-1 and Bcl-2 interacting and cooperating to prevent cell death. Future experiments may elucidate the mechanism by which the combined expression of BAG-1 and Bcl-2 proteins in the photoreceptor cell are consistent with BAG-1 and Bcl-2 interacting and cooperating to prevent cell death.
greater than 9 weeks was significant. These results suggest that the coexpression of BAG-1 and Bcl-2, at the appropriate ratio and overall levels, may serve as a potential therapeutic approach to treat retinal degenerative diseases.

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References


BAG-1 and Bcl-2 Slow Photoreceptor Cell Death 1961