Photoreceptor Cells in the Vitiligo Mouse Die by Apoptosis
TRPM-2/Clusterin Expression Is Increased in the Neural Retina and in the Retinal Pigment Epithelium

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Purpose. To determine the mechanism of photoreceptor cell death in the vitiligo mouse, a model of retinal degeneration in which the genetic defect is not retina specific but is instead caused by single point mutation in the microphthalmia (mi) gene that codes for a basic helix-loop-helix DNA transcription factor.

Methods. Detection of apoptotic cells was performed in fixed retinal tissue using the TUNEL assay in animals 1, 2, 4, 6, 8, 16, 32, 40, and 52 weeks. Electron microscopic analysis was used to confirm the morphologic hallmarks of apoptosis, and Southern blot analysis was used to detect internucleosomal DNA fragmentation. Additionally, the expression of a gene associated with apoptosis, TRPM-2/clusterin, was examined.

Results. At ages beyond the time of normal retinal programmed cell death, vitiligo retinas had significantly more TUNEL-positive photoreceptor cells and more photoreceptor cells with condensed chromatin than controls. DNA internucleosomal fragmentation ladders were present in vitiligo retinas even as late as 15 weeks, a time well beyond developmental apoptosis in controls. TRPM-2/clusterin mRNA levels in vitiligo neural retinas were similar to controls initially but were two times greater than controls by 12 weeks. Surprisingly, TRPM-2/clusterin mRNA levels were elevated in the retinal pigment epithelium in the mutant; the expression at one week was two times greater than normals and remained elevated for many months, even though retinal pigment epithelial cells showed no morphologic evidence of apoptosis.

Conclusions. The morphologic and biochemical data suggest that photoreceptor cells die by apoptosis in vitiligo mice. The increased retinal TRPM-2/clusterin mRNA levels may be a direct response to these events. The increased expression of this gene in the retinal pigment epithelium, however, may reflect its role in tissue regression and membrane remodeling. Mechanisms by which the mi gene defect might result in the vitiligo retinopathy are proposed. Investig Ophthalmol Vis Sci. 1995;36:2193–2201.

The vitiligo mouse is one of several mouse mutants that has a genetic defect at the microphthalmia (mi) locus of chromosome 6.1,2 This gene codes for a basic helix-loop-helix zipper DNA transcription factor that is expressed in skin, heart, skeletal muscle, and the outer layer of developing retina.3,4 The most noticeable phenotypic features of the vitiligo mouse include a progressive depigmentation of the skin and pelage5; uneven pigmentation of the retinal pigment epithelium (RPE), and a slowly progressing degeneration of retinal photoreceptor cells.6,7 The mechanism by which photoreceptor cells die in this mouse is unknown. It has been shown that apoptosis is the common pathway that underlies photoreceptor cell death in several other genetically distinct rodent models of retinal degeneration.8–13 As a first step in understanding the manifestation of retinal degeneration in the vitiligo mouse, we examined whether photoreceptor cell death in this mutant is mediated through an apoptotic mechanism.
Apoptosis is a process of active cellular self-destruction that requires the expression of a number of genes. This is in contrast to necrosis, which is the result of massive tissue damage. Apoptosis was first characterized at the ultrastructural level by the presence of chromatin condensation and the appearance of cell fragments termed apoptotic bodies. It has been characterized more recently at the molecular level by the detection of an internucleosomal DNA fragmentation ladder on Southern blots and by in situ detection of DNA fragments using the TUNEL (terminal dUTP nick end labeling) assay. In the current study, we used both morphologic and molecular characteristics to determine if vitiligo photoreceptor cells were dying by apoptosis. Additionally, we asked whether one of the genes associated with apoptosis in a number of model systems, TRPM-2/clusterin, is expressed differently in the neural retina and RPE of the vitiligo mouse. An increase in the expression of TRPM-2/clusterin mRNA or protein has been observed in several instances of tissues undergoing apoptosis, including retinal degeneration in rd and rd/s mice, two mouse models of human retinitis pigmentosa. The results of our experiments provide strong evidence that photoreceptor cells die by apoptosis in the vitiligo mouse. This article represents the first cell biologic assessment of the mechanism responsible for the retinal phenotypic outcome of a mutation in the mi gene.

**MATERIALS AND METHODS**

**Animals**

The vitiligo (C57BL/6-mi<sup>h</sup>/mi<sup>h</sup>) mice used in this study were the offspring from our colony of breeding pairs. Age-matched, wild-type controls (C57BL/6 +/+ ) were obtained from Harlan Sprague–Dawley (Indianapolis, IN). Housing and feeding conditions of the animals have been described. Care and use of the animals adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**TUNEL, In Situ Detection of DNA Fragmentation**

Three to four eyes from vitiligo mice ages 1, 2, 4, 6, 8, 16, 32, 40, and 52 weeks and two to four eyes from C57BL/6 controls ages 1, 2, 6, 8, 10, and 40 weeks were analyzed. Mice were killed by CO<sub>2</sub>, and eyes were fixed overnight at room temperature in 4% buffered formalin. A small piece of ileum (used as positive control for the TUNEL assay) was removed from each mouse and fixed in the same manner. Eyes and intestines were dehydrated through serial alcohols, processed through xylene, and embedded together in a single paraffin block. Sections were cut at 3 μm thickness and were mounted on silanized glass slides. Slides were deparaffinized in xylene, subjected to proteinase K (Sigma, St. Louis, MO) 20 mg/ml in phosphate-buffered saline digestion for 15 minutes and were washed in four changes of distilled water for 2 minutes per wash. The TUNEL assay, terminal dUTP nick end labeling, was performed using the ApopTag in situ Apoptosis Detection Kit–Flourescein (Oncor, Gaithersburg, MD). For negative-stain control, water was substituted for the TdT enzyme in the reaction buffer of the kit. Tissues were viewed by epifluorescence using standard fluorescein excitation and emission filters. To distinguish between structures that autofluoresced versus those that were TUNEL positive, all slides were examined first with the red filter, then with the green. Autofluorescent structures were visible under both filters, whereas TUNEL-positive cells were detectable only by using the green filter. Positively labeled cells were counted in the outer nuclear (photoreceptor cell), inner nuclear, and ganglion cell layers of retina. Data were expressed as number of positively labeled cells per 1000 μm length of retinal tissue. Statistical analysis used multifactor analysis of variance to determine if there was a difference in number of TUNEL-positive cells between mutant and control mice (factors: mouse group, age, cell type). P < 0.01 was considered significant. Tukey’s paired comparison test was the post hoc statistical test.

**Electron Microscopy**

For electron microscopy, eyes were obtained from vitiligo mice (1, 2, 4, 8, 16 weeks) and from controls (1, 2, 4, 6, 8, and 18 weeks). They were fixed in 3% glutaraldehyde in cacodylate buffer; postfixed in 1% osmium tetroxide, infiltrated in Spurr resin, sectioned at 80 nm, and mounted on copper grids. Sections were stained with uranyl acetate and lead citrate and examined using a Phillips (Eindhoven, Netherlands) 400 transmission electron microscope. The analysis of tissues involved scanning sections in search of cells demonstrating the morphologic criteria of apoptosis, which included marked condensation of the nucleus and cytoplasm, nuclear fragmentation, and separation of protuberances forming on the cell surface as described by Kerr et al.

**Southern Blot Analysis of Internucleosomal DNA Fragmentation**

At each age examined, retinal dissections were performed on six eyes. The neural retinas were pooled, and samples were flash frozen in liquid nitrogen and were stored at −80°C. Methods for separating neural retina from retinal pigment epithelial–choroid complex have been described. The methodology for DNA isolation and Southern blot analysis using mouse C<sub>57</sub>B<sub>1</sub> DNA has been described. Briefly, DNA was extracted from frozen retinas of mutant and control mice ages 1, 3, 6, 8–11, and 15 weeks by homogenizing...
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Six frozen retinas in 75 µl of homogenization buffer (15 mM Tris-HCl [pH 7.4], 2 mM EDTA, 0.5 mM spermidine, 0.15 mM spermine, 60 mM KCl, 15 mM NaCl). The sample was mixed with an equal volume of 3% low-melting agarose with proteinase K (8 µg/µl) and N-lauroylsarcosine (4% wt/vol). Samples were electrophoresed and transferred to GeneScreen Plus (NEN-Dupont, Boston, MA) membrane, prehybridized, and hybridized with the mouse Cot-1 DNA probe as described.25

Northern Blot Analysis of TRPM-2 Expression

At each age analyzed, 6 to 10 eyes from normal or vitiligo mice were used. Neural retinas were dissected from the RPE, and the two samples were frozen in the same manner as described above for Southern blot analysis. The following ages were analyzed: 1, 2, 4, 6, 8, and 12 weeks, 10, 11, and 13 months. The analysis was performed three times. In each case, total RNA was extracted using RNAzol B (Tel-test, Friendswood, TX). The ethanol-precipitated RNA was electrophoresed on 1.2% agarose gels in the presence of 1.2% formaldehyde. The RNA was capillary blotted onto an Immobilon N membrane (Millipore, Bedford, MA), ultraviolet light cross-linked for 5 minutes, and hybridized with a rat TRPM-2 cDNA probe labeled with [α-<sup>35</sup>P]-dCTP using the method of Feinberg and Vogelstein.27 Prehybridization and hybridization were carried out at 42°C for 16 to 18 hours in 5× SSPE, 5× Denhardt’s, 0.5% sodium dodecyl sulfate (SDS), 50% formamide, 10% dextran sulfate, and 0.1 mg/ml salmon sperm DNA. After hybridization, membranes were washed three times in 1× SSC containing 1% SDS for 20 minutes at room temperature and twice in 0.1× SSC containing 0.1% SDS for 15 minutes at 65°C, and they were exposed to Kodak X-AR film (Eastman Kodak, Rochester, NY). The membranes were scanned overnight using an Ambis (San Diego, CA) radioanalytic imaging system to determine the radioactivity associated with the TRPM-2 message. As a control, membranes were subsequently stripped and reprobed with a probe for 18S rRNA purified from guinea pig lens (the kind gift of Dr. T. Borras). The ratio of radioactivity in the TRPM-2 band to that in the 18S control band was determined at each age in neural retina and retinal pigment epithelial samples of normal and mutant mice at various ages.

RESULTS

TUNEL Assay

Examination of retinal tissue processed for detection of 3'-OH DNA ends by the TUNEL assay revealed two major findings. First, TUNEL-positive cells were detected in the normal retinas when examined at 1 week after birth, an observation consistent with the published description of normal histogenetic cell death of the developing retina.28 The apoptotic cells were observed in the three nuclear layers of the retina, but they were most numerous in the inner nuclear layer, the retinal layer containing the second-order neurons of the visual pathway and Müller cells that have glial function. This phenomenon was observed in the 1-week mutant retinas as well. As shown in Figure 1A, approximately 20 TUNEL-positive inner nuclear layer cells were detected per 1000 µm length of retina in normals and mutants, whereas fewer than 5 per 1000 µm were observed in the outer nuclear layer or ganglion cell layer at this age in either group of
animals. Statistical analysis indicated that the inner nuclear layer contained significantly more apoptotic cells than the other layers (analysis of variance, $F = 12.09, P = 0.0007$). There were, however, no significant differences between control and vitiligo mice.

The second major finding of this assay was that at ages beyond 1 week, normal retinas had few TUNEL-positive cells regardless of the cellular layer examined. Mutant retinas, however, continued to display positive cells for many weeks. The major difference was that TUNEL-positive cells were no longer present in the inner nuclear layer but were much more prevalent in the outer nuclear layer containing the nuclei of photoreceptor cells. There was not a significant difference between controls and mutants for the number of TUNEL-positive inner nuclear layer or ganglion cells. Between the ages of 2 and 16 weeks, however, there was a significant difference in the number of TUNEL-positive photoreceptor cell nuclei (analysis of variance, $F = 7.24, P = 0.01$). These data are shown in Figure 1B. Note that at 32, 40, and 52 weeks, the number of TUNEL-positive cells was extremely low in mutant retinas and controls. By this age, the number of photoreceptor cells is greatly reduced in this mutant, though there does appear to be a phenomenon of photoreceptor cell sparing in the periphery.

Light photomicrographs illustrating these data are shown in Figure 2. The similarity in TUNEL-positive inner nuclear layer cells of 1 week normal (Fig. 2A) and mutant (Fig. 2B) mice is apparent. The shift of TUNEL-positive cells to the photoreceptor cells of the outer nuclear layer is evident in the photomicrograph of the 16-week vitiligo retina (Fig. 2C). At this age, the number of rows of photoreceptor cell nuclei in the degenerating retinas is approximately 7 to 8 (rather than 10 to 11, typical of normal retinas). A more advanced stage of the degeneration of the mutant retina is shown in Figure 2D. By this age (40 weeks), the retina is thin, the outer segments (seen autofluorescing at the top of the figure) are considerably shorter, and the number of photoreceptor cells is reduced. In this particular field of a midperipheral portion of retina, two adjacent photoreceptor cells are positive for the TUNEL assay. Interestingly, retinal pigment epithelial cells were not positive for the TUNEL assay at any age in the mutant mice or in normals. Owing to the lack of any fluorescent labeling of these cells, photomicrographs are not shown.

**High-Power Microscopic Confirmation of Apoptotic Photoreceptor Cells**

Examination of TUNEL-labeled cells at higher magnification than used in the morphometric analysis revealed photoreceptor cell nuclei in various stages of apoptosis. Figures 3A and 3B (2-week mutant retina) show the fluorescently positive photoreceptor cell nuclei with the dye segregated at the boundaries of the nucleus. Figure 3C shows a photoreceptor cell in which the nucleus appears to be dissociating from itself. Transmission electron microscopic examination of retinas revealed photoreceptor cells that had condensed chromatin in their nuclei. This feature was observed only occasionally on any given section, and the altered nuclei were typically surrounded by normal-appearing nuclei. Figure 3D shows an example of this phenomenon in a 2-week vitiligo retina. An example of peripherally condensed nuclei in the vitiligo photoreceptor cells is shown in Figure 3E. Peripheral condensation is thought to precede the formation of crescent-shaped nuclei, which precedes nuclear condensation. Although these ultrastructural features were present in vitiligo photoreceptor cells at ages older than 2 weeks, they were rarely observed inagematched control photoreceptor cells.

**Southern Blot Analysis of Neural Retina and Retinal Pigment Epithelium**

DNA isolated from neural retinas of control and mutant mice was electrophoresed and transferred to a nylon membrane. The mouse CΔ-1 DNA probe revealed a DNA ladder in the 1-week retina samples of both control and vitiligo mice. Figure 4. This observation is consistent with normally occurring active cell death in the retina. Beyond this age, laddering was not observed in DNA of control retinas but was present in the DNA of mutant retinas ages 3, 6, 8, and 15 weeks. Figure 4 shows the blots of DNA from neural retinas of vitiligo and control mice at three ages. Duplication of this experiment, which included the 6- and 8-week time points, also revealed ladders in the DNA from mutant mice retinas but not from controls (data not shown).

**Northern Blot Analysis of TRPM-2 Expression**

Northern blotting was performed using total RNA isolated from vitiligo and normal mouse neural retina (Fig. 5) and RPE (Fig. 6) using a rat TRPM-2 cDNA probe, followed by rehybridization with a probe for 18S rRNA. Quantification of TRPM-2 message (ratio of TRPM-2/18S) in these blots using an Ambis radiographic imaging system is provided in the figures. In normal mouse retina at 1 week after birth, TRPM-2 message was detected at low levels. A sevenfold to eightfold increase was observed at 2 weeks, and the level of expression remained constant through 8 weeks. At 12 weeks, there was a decrease of approximately 50%. The level of TRPM-2 message then remained at a lower level through 13 months. In vitiligo mouse retina, TRPM-2 message was barely detectable at 1 week, but by 2 weeks the level of expression was similar to that observed in normal retina, remaining similar to normal through 8 weeks. In contrast to normal retina, however, there was no decrease in TRPM-2 message at 12 weeks, and levels remained $1\frac{1}{2}$ to 2...
FIGURE 2. In situ labeling of cells undergoing apoptosis using the TUNEL assay in formalin-fixed, paraffin-embedded mouse retinas. TUNEL-positive cells are indicated at the arrow. (A) Control retina at 1-week. Most TUNEL-positive cells are in the inner nuclear layer (INL), although two positive cells are observed in this field in the ganglion cell layer (GCL). No TUNEL-positive cells are observed in the outer nuclear layer (ONL) in this field, although they were occasionally observed in other sections. (B) Vitiligo retina at 1-week. TUNEL-positive cells are most prevalent in the INL, but a few are seen in the ONL of this particular field. (C) Vitiligo retina at 16 weeks. The retina is deteriorating at this age, which is why the outer segments (OS) are disrupted and vesiculated in appearance. TUNEL-positive cells are present in the ONL but are rare in either INL or GCL. (D) Vitiligo retina at 40 weeks. The retina is more disrupted, and no outer segments remain. A few TUNEL-positive cells are observed in the ONL, but in general they were rare by this age. Inner segments (IS) were autofluorescing in these sections. Bar = 30 μm.

times above normal through 13 months. Hence, it appears that the level of TRPM-2 message expression in retina correlates with the slow loss of the photoreceptor cells.

Surprisingly, the RPE of the vitiligo mice demonstrated a very different pattern of TRPM-2 expression when compared to age-matched normal mice. As shown in Figure 6, as early as 1 week, TRPM-2 message in the vitiligo mouse RPE was approximately two times greater than normal. Expression remained above normal levels through 10 months. Similar results were obtained in two additional separate blotting experiments using different groups of mice (data not shown). Thus, an elevation of TRPM-2 message is an early event in vitiligo RPE occurring by at least 1 week of postnatal development; while in the retina, greater than normal expression of the TRPM-2 message is not observed until after 8 weeks, a time coinciding with the initial detection of photoreceptor cell loss.

DISCUSSION

There are two important findings of the current study. The first is that photoreceptor cell death in the vitiligo mouse occurs through an apoptotic mechanism. This observation is significant because it represents the first report of this process in a retinal degeneration model in which the genetic defect is not restricted to photo-
receptor cells or RPE. Two groups have reported apoptotic photoreceptor cell death in the Royal College of Surgeons rat in which the genetic defect is thought to reside in the RPE. Three groups have reported comprehensive studies demonstrating that mice with defects in photoreceptor cell-specific genes (β subunit of cGMP-PDE, peripherin-EDS, and rhodopsin) lead to photoreceptor cell death by apoptosis. In addition, TRPM-2/clusterin expression increased concomitant with photoreceptor cell death in two of these mouse models, rd and rd. Chang and coworkers proposed that, although the mechanisms of pathogenesis were different in the three models they tested, the photoreceptor cells die by a final common pathway of programmed cell death. They point out, however, that "not all forms of photoreceptor cell death occur via apoptosis."

In the current work, we have had the opportunity to examine the mechanism of photoreceptor cell death in a mouse that does not have a retina-specific genetic defect, much less a defect of photoreceptor cells. Yet, our data clearly support an apoptotic mechanism of photoreceptor cell death in this mutant with a defect in the microphthalmia (mi) gene. We have shown that there is a prolonged period of elevated TUNEL positivity in the photoreceptor cells that parallels the slow, progressive loss of the photoreceptor cell nuclei in this model. The ultrastructural analysis revealed that the features characteristic of apoptosis, as described by Kerr et al., are evident in the photoreceptor cells of the vitiligo mouse, particularly between 2 and 16 weeks. The detection of the DNA internucleosomal fragmentation ladder confirms the presence of active cell death in the neural retina of this mutant. Finally, the expression of TRPM-2/clusterin mRNA isolated from neural retina of vitiligo mice correlates with the timing of photoreceptor cell loss. Increased retinal TRPM-2/clusterin mRNA levels have
FIGURE 4. Detection of DNA laddering in retinas after Southern blot analysis and probing with cOA-1 DNA. The three left lanes represent samples of DNA isolated from normal mice at 1, 3, and 11 weeks, and the three right lanes are from vitiligo mice at 1, 3, and 15 weeks. In 1-week-old normal mice, a ladder was observed and is correlated with the programmed death of cells in the INL of normal retinas. A similar ladder is observed in vitiligo retinas at 1 week. At older ages, no laddering is observed in DNA isolated from control mice, but it is present in the vitiligo retinas at 3 and 15 weeks.

been correlated with the period of photoreceptor cell loss in other known models of retinal degeneration, such as rd, rds, and light-induced retinal damage and may, therefore, represent a common feature of photoreceptor cell death.

The second important finding of this work is that expression of the TRPM-2/clusterin mRNA was much higher in the mutant RPE than in normals. It was upregulated at an age that precedes by several weeks the age when photoreceptor cell loss occurs, and it remained high for many weeks. The cause of the increased TRPM-2/clusterin is unknown, but several theories can be considered. One possible explanation for the increased TRPM-2/clusterin expression is that it reflects apoptosis in the RPE cells. Our studies of the RPE cells did not show evidence of this process. The cells were not positive for TUNEL assay, nor did they demonstrate the typical ultrastructural characteristics of apoptosis. It should be mentioned, however, that the current study examined TUNEL positivity in tissue sections. A comprehensive analysis of TUNEL-positive RPE cells in wholemount preparations might in fact reveal an occasional TUNEL-positive cell; such a finding would not be trivial. It is possible that at much later ages, the RPE cells in the vitiligo mouse die (either by apoptosis or by some other mechanism) because the retina deteriorates profoundly by 14 to 17 months.

A second explanation for increased TRPM-2/clusterin expression is that it may be accumulating in response to stress in the RPE cell. TRPM-2/clusterin is one of the gene transcripts that is upregulated in regressing, involuting, or injured tissues. It has been implicated in scavenging excess lipids from membrane debris, and it may be induced whenever there are perturbations of normal membrane structures. The RPE of the vitiligo mouse is phenotypically abnormal at birth; it is unevenly pigmented with hyperpigmented cells located adjacent to cells with sparse pigmentation. The RPE is also functionally abnormal by age 4 weeks. Phagocytosis of shed outer segment disks by vitiligo RPE cells, particularly those in the posterior area of the eye, is significantly reduced. Moreover, there is an elevation of retinoids (retinyl palmitate and retinol) in the RPE of these mutants. An accumulation of retinol may be important because of the membranolytic characteristics of this form of vitamin A. If indeed this accumulation is damaging to the RPE, it could account for increased TRPM-2/clusterin.

A third explanation for altered TRPM-2/clusterin expression is that its expression could be influenced by the presence of the mi gene product, which is a DNA-binding transcription factor that interacts with other related transcription factors, including TFEB, TFE3, and TFEC. The complex binds to the M box sequence (CATGTG, CACGTG, or CAGATG), a regu-
TRPM-2/clusterin gene expression through the M box gene regulation (enhancement or suppression) has in the normal mouse embryo at day 13.1 implicates ever, has not been determined. Its expression in the normal adult mouse eye, how-

mi expression of this gene. The number under each lane represents the ratio of AMBIS Detection of radioactivity in TRPM-2/clusterin mRNA to that of the 18S rRNA as determined using an AMBIS radiolytic imaging system.

FIGURE 6. Northern blot analysis of TRPM-2 expression in retinal pigment epithelial (RPE) samples of vitiligo (V) and control (C) mice at 1 week (wk) to 15 months (mo). Total RNA was isolated from 6 to 10 RPE samples at each age, as described in the Methods section. Blots were probed with a 32P-labeled TRPM-2 cDNA probe and with a 32P-labeled probe for 18S rRNA probe, as described in Methods. Fourteen micrograms of total RNA was applied in each lane. The number under each lane represents the ratio of AMBIS detection of radioactivity in TRPM-2/clusterin mRNA to that of the 18S rRNA as determined using an AMBIS radiolytic imaging system.

atory element that is highly conserved in the promoters of tyrosinase, tyrosinase-related protein 1, and tyrosinase-related protein 2, three major pigmentation enzyme genes, and mouse mast cell protease 6. It is possible that mi may control or influence the expression of these and other genes. The specific genetic mi defect in the vitiligo mouse is a G-to-A transition at bp 793 that leads to an Asp222Asn substitution in helix 1. This mutation does not affect the ability of subunit tetramerization or abolish the capacity for DNA binding. In fact, the vitiligo mi mutant binds DNA with a Ke that is 6% greater than that of the wild type of the protein. The net effect of the mutation on specific gene regulation (enhancement or suppression) has not been determined. We have scanned the available 5' upstream region of the TRPM-2/clusterin gene for the presence of the M box motif (CAGGTG, CATGTG, CACATG). There are seven putative M box sites; the motif and occurs at position -2207. The TRPM-

2 promoter is complex (21); thus, augmentation of TRPM-2/clusterin gene expression through the M box may represent one of many possible regulation points of this gene.

The mi gene is expressed in several tissues, including heart and skin. The precise cellular location of expression of mi in the normal adult mouse eye, however, has not been determined. Its expression in the outer (presumably pigment epithelial) layer of retina in the normal mouse embryo at day 13.1 implicates the RPE as the primary site of the vitiligo ocular defect, but this remains to be tested. A defect in mi could have far-reaching effects on normal RPE cell function, which in turn may have an impact on surrounding cell populations, notably the photoreceptor cell. Complications leading to decreased phagocytosis of shed photoreceptor disks by RPE cells, decreased production of survival factors, the abnormal expression or suppression of extracellular cell matrix components, or the expression of secreted enzymes that can distort the extracellular matrix could act as biologic signals for adjacent cells (e.g., photoreceptor cells) to undergo apoptosis.

Key Words
apoptosis, clusterin, microphthalmia, photoreceptor cells, retinal degeneration, TRPM-2, vitiligo

Acknowledgments
The original mi/mi mice breeding pairs were provided by Dr. Richard Sidman, New England Regional Primate Research Center, Southboro, MA (funded by National Eye Institute grant EY6859). The authors thank Dr. Gerald Chader for constructive criticisms of the manuscript; Dr. Paul McNeil for the use of his fluorescent microscope and Mr. Bob Khakee for training us to use this instrument; Dr. Ed Baggall and Mrs. Brenda Headrick for assistance in electron microscopy; Dr. Teresa Borras for the 18S rRNA probe; Mrs. Sue Johnson for assistance in preparing the manuscript.

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