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A unique pattern of photoreceptor degeneration in *cyclin D1* mutant mice

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ABSTRACT ***Cyclin D1*-deficient mice have small eyes with thin retinas. We observed that there was a lower level of retinal cell proliferation and a unique pattern of photoreceptor cell death. Death was first observed in scattered clusters of cells in the retina. It then appeared to spread from these few cells to nearby photoreceptors, eventually producing extensive holes in the photoreceptor layer. These holes appeared to be filled with interneurons from the inner nuclear layer. The death mainly occurred during the second to fourth postnatal weeks. Other models of photoreceptor degeneration in rodents differ in that they occur more uniformly across the retina, with death proceeding over a longer period of time until all, or nearly all, of the photoreceptors degenerate. We also tested whether expression of a *bcl-2* transgene could prevent the death and found that it could not.**

The vertebrate retina contains seven major cell types that are organized into three nuclear layers: the outer nuclear layer (ONL) comprising the nuclei of photoreceptor cells, the inner nuclear layer (INL) comprising the nuclei of interneurons and Müller glial cells, and the ganglion cell layer (1). Accompanying and following retinal cell differentiation, apoptosis can be observed in the retina (2, 3). The apoptosis presumably plays an important role in establishing the proper ratios of the cell types and in fine tuning the retinal connections. For example, differentiating photoreceptor cells that fail to migrate out of the INL to their normal position in the ONL die in the first 3 postnatal weeks (2). Photoreceptor cell death also can be caused by abnormalities in the retina, either due to a defect in the photoreceptors themselves or in other retinal cells that interact with the photoreceptors. Mutations in the phototransduction pathway components, such as rhodopsin and cGMP phosphodiesterase, can cause photoreceptor apoptosis by mechanisms that are still obscure (4–8). In such cases, photoreceptor loss occurs throughout the retina, such that eventually all photoreceptors degenerate (5). By contrast, focal degeneration can be induced in mouse retinas that are mosaic for a mutant rhodopsin and in the retinas of rats chimeric for the Royal College of Surgeons (RCS) *rd* mutation (5, 9). In these cases, the focal nature of the lesion is caused by the nonuniform distribution of the mutant cells. Human retinas also can undergo inherited degeneration in a heterogeneous fashion. In an extreme example, a 65-yr-old male with a T17M mutation of rhodopsin lost photoreceptors mainly in the lower half of his retina (10). In such a situation, mosaicism is not an obvious explanation. Similarly, most cases of human retinitis pigmentosa begin with a focal loss of visual acuity (a scotoma), although functional tests usually reveal widespread loss of rod function outside the scotoma that is below the threshold of sensation by the patients. The scotoma usually enlarges, especially in the mid-peripheral retina and may lead to destruction of the entire retina (11).

Apoptosis is a physiological or pathological cell death that occurs when a cell activates its suicide program in response to internal or external signals (12). Cells undergoing apoptosis show characteristic morphological and molecular changes, including chromatin condensation and the cleavage of chromosomal DNA at internucleosomal intervals (13). Important regulators of apoptosis include genes that promote apoptosis, such as the *ICE/Ced-3* family of proteases, and genes that inhibit apoptosis, such as some members of the *bcl-2* family (12, 14). Aberrations involving control of the cell cycle have been shown to cause apoptosis (15). For example, postmitotic neurons undergo apoptosis when they are forced to reenter mitosis because of the overexpression of *cyclin D1* or the loss of the retinoblastoma susceptibility gene *Rb* (16, 17).

Cyclin D1 is one of the three mammalian D type cyclins that promote progression through the G_1 phase of the cell cycle by activating the cyclin-dependent kinases, *cdk4* or *cdk6* (18–21). The phosphorylation of the Rb protein by these kinases allows the cell to enter the S phase of the cell cycle (21, 22). In addition to its role as an activating partner for the cyclin-dependent kinases, *cyclin D1* also can activate the estrogen receptor in a manner that is independent of the cyclin-dependent kinases (23, 24). *Cyclin D1* is highly expressed in the proliferating mouse neural retina (25). *Cyclin D1*-deficient mice show defects in eye, mammary gland, and nervous system development (25, 26). We show in this study that photoreceptor cells in *cyclin D1* mutant mice undergo apoptosis in a unique pattern.

MATERIALS AND METHODS

Mouse Strains. The *cyclin D1*-deficient mice in the B6/129 strain background were from P. Sicinski and R. Weinberg (25) and the Jackson Laboratory. The *opsin/bcl-2*-transgenic mice in the B6 and DBA-mixed background were from J. Chen and M. Simon (27). Genotypes were determined by PCR as described in (25, 27). The *or*^{J} and *Pax6* mutant mice were from the Jackson Laboratory.

[^{3}H]Thymidine Labeling. Retinal explants were cultured for 12 hr in medium containing 2 μCi/ml [^{3}H]thymidine (1 Ci = 37 GBq), as described in ref. 28.

Cell Death Assays. Retinas were dissected in DMEM (GIBCO/BRL), incubated in DMEM containing 1.6 μM acridine orange for 20 min, and examined with a Zeiss Axiophot microscope. Terminal deoxynucleotidyltransferase-

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Abbreviations: ONL, outer nuclear layer; INL, inner nuclear layer; TUNEL, terminal deoxynucleotidyltransferase-mediated dUTP end labeling; TEM, transmission electron microscopy; P, postnatal day.

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mediated dUTP end labeling (TUNEL) was performed according to manufacturer's instructions (Promega).

Electron Microscopy. For scanning electron microscopy, retinas were fixed in 4% paraformaldehyde, dehydrated, critical point dried, and examined with a Amray SEM machine (Bedford, MA) at 10 kV. For transmission electron microscopy (TEM), eyes were fixed in 4% paraformaldehyde in PBS, post-fixed in 1% OsO_4 in 0.15 M phosphate buffer (pH 7.0), dehydrated, and embedded in Durcupan ACM (araldite) (Polysciences, Warrington Park, PA). Sections were viewed with a Philips EM300 at 60 kV.

Immunohistochemistry. For whole mount staining, retinas were fixed in 4% paraformaldehyde, permeablized and blocked in PBS containing 10% normal donkey serum and 0.5% Triton X-100 (0.1% Triton X-100 for VC1.1 antibody), incubated overnight at 4°C in primary antibodies, washed with PBS, incubated for 2 hr in Texas Red-conjugated secondary antibodies, washed with PBS, and examined with a Zeiss Axiophot microscope or a Leica confocal microscope. Alternatively, retinas were sectioned at 20 μm and processed for antibody staining as described above. Sources of antibodies were: rabbit anti-recoverin, from J. Hurley (29); rabbit anti-CRALBP, from J. Saari (30); anti-neurofilament, from V. Lee (31); anti-rhodopsin and VC1.1 antibodies, from Sigma; rabbit anti-Fas-L, rabbit anti-Fas, and goat anti-tumor necrosis factor-receptor 1 antibodies, from Santa Cruz Biotechnology; and rabbit anti-tumor necrosis factor-α, from Genzyme. The secondary antibodies were from the Jackson Laboratory. The nuclear acid dye SYTO 11 was from Molecular Probes.

RESULTS

Reduction in Mitosis and Total Cell Number in *Cyclin D1* Mutant Retinas. It was reported previously that the *cyclin D1* mutant mice have hypocellular retinas (25, 26). The reduction in the number of cells in the *cyclin D1* mutant retinas could be caused by a decrease in mitotic activity, an increase in cell death, or both. [^{3}H]thymidine labeling of cells undergoing DNA synthesis was performed to determine whether the mitotic activity in the mutant retinas was lower than that of the wild-type. As shown in Fig. 1*A*, the mitotic activity was ≈threefold lower in the *cyclin D1* mutant retina relative to that of wild-type or heterozygous siblings. Quantification of the number of total retinal cells showed that the *cyclin D1* mutant retinas contained ≈one-third of the wild-type number at birth (Fig. 1*B*). Between the first postnatal day (P0) and P14, the total number of retinal cells increased >twofold in the wild-type or heterozygous mice, but in the *cyclin D1* mutant mice, there was only a slight increase (Fig. 1*B*). At P35, the difference

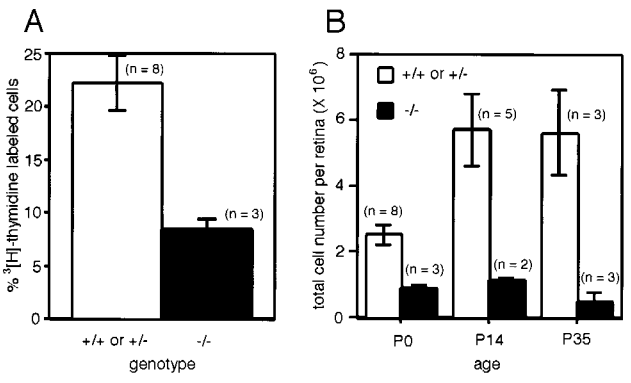

FIG. 1. Reduction in mitosis and total cell number in *cyclin D1* mutant retinas. (*A*) Neonatal (P0) retinas were labeled for 12 hr by [^{3}H]thymidine, and the percentages of ^{3}H-labeled retinal cells were scored. (*B*) The total number of retinal cells in mice of the indicated ages. Error bars show SD. The numbers in parentheses indicate the number of animals examined.

in total retinal cell number between the *cyclin D1* mutants and wild-type or heterozygous siblings increased to >tenfold (Fig. 1*B*). Because the majority of retinal cell mitosis occurs in the embryonic period (28), the >threefold further difference in the total number of retinal cells that occurred during the early postnatal period suggested that there was an increase in retinal cell death during the postnatal period in the mutants.

Increase of Photoreceptor Cell Death in the *Cyclin D1* Mutants. Consistent with the cell death hypothesis, starting at the end of the first postnatal week, the *cyclin D1* mutant retinas developed hypocellular "holes" (cf. Fig. 2 *B–D* with *A*). This process was progressive: holes were small at an early stage and increased in size in the subsequent several weeks (Figs. 2 *B–D* and 4 *A* and *B*). However, an increase in the hole size did not continue throughout the first several months. The expansion rate of the holes reduced after several weeks, so that the holes in 6-mo-old *cyclin D1* mutant retinas appeared similar to the large holes observed in 3-wk-old mutant retinas (cf. Fig. 2 *D* and *C*). The peak of the expansion of the holes was around P10, when *cyclin D1* is no longer expressed during normal development (data not shown). The initiation of the holes was not synchronous, so that during the phase when the holes were expanding, there were holes of various sizes in the same retina (Fig. 2 *B*, *C*, and *E*). There was a central to peripheral gradient in the distribution of the holes. Most of the holes were located in the central two-thirds of the retina. The peripheral one-third only occasionally developed holes (Fig. 2*B–D*).

To examine if the development of the holes in the *cyclin D1* mutant retinas was a consequence of a reduction of the total number of retinal cells, we analyzed the hypocellular retinas of the Small eye (*Pax* $6^{+/-}$) and the ocular retardation (*or*^{J}) mutant mice (32, 33). The hypocellular retinas of these mice did not develop any holes similar to those observed in the *Cyclin D1* mutant retinas (data not shown). No such pattern of retinal degeneration has been reported in previous studies on other mutants with small eyes or retinal degeneration. Therefore, this unique pattern of retinal cell loss appears to be specific to the *cyclin D1* mutant retina.

The development of the holes is not caused by the background of the particular mouse colony. The *cyclin D1* mutant mice from the Jackson Laboratory showed the same phenotype as the original stock obtained from P. Sicinski and R. Weinberg at the Massachusetts Institute of Technology (25). Eleven *cyclin D1* homozygous progeny were obtained by crossing these *cyclin D1* stocks to mice of a different strain background (B6/DBA). All of these *cyclin D1* homozygous mice developed holes in the retinas, whereas all the wild-type or heterozygous siblings had normal retinas. It is unlikely to be caused by a pathogen in our animal facility because the wild-type and heterozygous siblings and other mice raised in the same room never showed this retinal phenotype, whereas all *cyclin D1* homozygous animals showed this retinal phenotype, regardless of whether they were raised in this facility or in the Jackson Laboratory.

Ultrastructural and Immunohistochemical Characterization of the Holes in the *Cyclin D1* Mutant Retinas. Scanning electron microscopy of the mutant retinas' photoreceptor outer segment surface revealed several stages in the formation of the holes. Clusters of cells protruding into the outer segment layer were observed (small arrow in Fig. 2*E*) and were followed by the appearance of larger clusters of cells, with the center of the clusters losing cells and leading to the formation of holes (arrowhead in Fig. 2*E*; Fig. 2*F*). The continued cell loss in the holes coupled with the eruption of more cells to the outer segment layer in the periphery of the holes generated the crater-shaped holes (large arrow in Fig. 2*E*).

TEM revealed that most of the cells in the holes were INL cells. Normally, the ONL contains only rod and cone photoreceptors' nuclei. Over 90% of the photoreceptor cells are rod photoreceptor cells, which are smaller and more condensed

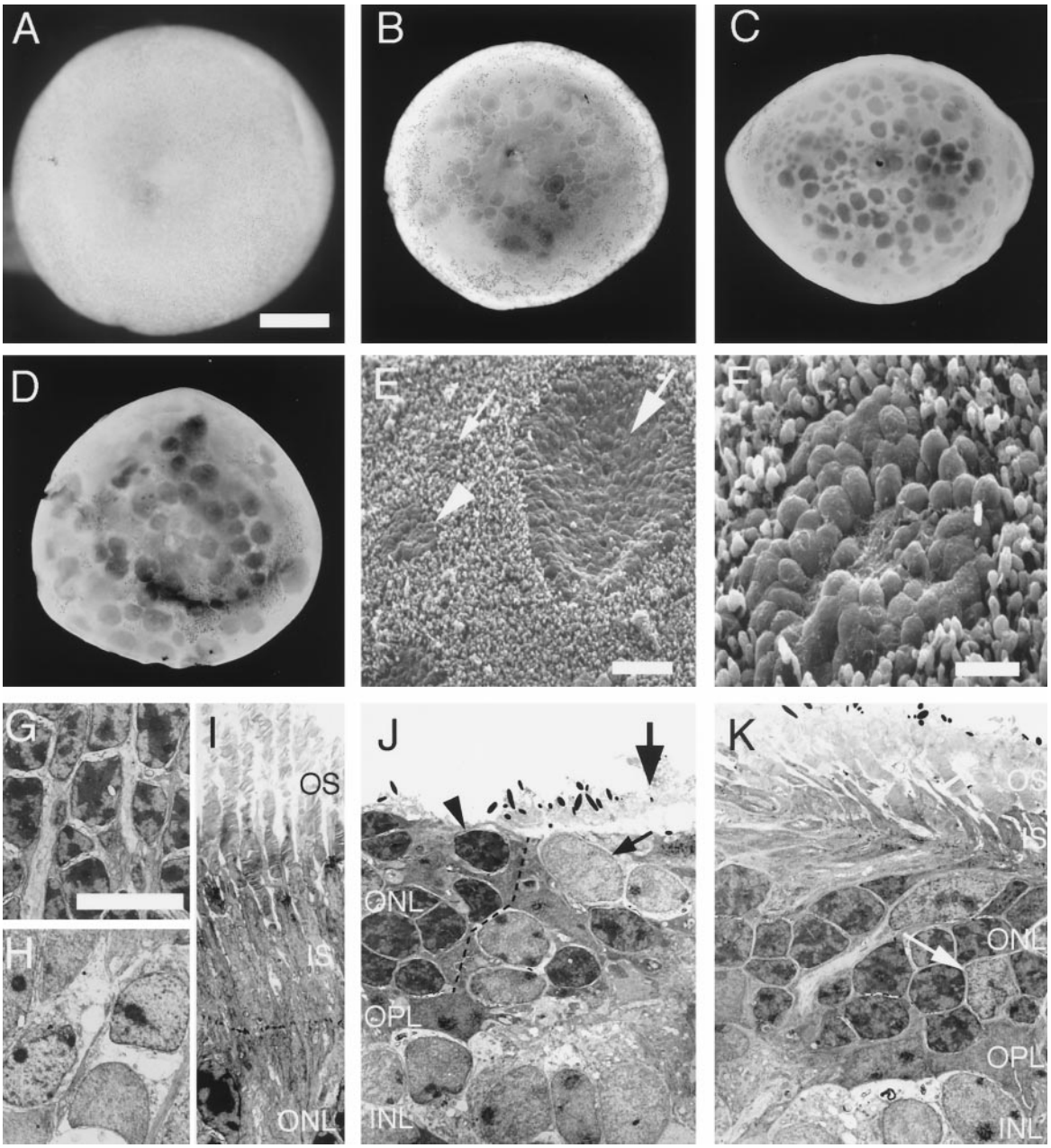

FIG. 2. Cell death in the *cyclin D1* mutant retinas. (*A–D*) Dark field images of intact retinas. (*A*) Wild-type, P10. (*B*) *Cyclin D1*$^{-/-}$, P10. (*C*) *Cyclin D1*$^{-/-}$, P21. (*C*) *Cyclin D1*$^{-/-}$, P180. (*E* and *F*) Scanning electron microscopy images of the outer segment surface of a P21 *cyclin D1*$^{-/-}$ retina. (*F*) High magnification view of the area indicated by the arrowhead in *E*. Small arrow in *E* points to a small cluster of cells that protrude into the outer segment layer. Arrowhead in *E* points to a large cluster of cells that appear to be in an expanding hole. Large arrow in *E* points to a large hole. (*G–K*) TEM images of retinal sections. (*G–I*) P21 wild-type retina. (*G*) Nuclei in the ONL. (*H*) Nuclei in the INL. (*I*) Inner and outer segments of photoreceptors. (*J* and *K*), P21 *cyclin D1*$^{-/-}$ retina. (*J*) A hole (indicated by the large arrow) and the boundary of the hole (indicated by the dashed line). Note the presence of cells with INL cell morphology (small arrow) in the hole at the ONL level and the photoreceptor cells without inner or outer segments (arrowhead) at the boundary of the hole. (*K*) An area that is not a hole. Note the presence of many pale nuclei in the ONL (arrow) and reduction in the length of the inner and outer segments of the photoreceptors. OPL, outer plexiform layer. OS, outer segment. IS, inner segment. (Scale bar in *A* is 500 μm; *A–D* are to the same scale. Scale bar in *E* is 20 μm. Scale bar in *F* is 5 μm. Scale bar in *G* is 5 μm; and *G–K* are to the same scale.)

compared with the nuclei of the cone photoreceptor cells or INL cells. In TEM images, the rod photoreceptor cell nuclei are dark and small and the INL cell nuclei are pale and large (cf. Fig. 2 *G* and *H*). In the holes of the mutant retinas, most of the nuclei were large and pale, appearing to be INL nuclei dislocated to the ONL (large arrow in Fig. 2*J*). The photoreceptor cells around the holes were without inner or outer segments, and their nuclei were more condensed than those in areas without holes or in wild-type retinas (arrowhead in Fig. 2*J*). Loss of the outer segments is a hallmark of photoreceptor degeneration (34), and nuclear condensation is a hallmark of apoptosis (13). These observations were consistent with the loss of cells at the edge of the holes and thus the expansion of the holes. Even in areas without holes, there were many pale nuclei in the ONL in the *cyclin D1* mutant retinas (Fig. 2*K*), indicating that there was either an increase in the percentage of cone photoreceptor cells or the presence of INL cells in the ONL. Immunohistochemical analysis revealed that at least some of these pale nuclei were nuclei of the INL cells (see below). The length of the inner and outer segments of pho-

toreceptor cells in the *cyclin D1* mutant was only ≈one-third that of the wild-type siblings (cf. Fig. 2 *K* with *I*), indicating perhaps a differentiation defect of the photoreceptor cells in the *cyclin D1* mutant.

Immunohistochemical analysis with retinal cell type-specific antibodies confirmed the absence of both rod and cone photoreceptors in the holes. Both rod and cone photoreceptors express the calcium-binding protein recoverin (Fig. 3*A*) (29). In *cyclin D1* mutant retinas, recoverin was expressed in photoreceptors but was not detected in the holes (Fig. 3*B*). Near the holes, the recoverin-positive photoreceptors tapered off toward the holes, indicating a gradual loss of photoreceptor cells spreading outward from the holes (Fig. 3*B*). Staining with an antibody recognizing the rod photoreceptor-specific product, rhodopsin, revealed the same pattern (Fig. 3*C*). Horizontal cells, which can be recognized by an antibody against neurofilament, also were not observed inside the holes (Fig. 3*D*). Consistent with the TEM observations, the holes appeared to be filled by INL cells, and there was an increase of cells that stained with the amacrine cell marker, VC1.1 (Fig. 3 *E* and *G–I*). At the early stage of hole formation, when clusters of cells protruded into the outer segment layer, most of the cells in the clusters were VC1.1-negative (Fig. 3*F*). The subsequent expansion of the holes was accompanied by the accumulation of VC1.1-positive cells inside the holes (Fig. 3*G*). The high density of VC1.1-positive cells inside the holes was associated with a reduction of VC1.1-positive cells adjacent to the holes, suggesting that the abundance of VC1.1-positive cells inside the holes was at least in part caused by the migration of VC1.1-positive cells into the holes from the surrounding tissue (Fig. 3 *E* and *G–I*).

As Müller glial cells have been proposed to perform a maintenance function in the retina (35–37), the formation of the holes could be a consequence of a lack of Müller glial cells. Staining with an antibody against the Müller glial cell-specific protein, CRALBP, revealed the presence of an approximately normal density of Müller glial cells in the mutant retinas, even in the areas that developed holes (Fig. 3*K*). Therefore the holes were unlikely to be caused by a simple lack of Müller glial cells. However, it is still possible that the Müller glial cells in the *cyclin D1* mutant retinas were abnormal, and this problem underlies photoreceptor degeneration. The location of the cell bodies of the Müller glial cells in the *cyclin D1* mutant retinas was abnormal. In the wild-type retinas, Müller glial cell bodies were located in the middle of the INL (arrow in Fig. 3*J*). In the *cyclin D1* mutant retinas, the cell bodies of many Müller glial cells were abnormally located in the ONL (arrows in Fig. 3*K*), consistent with the observations by TEM described above (Fig. 2*K*).

Apoptosis of Photoreceptor Cells Leads to the Formation of Holes in the ONL. Acridine orange staining confirmed that cell death accompanied the development of the holes. At the end of the first postnatal week, clusters of cells were labeled by acridine orange (Fig. 4*A*). The development of such cell clusters was followed by the appearance of rings of cells that stained with acridine orange around the holes (Fig. 4*B*). This pattern of acridine orange staining was not observed in wild-type retinas (Fig. 4*C*). TUNEL assay revealed that there were TUNEL-positive cells surrounding the holes, indicating that the cell death was through the apoptosis pathway (Fig. 4*D–H*). We crossed the *cyclin D1* mutant mice to transgenic mice carrying the apoptosis inhibitor *bcl-2* under the control of the rhodopsin promoter (*opsin/bcl-2*) (27), to generate *cyclin D1*$^{-/-}$; *opsin/bcl-2* mice. Six *cyclin D1*$^{-/-}$; *opsin/bcl-2* mice were examined. The *bcl-2* transgene failed to reduce the apoptosis in the *cyclin D1* mutant retinas (Fig. 4*I*). This *bcl-2* transgene was shown to reduce photoreceptor apoptosis caused by expression of mutant opsin in photoreceptors, mutation in the cGMP phosphodiesterase gene, and exposure to constant light in albino mice (27). Its failure in reducing the

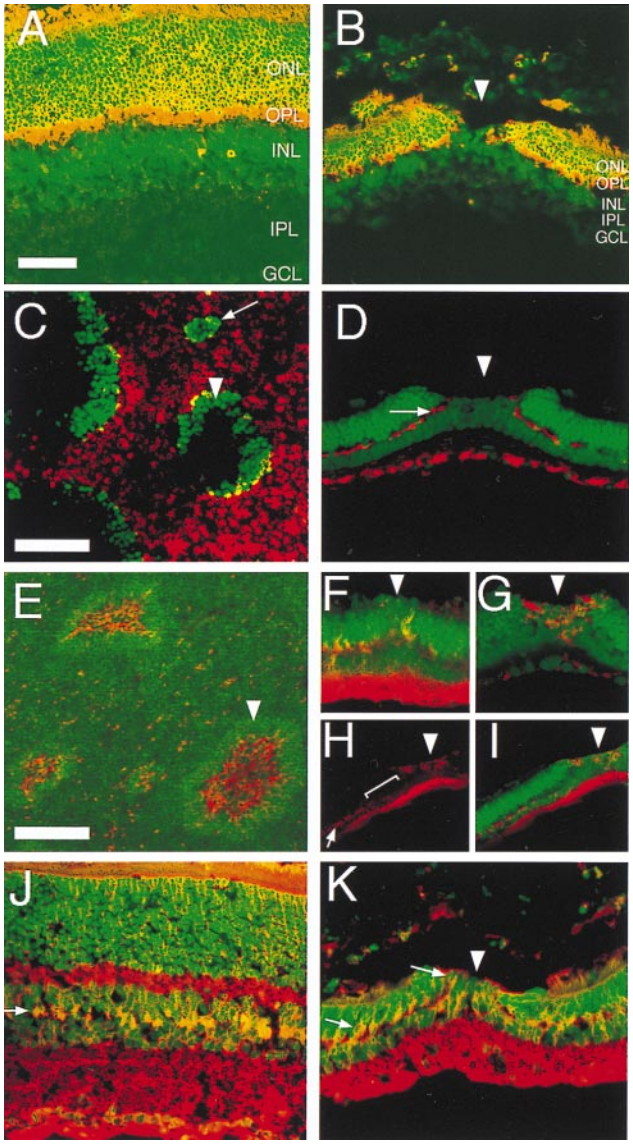

FIG. 3. Immunohistochemical characterization of holes in the *cyclin D1* mutant retinas. (*A* and *J*) Sections of P21 wild-type retinas. (*B*, *D*, *F–I*, and *K*) Sections of P21 *cyclin D1*$^{-/-}$ retinas. (*C* and *E*) Whole mount of P21 *cyclin D1*$^{-/-}$ retinas. Red, signals of antibody staining; green, nuclei. Arrowheads point to examples of holes. (*A* and *B*) Anti-recoverin; note the absence of recoverin-positive cells in the hole *B*. (*C*) Anti-rhodopsin, showing the outer segment surface of a mutant retina; note the absence of rhodopsin inside the holes. The arrow points to a small cell cluster that protrudes into the outer segment layer. (*D*) Anti-neurofilament; note the absence of neurofilament-positive cells in the hole. The arrow points to the neurofilament-positive horizontal cells outside the hole. (*E–I*) VC1.1 antibody; note the high density of VC1.1-positive cells inside the holes and reduction of VC1.1-positive cells adjacent to the holes. (*E*) A whole mount retina stained with VC1.1 antibody. (*F–I*) VC1.1 antibody staining of sections. (*F*) A hole at early stage. (*G*) A hole at late stage. (*H*) VC1.1 antibody staining of a hole at low magnification. Arrow in *H* points to the VC1.1-positive cells at a distance to the hole. Bracket in *H* indicates the area that contains fewer VC1.1-positive cells. (*I*) Merged image of the VC1.1 antibody staining in *H* and the nuclear stain. (*J* and *K*), anti-CRALBP; note the location of the CRALBP-positive Müller glial cells in the middle of the INL in the wild-type (arrow in *J*) and the abnormal location of the Müller glial cells in the ONL in the *cyclin D1*$^{-/-}$ mutant (arrows in K). OPL, outer plexiform layer. IPL, inner plexiform layer. GCL, ganglion cell layer. (Scale bar in *A* is 50 μm; *A*, *B*, *J*, and *K* are to the same scale. Scale bar in *C* is 50 μm; *C*, *D*, *F*, and *G* are to the same scale. Scale bar in *E* is 25 μm; *E*, *H*, and I are to the same scale.)

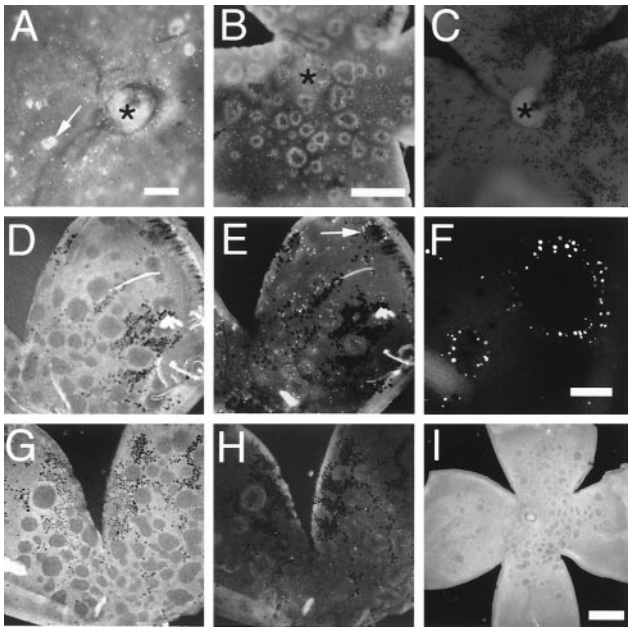

FIG. 4. Apoptosis in the *cyclin D1*$^{-/-}$ mutant retinas. In all panels, the photoreceptor side of the whole mount retina is shown (vitreous side facing down). (*A–C*) Acridine orange staining. (*A*) *Cyclin D1*$^{-/-}$, P6. Arrow points to a cluster of acridine orange-positive cells. (*B*) *Cyclin D1*$^{-/-}$, P10; note the rings of acridine orange-positive cells and some scattered acridine orange-positive cells. (*C*) Wild-type. Asterisks indicate the optic nerve head. (*D–F*) TUNEL assays of P21 *cyclin D1*$^{-/-}$ mutant retinas. (*D*) Dark field image of the retina. (*E*) TUNEL assay viewed under UV illumination; note the presence of TUNEL-positive cells at the edge of the holes. One of the holes is indicated by the arrow and is shown at a higher magnification in *F*. (*G* and *H*) Negative control for TUNEL assay (without adding terminal transferase in the assay). (*G*) Dark field image. (*H*) UV illumination. (*I*) P10 *cyclin D1*$^{-/-}$; *opsin/bcl-2*. Note the presence of holes. Black speckles in retinas were cells or pigment granules from the pigmented epithelium that adhered to the retinas. (Scale bar in *A* is 100 μm. Scale bar in *B* is 250 μm; *B*, *C*, *D*, *E*, *G*, and *H* are to the same scale. Scale bar in *F* is 50 μm. Scale bar in *I* is 500 μm.)

photoreceptor cell death in the *cyclin D1* mutant mice indicates a different cell death mechanism, although it is possible that the dosage of the transgene is not sufficient to prevent the cell death in the *cyclin D1* mutant.

The outward expansion of the holes suggests an inductive mechanism of cell death. It is possible that the cells in the holes, or the dying cells at the edge of the holes, secrete death-inducing factors to induce apoptosis of the neighboring cells. We examined the expression of the death-inducing factors, Fas ligand and tumor necrosis factor-α, and their receptors, Fas and tumor necrosis factor receptor 1 (12). We did not find any evidence of their overexpression in the cells in or around the holes (data not shown).

DISCUSSION

We report here a unique pattern of photoreceptor degeneration in *cyclin D1*-deficient mice. Unlike other kinds of photoreceptor degeneration in rodents, which occur more or less uniformly across the entire retina, the *cyclin D1*-associated photoreceptor degeneration occurs in a sporadic and focal fashion. There appears to be a temporal and spatial pattern for the degeneration: it mainly occurs within a small time window (postnatal second to fourth week) and in a central-peripheral gradient. The time window of photoreceptor degeneration overlaps with the time window of normal photoreceptor cell death during development (2). It is possible that there exists a common control on both cell death processes. The central–peripheral gradient of photoreceptor degeneration in the *cyclin D1* mutant retinas may be correlated with the central–peripheral gradient of retinal maturation (38, 39). Because the *cyclin D1* mutant mice also show neurological abnormalities, it is possible that cell death occurs in other brain regions where the *cyclin D1* gene is normally expressed (25). Focal loss of retinal cells is not unprecedented. In an infant with "leopard spot" retinopathy and Warburg syndrome, focal thinning of the INL led to variation in retinal thickness that allowed the retinal pigmented epithelium to show through as small dark spots (40). In that case, however, the photoreceptors were normal, unlike the phenotype of the *cyclin D1* mutant mice.

Although this pattern of photoreceptor degeneration is specific to the *cyclin D1* mutant, it is not obvious how the lack of *cyclin D1* function causes photoreceptor degeneration. The peak of the degeneration is at a time when the normal expression of the *cyclin D1* gene is absent, so *cyclin D1* cannot normally play a direct role in the prevention of degeneration at this time. It may be that the *cyclin D1* gene is required earlier in development to prevent the subsequent degeneration. The short photoreceptor inner and outer segments in the *cyclin D1* mutant suggest a developmental defect of the photoreceptor cells. It was reported previously that disturbing the cell cycle machinery can lead to cell death (15–17). The cell death can happen when the cell cycle is either inappropriately accelerated or inappropriately blocked. Ectopic expression of the cell cycle-promoting gene *cyclin D1* in postmitotic neurons, or loss of the cell cycle inhibitory gene *Rb*, can cause apoptosis (16, 17). Overexpression of some oncogenes can also lead to apoptosis (41). For example, overexpression of SV40 T antigen under the control of the rhodopsin promoter or HPV16 E7 under the control of the IRBP promoter causes rod photoreceptor degeneration (42, 43). On the other hand, overexpression of *p27*, a negative regulator of cell cycle progression, also can cause apoptosis (44, 45). It has been reported that *cyclin D1* is a mediator of neuronal apoptosis (17). Our results show that photoreceptor neurons lacking the *cyclin D1* gene degenerate, indicating that *cyclin D1* is not a universal mediator of neuronal apoptosis. It is possible that in the absence of *cyclin D1*, the other closely related D type cyclins, *cyclin D2* and *cyclin D3*, can replace *cyclin D1* as the mediator.

What triggers the photoreceptor degeneration? The *cyclin D1* mutant retinas contain Müller glial cells in the ONL. It has been shown that Müller glial cells secrete ciliary neurotrophic factor that can inhibit rod photoreceptor differentiation (46, 47). It is possible that some factors secreted from the Müller glial cells cause the photoreceptor degeneration. Another possible cause of photoreceptor degeneration is improper interneuronal connections. However, electroretinograms showed that the *cyclin D1* mutant retinas were able to respond to light, indicating that retinal neurons are at least approximately properly connected (25).

What causes the expansion of the holes? The holes in the *cyclin D1* mutant retina contain a high density of amacrine cells. The high density of amacrine cells in the holes may create an environment that is detrimental to photoreceptor cells. The apparent inductive mechanism underlying the expansion of the holes is intriguing. Cone photoreceptors degenerate in animals with mutations in genes expressed only in rod photoreceptors, such as mutations in the rhodopsin gene (5). This phenomenon also can be observed in certain forms of retinitis pigmentosa, a disease of rod photoreceptors in humans, and in Stargart's disease, a form of macular degeneration of central retinal cones induced by a mutation of the *ABCR* gene that is expressed in rods (11, 48–51). Both the induction of death in neighboring photoreceptors and the arrest of death reported here are thus of great interest, with respect to understanding and potentially controlling retinal degeneration in humans.

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