Calcium-induced Calpain Mediates Apoptosis via Caspase-3 in a Mouse Photoreceptor Cell Line*

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The rd mouse, an accepted animal model for photoreceptor degeneration in retinitis pigmentosa, has a recessive mutation for the gene encoding the β-subunit of the rhodopsin (5). Extensive studies of retinal degeneration in an rd photoreceptor model of photoreceptor degeneration leads to rapid photoreceptor degeneration, which is postulated to be the cause of cell death in the rd mouse (8). Although the molecular mechanisms underlying the apoptotic pathway in the rd model remain unknown, there are two possible (but not mutually independent) triggering mechanisms: one is Ca^{2+} overload, and the other is the generation of reactive oxygen species.

This article has been withdrawn by the authors after they discovered that Figs. 6 and 8 erroneously have panel duplications. A corrigendum cannot be issued by replacement with the correct panels as the original digital figures from prior to 2004 are no longer available. Although the overall conclusions of the study were validated by other approaches in the publication (i.e. Figs. 5, 7, and 9) and have since been confirmed by other investigators (e.g., Sanvicencs, N., Gómez-Vicente, V., Masip, I., Messeguer, A., and Cotter, T. G. (2004) Oxidative stress-induced apoptosis in retinal photoreceptor cells is mediated by calpains and caspases and blocked by the oxygen radical scavenger CR-6. J. Biol. Chem. 279, 39268–39278 and Gómez-Vicente, V., Donovan, M., and Cotter, T. G. (2005) Multiple death pathways in retina-derived 661W cells following growth factor deprivation: Crosstalk between caspases and calpains. Cell Death Differ. 12, 796–804), the authors are voluntarily withdrawing the article to respect the highest standards of transparency and reliability of research.
rd mouse. Based on that and other observations in various systems, we propose the following pathway of apoptosis in photoreceptor degeneration (Fig. 1): an increase in intracellular Ca\(^{2+}\) activates calpain, a Ca\(^{2+}\)-dependent cysteine protease (17), which can cleave the proapoptotic Bcl-2 family protein bid. Interaction of truncated bid (t-bid) with the mitochondrial permeability transition pore (PTP)\(^{2}\) causes mitochondrial membrane potential loss (\(\Delta \Psi _{m}\)), leading to the release of cytochrome c. The increase in cytoplasmic cytochrome c catalyzes the activity of the apoptosome (18), leading to the activation of caspases, which cleave downstream death substrates and acti-

In this study, we first used 661W photoreceptor cells exposed to the Ca\(^{2+}\)-activated apoptotic pathway outlined in the hypothesis. To further the Ca\(^{2+}\)-activated apoptotic systems, we propose the following pathway of apoptosis in photoreceptor degeneration (Fig. 1): an increase in intracellular Ca\(^{2+}\) activates calpain, a Ca\(^{2+}\)-dependent cysteine protease (17), which can cleave the proapoptotic Bcl-2 family protein bid. Interaction of truncated bid (t-bid) with the mitochondrial permeability transition pore (PTP)\(^{2}\) causes mitochondrial membrane potential loss (\(\Delta \Psi _{m}\)), leading to the release of cytochrome c. The increase in cytoplasmic cytochrome c catalyzes the activity of the apoptosome (18), leading to the activation of caspases, which cleave downstream death substrates and acti-

Materials and Methods

Animals—The rd mouse line was generously provided by Dr. Debra Farber (Jules Stein, UCLA), and wild type animals with the same genetic background (C57BL/6) were purchased from Taconic Farms (Germantown, NY) and maintained in the Animal Facility at the Medical University of South Carolina with food and water ad libitum. Animals were handled in accordance with institutional guidelines and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

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Measurements of Intracellular Calcium—The intracellular levels of Ca$^{2+}$-sensitive fluorescent dye Fluo-3 (4 mmol/liter) in a dye loading buffer (Hanks' buffer without phenol red + 20 mmol/liter HEPES and 1% bovine serum albumin) and incubated at 37 °C for 1 h. After incubation, the cells were washed three to four times with the wash buffer (Hanks' buffer and 20 mmol/liter HEPES), loaded with the ionophore A23187, 8-Br-cGMP, or IBMX with varying concentrations in different wells, and intracellular Ca$^{2+}$ levels were assayed in the FLIPR system equipped with an 488 nm excitation filter and 570 nm emission filter. MTT Assay—Cells were washed twice with cold phosphate-buffered saline (PBS) after appropriate treatment periods and incubated in culture medium with 0.5 mg/ml MTT dye (Sigma) for 2 h. After aspiration of the medium, the dark blue crystals formed were dissolved with 0.1 N HCl in isopropyl alcohol, and absorbance was measured at 570 nm (background wavelength 630 nm) using a spectrophotometer. Results are presented as percentage of survival, taking control as 100%.

Detection and Quantification of Apoptosis—To detect and quantify the number of cells undergoing apoptosis, cells were harvested after appropriate treatments and washed in cold PBS, recentrifuged, and resuspended in annexin binding buffer (50 mmol/liter HEPES, 700 mmol/liter NaCl, 12.5 mmol/liter CaCl$_2$, pH 7.4) and stained with Alexa Fluor 488 annexin V and propidium iodide dyes (Apoptosis Assay Kit, Molecular Probes). Cells were then followed by flow cytometric analysis on a FACScan instrument maintained by the Medical University of South Carolina core facility, measuring the fluorescent emission at 530 nm and 575 nm to detect and quantify the population of live and apoptotic cells.

Immunocytochemistry Analysis—Cells were fixed in 4% paraformaldehyde in PBS, pH 7.4, on chamber slides after the appropriate treatment periods. Primary antibodies were added in PBS-TX (0.1% Triton X-100 (TX)) for 2 h at room temperature. The primary antibodies, rabbit polyclonal anti-activated caspase-3 (1:200, Biosource), rabbit polyclonal anti-cytochrome c (1:100, BIOSOURCE International), mouse monoclonal anti-cytochrome c (1:100, Santa Cruz Biotechnology) and a sheep polyclonal anti-cyt c (1:100, BIOSOURCE International) were incubated with the respective substrates Ac-LLY-AU and 5-AFC and z-DEVD-AFC. A multifold increase in both activity levels was observed at various time points. Calpain activation could be measured using JC1 dye (Molecular Devices). The cells were seeded in a black wall 96-well plate with a correction wavelength at 540 nm and 575 nm to detect and quantify the population of live and apoptotic cells.

**Western Blot Analysis**—To detect the expression of calpain, rabbit IgG (Jackson Immunoresearch Laboratories) and monoclonal anti-cytochrome c (Bio-Rad) were visualized using a Nikon microscope and a digital camera driven by Axioscope software.

**Calpain and Caspase-3 Activity in the rd Mouse Retina**—To identify the mitochondrial and cytosolic fractions, cells were fractionated as described in the Apoalert cell fractionation protocol (Clontech). The pelleted cells were resuspended in fractionation buffer mix (including 500X protease inhibitor mixture and 1 mol/liter dithiothreitol), incubated on ice for 10 min, homogenized, and centrifuged at 10,000 × g for 25 min at 4 °C. The supernatant was collected, and the protein concentration was determined using a cytochrome c immunoassay (R&D Systems). Equal amounts of protein from the cytosolic and enriched mitochondrial fractions were plated in microplate wells containing 100 μl of substrate solution. The plate was incubated for 30 min at room temperature, 100 μl of stop solution was added, and the plate was read at A$_{550}$ nm (correction wavelength at 540 nm). Results are expressed as a measure of cytochrome c concentration (ng/ml) as arbitrary units compared with the untreated controls, in the mitochondrial and cytoplasmic subcellular fractions of the cells.

**Statistical Analysis**—All experiments described were performed at least in triplicate. Statistical significance was determined using Student’s t test, with a significance level of 𝑝 < 0.05.

**RESULTS**

**Calpain and Caspase-3 Activity in the rd Mouse Retina**—To establish the cornerstones of our hypothesis of the apoptotic pathway in the rd mouse photoreceptors, calpain and caspase-3 activities were measured in rd and wild type mouse retinas. As shown in Fig. 2A, calpain activity in the rd mouse retina was elevated at P7 compared with wild type retinas; activity levels peaked by P10; and after completion of degeneration of photoreceptors (P21), the activity levels were comparable with those found in the wild type mouse retina. Similarly, caspase-3 levels (Fig. 2B) were elevated in the rd mouse retina starting at P7, peaked at P15, and dropped to wild type levels by P21. The
mice, in parallel with the massive Ca\(^{2+}\) influx observed in the rd retinas beginning at P5 and stayed elevated until P17 (12). The markedly increased activity levels of intracellular Ca\(^{2+}\) in 661W cells were measured by Fluo-3 dye using the FLIPR method. Intracellular Ca\(^{2+}\) levels were monitored after a 2-h incubation at 37°C with 0.5 mg/ml MTT dye. Cell viability was decreased 40% (±5.8%) by 24 h after a 5 mmol/liter ionophore treatment. C, a significant decrease in cell viability was also observed after treatment with 8-Br-cGMP or IBMX for different time intervals. Pretreatment with 100 μmol/liter SJA6017 completely attenuated the ionophore-induced cell death (92.2 ± 3.4%), whereas 2 μmol/liter z-devd-fmk offered only partial protection (72 ± 5.8%). A similar increase in cell viability after 24 h with SJA6017 and z-devd-fmk pretreatment was observed in the case of 8-Br-cGMP (88.8 ± 4.5% and 72.3 ± 3.4%) and IBMX (90.4 ± 5.6% and 73 ± 4.6%) with SJA6017 and z-devd-fmk pretreatment, respectively. The results are shown as a percentage of control values (arbitrarily set at 100%). All experiments were performed in triplicate; mean and data are presented as the mean ± S.E.

Calcium-induced Apoptosis in 661W Cells—After having established in vivo that both calpain and caspase-3 are activated in the rd retina, we sought to establish a photoreceptor cell culture model to investigate the mechanisms of Ca\(^{2+}\)-induced cell death. For this purpose, 661W cells were treated with A23187, 8-Br-cGMP, or IBMX, all of which were used to mimic the massive Ca\(^{2+}\) influx observed in the rd mouse model. Intracellular Ca\(^{2+}\) levels were measured by loading the 661W cells with the Ca\(^{2+}\) indicator Fluo-3 and measuring the fluorescence changes after treatment with the compounds by the FLIPR method. 661W cells showed a multifold increase in the intracellular Ca\(^{2+}\) after treatment with A23187, 8-Br-cGMP, or IBMX compared with the untreated controls (Fig. 3A). Cell viability in response to the prolonged rise in intracellular Ca\(^{2+}\) was measured using the colorimetric MTT assay. 661W were found to undergo cell death in a time- and dose-dependent manner after treatment with A23187 (Fig. 3B). The 5 μmol/liter dose of ionophore A23187, which caused 40 ± 5.8% cell death after 24 h, was chosen for all further experiments. We will refer to the 5 μmol/liter calcium ionophore treatment as A23187 or ionophore treatment. Likewise, when 661W cells were subjected to 1 mmol/liter 8-Br-cGMP or 1 mmol/liter IBMX for different time periods, a pattern of cell death was observed similar to that seen for the Ca\(^{2+}\)-ionophore (57.7 ± 2.3% and 66.7 ± 4.8% cell death, respectively) (Fig. 3C). To confirm that ionophore-induced cell death in 661W cells was associated with apoptosis, flow cytometry analysis of fluorescently labeled annexin V binding (plotted on each x axis of Fig. 4) and propidium iodide uptake (plotted on each y axis of Fig. 4) was performed. The untreated cells remained viable as observed in the lower left panel (R1) after 18 h (Fig. 4A) and 24 h (Fig. 4B). Ionophore-treated cells underwent apoptosis as seen by a shift toward the lower and upper right panels (R2 and R4) of Fig. 4, C and D, with a considerable increase in the population of annexin-positive cells showing that 25 ± 2.4% of the cells underwent apoptotic cell death after 18 h, and 40 ± 4.6% underwent apoptotic cell death after 24 h, respectively.

Involvement of Calpain and Caspases in Ca\(^{2+}\)-induced Apoptosis—To confirm that the same proteases that are activated in the rd mouse retina (i.e. calpain and caspase-3) were
also activated by Ca\textsuperscript{2+} influx in 661W cells, pretreatment with both the enzyme inhibitors was used for the cell survival assays, enzymatic activity assays, and immunocytochemistry.

To examine the effects of the calpain inhibitor SJA6017 and the caspase-3 inhibitor z-devd-fmk on the Ca\textsuperscript{2+} influx-induced cell death, cells were pretreated with these inhibitors, and the cell viability was measured by MTT assay. Inhibiting calpain by SJA6017 significantly increased the cell survival (Fig. 3D) compared with z-devd-fmk pretreatment (92.2 \pm 3.4\% and 72 \pm 5.8\% cell viability, respectively) after A23187 treatment for 24 h. The cell death triggered by 5 \textmu mol/liter A23187 could be attenuated by SJA6017 in a dose-dependent manner (data not shown), with a maximal effect at 100 \textmu mol/liter, but only a partial rescue could be achieved with z-devd-fmk pretreatments (Fig. 3D), with a maximal effect at 2 \textmu mol/liter. These two doses for both inhibitors were subsequently used for all experiments and will therefore only be referred to by name, excluding the dose. Likewise, cell viability was significantly higher after SJA6017 pretreatment (88.8 \pm 4.5\% and 90.4 \pm 5.6\%) than z-devd-fmk (72.3 \pm 3.4\% and 73 \pm 4.6\%), when cells were exposed to 8-Br-cGMP or IBMX for 24 h. Similarly, using annexin V binding and propidium iodide uptake, pretreatment with SJA6017 (Fig. 4, E and F) was found to offer complete protection (6.8 \pm 1.8\% and 8 \pm 2.2\% apoptotic cells, respectively) from the ionophore-induced apoptotic cell death, whereas z-devd-fmk pretreatment (Fig. 4, G and H) offered only a partial rescue at both the time points (17.7 \pm 2.3\% and 24 \pm 4.0\% apoptotic cells, respectively). Because caspase-3 activation is a relatively late event in the apoptotic cascade, a significant number of cells may already be in the late stage of apoptosis, detectable by annexin V staining (Fig. 4, G and H). On the other hand, SJA6017 presumably blocks the events upstream of mitochondrial changes and the caspase cascade activation, thereby completely blocking the proapoptotic events induced by the ionophore. These findings demonstrate that raising intracellular Ca\textsuperscript{2+} by A23187, 8-Br-cGMP, or IBMX induces apoptosis in 661W cells, and prevention of calpain activation is a key event to rescue the photoreceptor cells from Ca\textsuperscript{2+} influx-induced cell death.

Enzymatic activity of the two protease systems in the cells was monitored further in the presence of inhibitors to establish the activation cascade. Fluorometric measurement of the calpain substrate Ac-LLY-AFC was used to measure calpain activity, which was activated by the ionophore, 8-Br-cGMP, or IBMX treatment and could only be inhibited by SJA6017 but not with z-devd-fmk (Fig. 5A), suggesting an upstream role of

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**Fig. 4. Ionophore-induced apoptosis in 661W cells as measured by flow cytometry analysis.** 661W cells were treated with A23187 alone and after pretreatment with SJA6017 or z-devd-fmk. Cells were subjected to flow cytometry analysis after being stained with annexin V-fluorescein (y axis) and propidium iodide dyes (x axis) following the basic culture protocol. The population of cells undergoing apoptosis is localized in R2 and R4, representing annexin-positive cells for phosphatidylserine externalization, whereas the viable population of cells is observed in R1. The untreated, viable cells are predominantly present in R1. Cells treated with the A23187 ionophore for 18 h (C) showed a shift of 25 \pm 2.4\% cells toward apoptosis, indicative of the uptake of annexin V and signifying apoptosis. The shift was enhanced further by a 24-h treatment with ionophore (D), at which time 40 \pm 4.6\% of the cells were annexin-positive. Pretreatment with SJA6017 offered complete protection from the ionophore-induced apoptosis with only 6.8 \pm 1.8\% (E) and 8 \pm 2.2\% (F) annexin-positive cells, whereas z-devd-fmk offered only partial protection with 17.7 \pm 2.3\% (G) and 24 \pm 4.0\% (H) cells undergoing apoptosis. FITC, fluorescein isothiocyanate.
the Ca\textsuperscript{2+}–activated cysteine protease, calpain. Conversely, analysis of the cleavage of the caspase-3 substrate, Ac-DEVD-AFC, induced by A23187, 8-Br-cGMP or IBMX, demonstrated an increase in caspase-3 activity, which could be inhibited by both SJA6017 and z-devd-fmk (Fig. 5C). These results suggest that the Ca\textsuperscript{2+}–induced calpain activation mediates cell death by regulating caspase-3 activity in an SJA6017-inhibitable manner.

Immunocytochemistry was performed using specific antibodies for calpain and activated caspase-3 after ionophore induction of 661W cells. The untreated cells showed minimal fluorescence compared with a markedly increased signal for calpain after ionophore treatment (Fig. 6A). A similar observation was made for the presence of activated caspase-3, which, as expected, had a relatively weak fluorescent signal for untreated cells compared with the bright intense fluorescence seen in ionophore-treated cells (Fig. 6D).

Taken together, our results indicate that (i) Ca\textsuperscript{2+}–induced cell death is caused by apoptosis, involving the activation of both calpain and caspase-3; and (ii) the acute rise in Ca\textsuperscript{2+} can be reproduced by different means (Ca\textsuperscript{2+} ionophore, 8-Br-cGMP, or IBMX) resulting in comparable levels of cell death and enzyme activation. Thus, the remainder of the pathway was analyzed using Ca\textsuperscript{2+} ionophore treatment.

### Analysis of the Caspase Cascade Involved in Ionophore-induced Apoptosis

Subsequent to the initial trigger of massive Ca\textsuperscript{2+} influx, there could be two possible pathways for the initiation of the apoptotic mechanism. One of them is a Ca\textsuperscript{2+}–activated cysteine protease calpain; the other way could be caspase-8 activation. As hypothesized in Fig. 1, the effector caspase-3 could be initiated by bid cleavage-mediated mitochondrial events, which in turn are triggered by either calpain or caspase-8. To delineate this part of the pathway involved in Ca\textsuperscript{2+}–induced apoptosis in 661W cells, caspase-8 and -9 enzyme activities were examined (Fig. 5, B and D). The proposed alternate route of Ca\textsuperscript{2+}–induced caspase-8 activation was not detected in our model because no changes in the Ca\textsuperscript{2+}–induced caspase-8 activity were detected in the 661W cells (Fig. 5B) or in the rd mouse retinas (data not shown). However, ionophore treatment leads to an increase in caspase-9 activity, which was attenuated by SJA6017, but z-devd-fmk did not change the activity levels (Fig. 5D).

### Calpain Cleaved Bid-induced Cytochrome c Release from the Mitochondria

So far, the results demonstrated that iono-
**Fig. 6. Immunofluorescence analysis of ionophore-induced apoptotic components in 661W cells.** Fluorescence microscopy was used to analyze the effect of ionophore on components of the apoptosis cascade (i.e. the presence of activated caspase-3, expression of calpain, bid cleavage, and cytochrome c release) in 661W cells. Cells were treated with A23187 for appropriate time periods and incubated with antigen-specific polyclonal antibodies followed by fluorescein isothiocyanate-conjugated secondary antibodies, and observed under a fluorescence microscope. The untreated cells showed a very weak fluorescent signal for calpain compared with the cells treated with the ionophore for 3 h. SJA6017 blocked the increase in calpain, whereas z-devd-fmk failed to do so. A dramatic increase in the intensity of fluorescence in the cells treated with the ionophore for 3 h was attenuated by SJA6017 pretreatment, but not by z-devd-fmk (punctate staining) but was found to be distributed throughout the cytosol in the untreated cells. This increase in t-bid was attenuated by SJA6017 pretreatment, but not by z-devd-fmk (punctate staining) but was found to be distributed throughout the cytosol in the untreated cells.

**Fig. 7. Ionophore-induced calpain cleavage of the proapoptotic Bcl-2 member protein bid.** Truncated bid (15-kDa subunit) induced by A23187 treatment for 3 h was observed by Western blot analysis on a 12% SDS gel using a specific rabbit anti t-bid primary antibody. Bid cleavage could be blocked by pretreatment with calpain inhibitor, SJA6017, but not by z-devd-fmk. Lane 1, untreated control; lane 2, A23187; lane 3, A23187 + SJA6017; lane 4, A23187 + z-devd-fmk; lane 5, A23187.

**DISCUSSION**

Caspases and calpain represent two cytosolic proteolytic systems, which are capable of producing cleavage of various endogenous proteins. The role of caspase-3 in the execution of apoptosis in two models of photoreceptor degeneration (i.e. rd mouse and lead-induced photoreceptor apoptosis) has been generally accepted (16, 22). Yet, a recent report postulates a caspase-independent, but possibly calpain-dependent, mechanism in the rd mouse model of photoreceptor degeneration (23). By contrast, however, in both the *in vivo* and *in vitro* models used in our studies we observed the activation of both calpain and caspase-3, using a variety of techniques. It is well estab-
Pharmacological manipulations of the rd mouse retina in vivo are difficult to achieve because animals would have to be treated at a very early age (prior to P6; see Fig. 2) and are difficult to interpret because of complex interactions. For this reason, we determined the cornerstones of the apoptotic pathway in vivo and followed up the pharmacology of the pathway analysis in vitro. Using enzymatic assays, we established that both calpain and caspase-3 are in a highly activated state during the phase of photoreceptor degeneration (P7–21) in the rd mouse, and the time course is highly indicative of a sequential activation of the two respective enzymes. To be able to analyze the apoptotic pathway in vitro, it was necessary to recapitulate Ca$^{2+}$-induced apoptosis in a photoreceptor cell line. For this purpose, 661W cells, which are derived from an ocular tumor and exhibit several markers of differentiated photoreceptors (20), were exposed to compounds known to increase intracellular Ca$^{2+}$, i.e., A23187, 8-Br-cGMP, and IBMX. Subsequent to the multifold increase in Ca$^{2+}$ induced by these compounds (Fig. 3A), the 661W cells were found to undergo apoptosis in a calcium and caspase-3-dependent manner. After having established the model system, we present evidence that ionophore-induced apoptosis involves the sequential activation of the two proteases and not two parallel pathways. The obvious candidates to link the upstream protease (i.e., calpain) to the downstream protease (i.e., caspase-3) are the mitochondrial receptors for Ca$^{2+}$.

Established that several caspases are involved in, if not always essential for, executing apoptosis, but the cross-talk between calpain and caspases remains unclear. Various studies have shown that activation of calpain induced by different apoptotic stimuli precedes cell death in different cell systems. Calpains can presumably activate the apoptotic phenotype by utilizing the existing latent death machinery, mainly via involvement of the caspase cascade. Hence, we set out to investigate the cross-talk between calpain and caspase-3 via the crucial endogenous regulators of the intrinsic apoptotic pathway (e.g., the proapoptotic factors such as cytochrome c) as represented in Fig. 1.

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In the experiments reported here, ionophore was found to induce an increase in bid cleavage, which could be inhibited by the calpain inhibitor, SJA6017. Likewise, Ca$^{2+}$-ionophore treatment resulted in a collapse of the mitochondrial membrane potential, in cytochrome c release from the mitochondria (see Fig. 1). Activation of calpain is known to cleave bid (24). Once released into the cytosol, cytochrome c is postulated to promote the formation of the caspase-9-Apaf-1 complex, which forms the apoptosisosome, which in turn activates caspase-3 (18). In the experiments reported here, ionophore was found to induce an increase in bid cleavage, which could be inhibited by the calpain inhibitor, SJA6017. Likewise, Ca$^{2+}$-ionophore treatment resulted in a collapse of the mitochondrial membrane potential, in cytochrome c release from the mitochondria, and in caspase-3 activation, all three of which could be blocked by the calpain inhibitor SJA6017. Thus, it appears that in the cell culture model of photoreceptor degeneration, caspase activation proceeds through the intrinsic (mitochondrial) apoptotic pathway. A similar model of ionophore-induced calpain activation triggering intracellular changes involving the release of cytochrome c from the mitochondria has been postulated in a large cell carcinoma cell line (25). Although massive Ca$^{2+}$ influx may target the mitochondria directly, in our model we observed that inhibition of calpain activation offers protection by blocking the downstream mitochondrial events and activation of the caspase cascade, thereby ruling out a direct mechanism for Ca$^{2+}$ influx affecting the mitochondria. An alternate route of bid cleavage and mitochondrial depolarization is presumed to be via caspase-8 activity (26). However, in our model the ionophore induction did not induce caspase-8 activity in either the cell culture model (Fig. 5B) or the in vivo model of the rd mouse retina (data not shown).

The results from this study identified selective targets of increased Ca$^{2+}$ influx in the rd model of photoreceptor degeneration and delineated the biochemical mechanisms and temporal sequence of the apoptotic signaling cascade events. Our study demonstrates that extrinsic agents can block the upstream events of the apoptotic pathway regulating the progress...
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sion of retinal degeneration. The ability to modulate key regulators of apoptotic cell death in the context of a mammalian photoreceptor‐derived cell line, and more importantly, in the context of a known human retinal degenerative disorder, will be useful for developing novel therapeutic strategies.

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WITHDRAWN
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