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Gene expression in the mouse retina: The effect of damaging light

Christian Grimm, Andreas Wenzel, Farhad Hafezi, Charlotte E. Remè

Department of Ophthalmology, University Eye Clinic, Zurich, Switzerland

Purpose: High levels of visible light induce apoptotic cell death of photoreceptors, a process depending on the activation of the transcription factor AP-1. This suggests that regulation of gene expression might be important for light-induced photoreceptor cell death. We measured expression of AP-1 family members and of several apoptosis-related genes to test their potential involvement in photoreceptor apoptosis.
Methods: Wildtype and *c-fos*$^{-/-}$ mice were exposed to low (roomlight) or high levels of visible light for up to two hours. Total RNA was prepared from isolated retinas during and after light exposure. Relative mRNA levels were determined semiquantitatively using either competitive or exponential RT-PCR.
Results: Expression of *c-fos*$^{-/-}$ was upregulated by intense light as early as 15 min after lights on. Highest levels (6-fold induction) were detected at 2 h after lights off declining thereafter to basal levels 20 h after the end of exposure. *c-jun* mRNA was induced at 30 min after lights on and high expression levels (fourfold induction) persisted at least for 8 h. Similarly, expression of *caspase-1* was six to 9-fold increased at 6 to 8 h after light exposure in wildtype but not in c-fos knockout mice. The latter mice are protected against light-induced photoreceptor apoptosis. Expression of other apoptosis-related genes (*bcl-2, bcl-X$_L$, bax, bad, caspase-3*) was not affected by light exposure or the lack of c-Fos in knockout mice.
Conclusions: Expression of *c-fos* and *c-jun* mRNA is transiently induced by exposure to damaging light. Induced expression of *c-jun* persists longer than expression of c-fos. Among the apoptosis-related genes, only *caspase-1*expression was upregulated by light exposure and Caspase-1 might therefore be involved in light-induced retinal degeneration.

Exposure to high levels of visible light induces apoptotic cell death of photoreceptors [1-3]. Photons of the damaging light are absorbed by the visual pigment rhodopsin [1,4] creating an intracellular death signal that leads to activation of the transcription factor AP-1. AP-1 DNA binding activity increases as soon as 15 min after the start of light exposure, reaching a maximum at about 6 h after a 2 h illumination [5]. Activation of AP-1 is essential for light-induced photoreceptor apoptosis since transrepression of AP-1 by activated glucocorticoid receptor protects photoreceptors from light damage (A. Wenzel, personal communication). AP-1 is a complex that consists either of heterodimers of members of the Fos (c-Fos, FosB, Fra-1, Fra-2) and the Jun (c-Jun, JunB, JunD) family of proteins or of homodimers of members of the Jun family of proteins [6,7]. Light-induced complexes are mainly composed of c-Fos, c-Jun and JunD proteins [8]. Whereas JunD is not essential for light-induced photoreceptor apoptosis [9], the lack of c-Fos completely protects the mouse retina against light damage [10].

Considerable evidence suggests a role for altered gene expression during apoptosis. Inhibition of both RNA and protein synthesis blocks the onset of apoptosis in a wide variety of systems [11,12] suggesting that specific genes need to be induced and controlled by transcription factors like AP-1. On the other hand, several cell types can express the cell death machinery constitutively at all times. Upon removal of survival signals that seem to suppress the intrinsic death program, such cells die by apoptotic mechanisms without de novo gene expression [13].

Execution of apoptosis frequently depends on the Bcl-2 family of proteins. Both death antagonists (e.g., Bcl-2, Bcl-X_L, Bcl-w, Bfl-1, Bag-1, Mcl-1, A1) and agonists (e.g., Bax, Bak, Bcl-X_S, Bad, Bid, Bik, Hrk) belong to the Bcl-2 family of proteins. Most of these proteins contain a transmembrane domain which localizes them predominantly to the outer mitochondrial membrane [14] where they might be involved in the regulation of the transmembrane potential controlling the release of pro-apoptotic factors like cytochrome c into the cytoplasm.

In the retina, several of these pro- and anti-apoptotic genes are expressed [15,16] and may affect retinal degeneration. Overexpression of Bcl-2 delayed photoreceptor apoptosis in the retinal degeneration slow (rds) mouse [17] and in the homozygous Pdegtm1 mouse [18] but not in a mouse carrying a dominant opsin mutation (K296E) [19,20]. Retinal degeneration induced by another rhodopsin mutation (S334ter), however, was delayed by the ectopic expression of Bcl-2 [21], an effect that was ameliorated by the coexpression of Bag with the Bcl-2 transgene [22]. Mixed results were reported for rescue of retinal degeneration in the retinal degeneration (rd) mouse: opsin driven overexpression of Bcl-2 did not affect photoreceptor apoptosis in a transgenic animal, whereas Bcl-2 delayed the degenerative process when delivered by adenovirus mediated transfer in a gene therapy approach [23]. Furthermore, Bcl-2 overexpression delayed apoptosis induced by constant light [21] or short term exposure to high intensity green light [20].

Correspondence to: Christian Grimm, Ph.D., Department of Ophthalmology, University Eye Clinic, University Hopsital, Zurich, Switzerland; Phone: 1-255-3905; FAX: 1-255-4385; email: cgrimm@opht.unizh.ch

In a variety of tissues, execution of apoptosis frequently relies on the activation of cysteine proteases (caspases) [24,25]. In the retina, several different caspases, mostly including Caspase-3, are activated during apoptosis induced by a variety of stimuli including ischemia, excitotoxicity, treatment with antibodies to heat shock protein 27 [26], lead and calcium overload [27], mutations in the opsin gene [28] or during the degenerative process in the Royal College of Surgeon (RCS) rat [29]. However, retinal degeneration involving oxidative stress could not be prevented by inhibitors of caspase activity [30] suggesting that both caspase-dependent and caspase-independent apoptosis can occur in the retina. Here, we tested activation of several apoptosis-related genes during light-induced degeneration of photoreceptors in wildtype mice. Besides *c-fos* and *c-jun*, *caspase-1* was the only apoptosis-related gene upregulated upon light exposure. Gene expression was compared to *c-fos*$^{-/-}$ mice which are protected against light-induced photoreceptor apoptosis. These mice lack functional c-Fos and may therefore have an AP-1 composition different from wildtype mice. This might affect expression of AP-1 target genes. Therefore, we tested whether any of the common apoptotic genes would be differentially expressed in the protected knockout mice. We show that this was not the case. Except for *c-fos*, all genes tested were similarly expressed in both wildtype and *c-fos*$^{-/-}$ mice. This suggests that the protection of *c-fos*$^{-/-}$ mice against light-induced photoreceptor apoptosis was not due to a generally altered gene expression. Furthermore, our results also suggest that light-induced photoreceptor apoptosis involves upregulation of *caspase-1* but not activation of other common pro- or anti-apoptotic genes tested.

METHODS

Animals: All experiments conformed to the ARVO statement for care and use of animals in research and to the guidelines of the Veterinary Authority of Zurich. Wildtype (129SV/Bl6(N2), pigmented; BALB/c, albino) or *c-fos*$^{-/-}$ mice (genetic background: 129SV/Bl6(N2), pigmented) were raised in cyclic light (12:12 h; 60 lux at cage level) for at least 10 days. The 129SV/Bl6(N2) mice have a mixed 129SV and C57/Bl6 background. They were bred on this background for more than 10 generations.

Light exposure and retinal morphology: Six to 10 week old mice were dark adapted overnight (16 h) and pupils of pigmented mice were dilated under dim red light with 1% Cyclogyl and 5% Phenylephrine 45 min prior to exposure (start at 10 am) to diffuse, white fluorescent light (TLD36 W/965 tubes, Philips; ultraviolet-impermeable diffuser) in cages with reflective interior. After light exposure, mice were either kept in darkness until retinal morphology was analyzed or until retinas were prepared for RNA isolation. For morphological analysis of retinal tissue, enucleated eyes were fixed in 2.5% glutaraldehyde and embedded in Epon 812. Sections were analyzed from both the superior and the inferior central regions of the retina. Shown in Figure 1 are only the inferior central regions, the most affected area in our light damage system.

RNA isolation, cDNA synthesis and PCR: Retinas were removed through a slit in the cornea and immediately frozen in liquid nitrogen. Retinas were stored at -70 °C until RNA preparation. Total retinal RNA was prepared using the RNeasy RNA isolation kit (Qiagen, Hilden, Germany). Reverse tran-

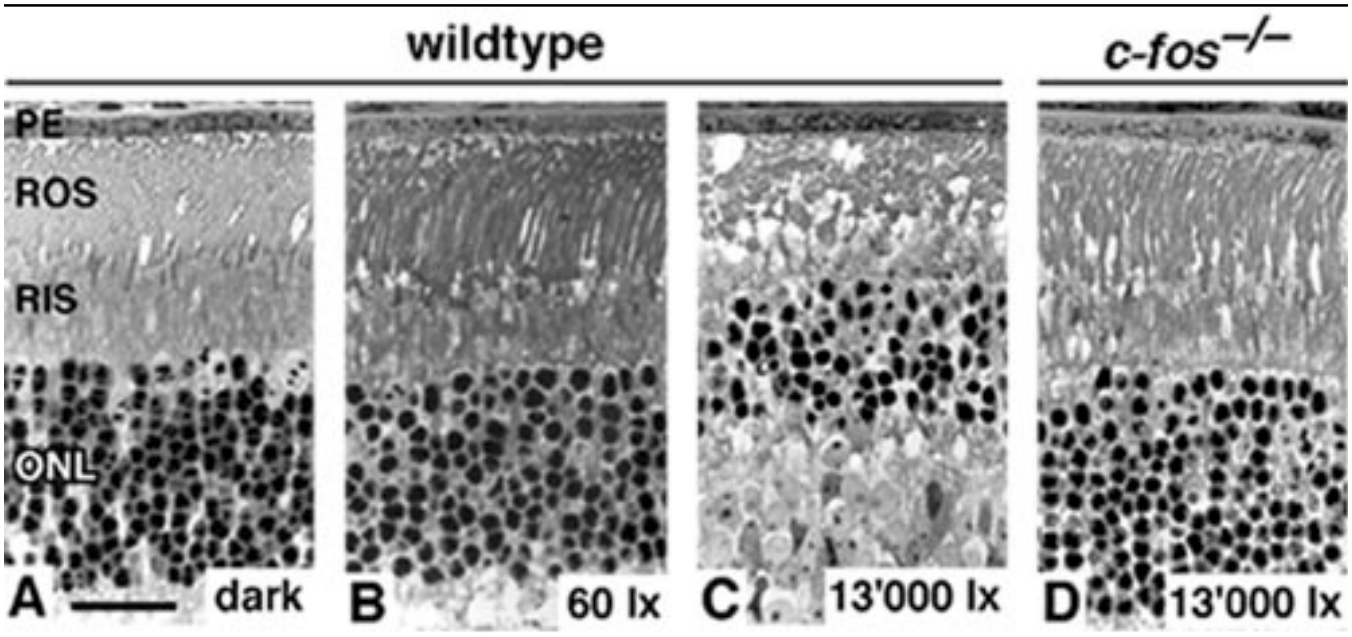

Figure 1. Photoreceptor apoptosis induced by high levels of white light. Light microscopic analysis of sections of central inferior retinal tissues of wildtype (129SV/Bl6) mice (**A** through **C**) and of *c-fos*$^{-/-}$ mice (**D**) before (**A**) and at 48 h after exposure to 13,000 lux (**C**, **D**) or to 60 lux (**B**) of white fluorescent light for 2 h. Scale bar: 25 µm. PE: pigment epithelium; ROS: rod outer segment; RIS: rod inner segment; ONL: outer nuclear layer.

scription was performed on 400 ng of total retinal RNA using oligo(dT) and M-MLV reverse transcriptase (Promega, Madison, USA). cDNAs corresponding to 10 ng of total RNA were amplified with primers specific for β-actin (see below). Amplification products were quantified on a PhosphorImager (Fuji, Tokyo, Japan) for standardization. Standardized cDNAs corresponding to 10 to 20 ng of total RNA were amplified by PCR using the following primer pairs and cycle numbers (linear range of amplification was determined for each amplified fragment in pre-experiments, data not shown): β-actin: 24 cycles; up: 5'-CAA CGG CTC CGG CAT GTG C-3'; down: 5'-CTC TTG CTC TGG GCC TCG-3'. Caspase-3: 30 cycles; up: 5'-AGT CAG TGG ACT CTG GGA TC-3'; down: 5'-GTA CAG TTC TTT CGT GAG CA-3'. Bad: 32 cycles; up: 5'-AGA GTA TGT TCC AGA TCC CAG-3'; down: 5'-GTC CTC GAA AAG GGC TAA GC-3'. Bax: 29 cycles; up: 5'-GCT CTG AAC AGA TCA TGA AG-3'; down: 5'-GAT GGT CAC TGT CTG CCA TG-3'. Bcl-2: 30 cycles; up: 5'-TTG TGG CCT TCT TTG AGT TCG-3'; down: 5'-ATT TCT ACT GCT TTA GTG AAC C-3'. Bcl-X_L: 30 cycles; up: 5'-GAC TTT CTC TCC TAC AAG C-3'; down: 5'-CGA AAG AGT TCA TTC ACT AC-3'. Caspase-1: 34 cycles; up: 5'-GAG AAG AGA GTG CTG AAT CAG-3'; down: 5'-CAA GAC GTG TAC GAG TGG TTG-3'.

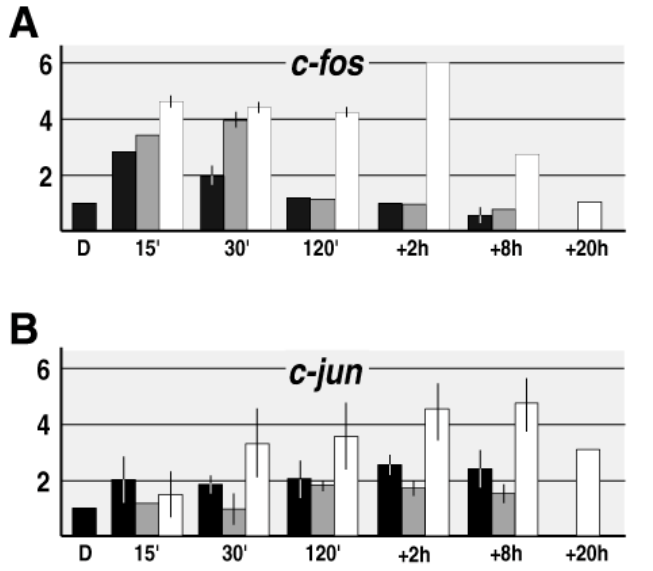

Figure 2. Retina levels of *c-fos* and *c-jun* mRNA. Analysis of *c-fos* and *c-jun* mRNA levels in retinas of 129SV/Bl6 mice by RT-PCR. **A**: Bar diagram of relative levels of *c-fos* RNA as estimated by competitive RT-PCR. **B**: Bar diagram of relative *c-jun* RNA levels as estimated by exponential RT-PCR. Mice with dilated pupils were either kept in darkness (black bars) or exposed to 60 lux (grey bars) or to 13,000 lux (white bars) of white light for 15 min (15'), 30 min (30') or 120 min (120') or for 120 min with a subsequent recovery period in darkness of 2 h (+2h), 8h (+8h) or 20 h (+20h) as indicated. Average values of two amplification reactions of RNA isolated from one retina per timepoint and condition are shown. The ranges of the two individual amplification values are shown whenever the range exceeded a value of 0.3. Second independent experiments with another group of mice are shown in Figure 5.

c-Jun: 28 cycles; up: 5'-GCA ATG GGC ACAT CAC CAC-3'; down: 5'-GAA GTT GCT GAG GTT GGC G-3'. c-Fos: 25 cycles; up: 5'-CAA CGC CGA CTA CGA GGC GTC AT-3'; down: 5'-GTG GAG ATG GCT GTC ACC G-3'. Semiquantitative PCR amplification of a 189 bp fragment of c-fos cDNA was done in reactions containing decreasing amounts (5-fold dilutions per step) of a 219 bp long competitor (mimic) DNA. Amplification was done during 30 cycles using the primer pair described above. Downstream primers in all amplification reactions were ^{32}P-end labeled. Amplification products were resolved on a 6% polyacrylamide gel and stained with ethidium bromide. Products were quantified on a PhosphorImager (Fuji).

RESULTS

Expression of c-fos *and* c-jun*:* Exposure to high levels of white light induces apoptotic cell death of photoreceptors in wildtype but not in *c-fos* knockout mice (Figure 1; [10]). Execution of cell death depends on activation of AP-1 [5,8]. Main components of activated AP-1 are c-Fos, c-Jun and JunD [5,8]. Retinal expression of immediate early genes like *c-fos* can be regulated by a variety of signals including stress [31] and light [32,33]. Accordingly, levels of *c-fos* mRNA increased 3.5- to 4.5-fold at 15 min after dark-adapted mice were exposed to normal roomlight (60 lux; Figure 2A, grey bars) or to damaging light of 13,000 lux (Figure 2A, white bars). In mice exposed to 60 lux, elevated *c-fos* levels persisted for at least 30 min after lights on, but declined to control levels after 2 h. In contrast, retinal *c-fos* mRNA levels remained elevated throughout the exposure time of two hours and at least until 2 additional hours after lights off. At 8 h after lights off, retinal *c-fos* mRNA levels of mice exposed to 13,000 lux declined to levels 2.5-fold above control. After 20 h in darkness, *c-fos* mRNA levels returned to control levels.

Surprisingly, *c-fos* mRNA levels of mice not exposed to light also increased transiently (Figure 2A, black bars). Although these mice remained in darkness during the time when experimental mice were exposed, they received low doses (ca. 10 lux) of red light (above 600 nm) during the dilation of their pupils. To test, whether the increase of *c-fos* mRNA levels in the dark control mice could have been due to handling, pupil dilation and red light illumination, we measured *c-fos* mRNAs by competitive RT-PCR (i) in retinas of eyes with non-dilated pupils isolated from mice that were dark adapted in separate cages for 16 h, (ii) in retinas of dark adapted mice that were exposed for 30 min to red light (10 lux) and (iii) in retinas of dark adapted mice that had dilated pupils and that were exposed to 30 min of red light (10 lux). Relative *c-fos* expression in control animals was set as 1 (n=3). Exposure to red light resulted in a 2.3-fold elevation of the RNA levels (n=3). Pupil dilation prior to red light exposure further increased the relative *c-fos* mRNA levels to a factor of 5.7 (n=3) as compared to the controls.

c-jun mRNA levels were determined by exponential PCR. In contrast to c-fos, *c-jun* RNA levels were not increased by handling of the animals (data not shown) and only marginally by exposure to room light (Figure 2B, grey bars). *c-jun* mRNA

levels of dark-maintained animals were somewhat elevated during the course of the experiment. The reason for this is not clear but it might be that the control levels (D) were slightly underestimated. Exposure to 13,000 lux resulted in a 3- to 4.5-fold increase of *c-jun* mRNA levels starting after 30 min of illumination and persisting for more than 8 h post-illumination. Even at 20 h after illumination, *c-jun* mRNA levels were elevated 3-fold (Figure 2B).

Expression of apoptosis-related genes: Retinal mRNAs of the two anti-apoptotic genes, bcl-2 (Figure 3A) and bcl-X_L (Figure 3B), and of the three pro-apoptotic genes bax (Figure 3C), bad (Figure 3D) and *caspase-3* (Figure 3E) were expressed at similar levels, independent of the light intensity and duration of exposure. Similarly, RNA levels of dark-maintained animals measured at different timepoints throughout the experiment were not different from control levels (not

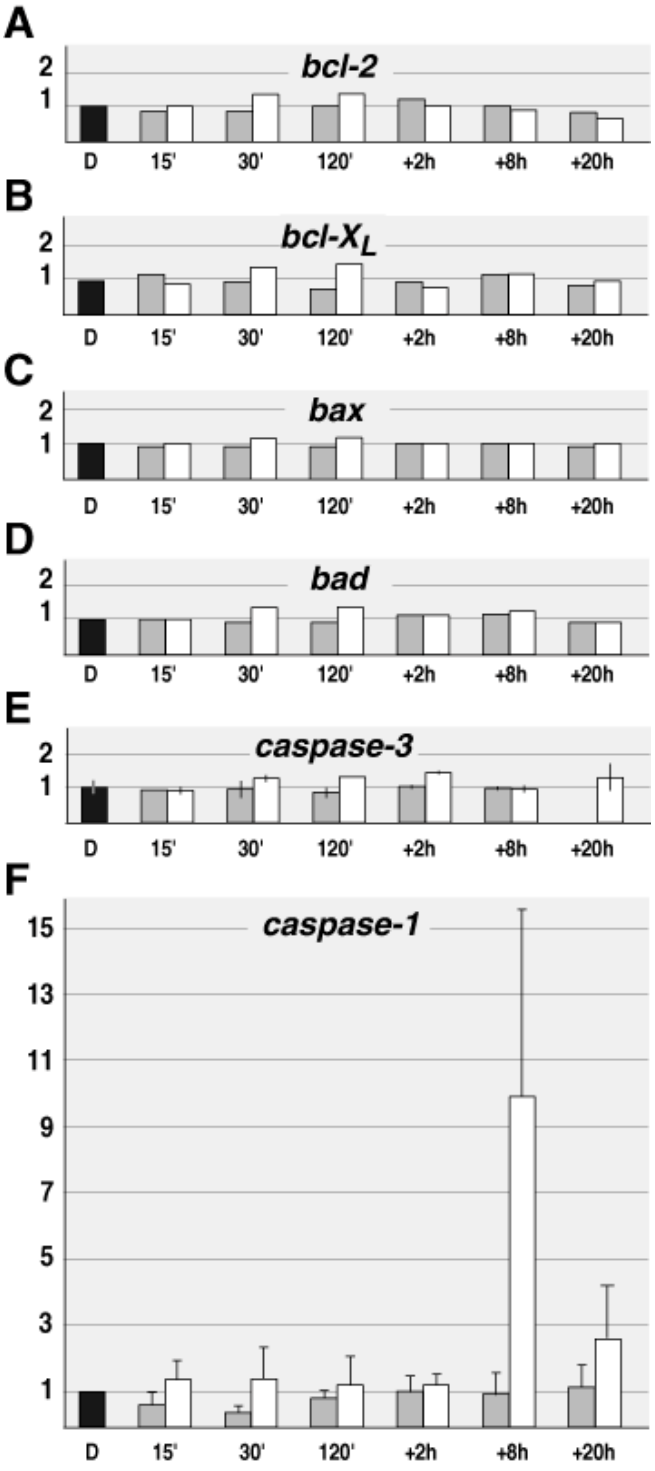

Figure 3. Relative levels of apoptosis-related mRNAs. Data are relative levels of apoptosis-related mRNAs in retinas of 129SV/Bl6 mice as determined by exponential RT-PCR (**A** through **F**; as indicated). Mice with dilated pupils were either kept in darkness (black bars) or exposed to 60 lux (grey bars) or to 13,000 lux (white bars) of white light for 15 min (15'), 30 min (30') or 120 min (120') or for 120 min with a subsequent recovery period in darkness of 2 h (+2h), 8h (+8h) or 20 h (+20h) as indicated. Amplification was done using the same RNA preparations as in Figure 2. Amplifications were done once (**A** through **D**), twice (**E**) or 3 times (**F**). The ranges of the two individual amplification values (**E**) and the standard deviation (**F**; bars), respectively, are shown. RNAs were isolated from one retina per timepoint and condition. A second independent experiment with another group of mice is shown in Figure 5.

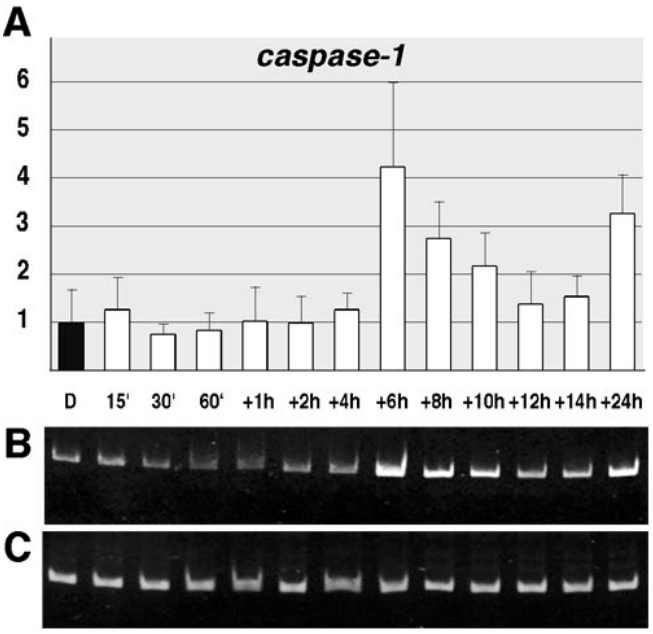

Figure 4. Relative levels of caspase-1 mRNA. Relative levels of caspase-1 mRNA in retinas of BALB/c mice as determined by exponential RT-PCR. Mice were either kept in darkness (D, black bar) or exposed to 13,000 lux of white light for 15 min (15'), 30 min (30'), 60 min (60') or to 60 min' with a subsequent recovery period in darkness of 1 h (+1h), 2 h (+2h), 4 h (+4h), 6 h (+6h), 8 h (+8h), 10 h (+10h), 12 h (+12h), 14 h (+14h) or 24 h (+24h) as indicated. Levels of caspase-1 RNA from dark control mice were set as 1. **A**: Average of 2 independent experiments (RNAs were isolated separately from one eye of two different mice). Each RNA of each experiment was amplified in triplicates. Bars: standard deviations of the six PCR amplifications per timepoint. RNA levels of unexposed mice (dark, D, black bar) were set as 1. **B**: Example amplification of caspase-1 RNA. **C**: Control amplification of β-actin RNA.

shown). However, mRNA of *caspase-1*was induced more than 9-fold at eight hours after a two hour exposure to 13,000 lux (Figure 3F, white bars). Exposure to 60 lux light did not induce *caspase-1* expression (grey bars) and RNA levels of dark-maintained animals remained unchanged throughout the experiment (not shown).

The generality of the induction of *caspase-1* expression by damaging light was verified in additional independent experiments using a different mouse strain (BALB/c) and shorter illumination periods (1 h illumination at 13,000 lux instead of 2 h). Although to a lesser extent than in 129SV/Bl6 mice, mRNA levels of *caspase-1* were again strongly induced at 6 h after illumination (Figure 4A,B). After the peak of activation, mRNA levels declined steadily and reached almost dark levels at 12 to 14 h after illumination. At 24 h after illumination, however, *caspase-1*mRNA levels increased again. This effect was observed also in the experiment shown in Figure 3. In contrast to *caspase-1*, levels of β-actin mRNA were comparable in all RNA samples (Figure 4C) demonstrating that the induction of *caspase-1* was not due to differences in quantity or quality of the RNA preparations.

Lack of c-Fos does not generally alter expression of pro- and anti-apoptotic genes: Since c-Fos is part of the transcription factor AP-1, lack of c-Fos could severely alter expression of pro- and anti-apoptotic genes. This could lead to the observed protection against damaging light. However, with the exception of *c-fos* (Figure 5A), all genes tested, including *caspase-1*, were similarly expressed in the retina of dark adapted wildtype and of dark adapted *c-fos*$^{-/-}$ mice (Figure 5B-H). Immediately after the illumination to 13,000 lux for 2 h, levels of *c-fos* and *c-jun* mRNAs were induced in wildtype animals 4- and 2-fold, respectively (Figure 5A,B). At this timepoint, *c-jun* mRNA was similarly induced (about 2-fold) also in *c-fos*$^{-/-}$ mice. At 8 h after light exposure, retinal mRNA levels in wildtype mice were elevated 2-fold for *c-fos*, 4-fold for *c-jun* and 8-fold for *caspase-1*; in accordance with the results of the experiments shown in Figure 2. In *c-fos*$^{-/-}$ mice, however, none of the RNAs tested were elevated above control levels at this timepoint.

DISCUSSION

Light doses above threshold induce photoreceptor apoptosis in the vertebrate retina. Here we show that exposure to damaging light but not to physiological light levels induces mRNAs of the proto-oncogenes, *c-fos* and *c-jun*, and of the cysteine protease *caspase-1*. Other apoptosis-related genes tested (Bcl-2, Bcl-X_L, Bad, Bax, caspase-3) were neither up- nor downregulated by light exposure. Furthermore, all genes tested (except for *c-fos*) were similarly expressed in dark-adapted wildtype and dark-adapted *c-fos*$^{-/-}$ mice excluding the possibility that the lack of c-Fos in the knockout mice generally prevents the transcription of apoptosis relevant genes.

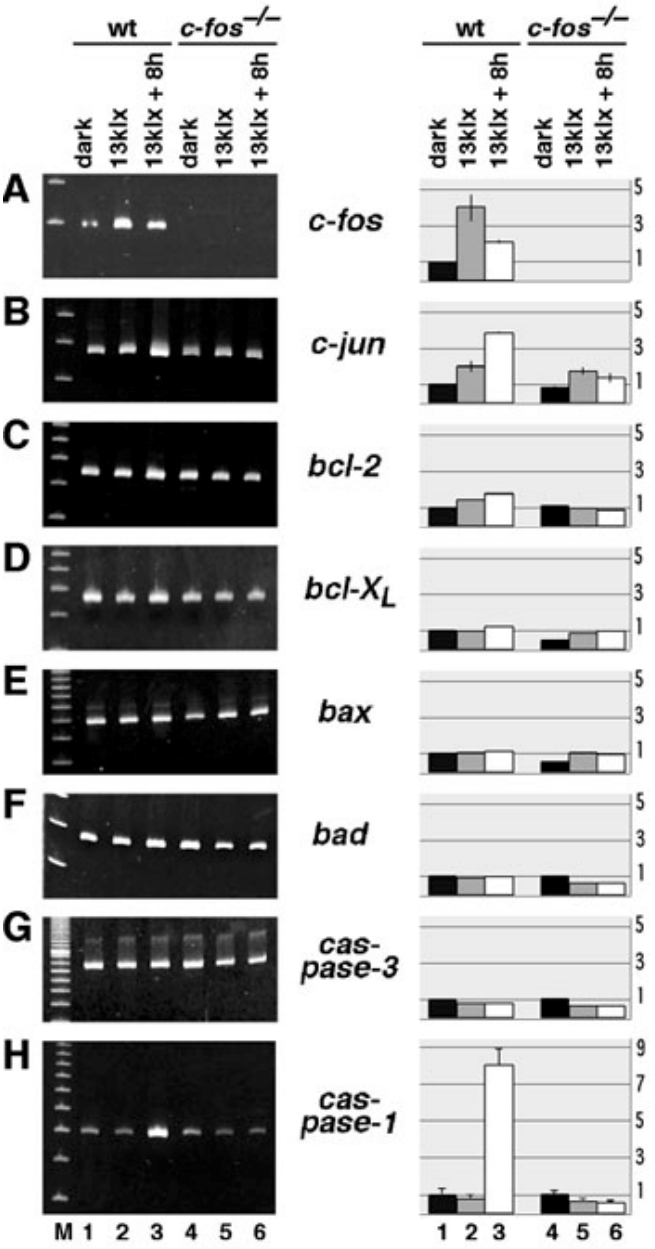

Figure 5. Gene expression in wildtype and *c-fos*$^{-/-}$ mice. Relative levels of indicated RNAs (**A** through **H**) in retinas of 129SV/Bl6 wildtype (lanes 1 through 3) and 129SV/Bl6 *c-fos*$^{-/-}$ mice (lanes 4 through 6) as determined by exponential RT-PCR. Panels on the left: example amplifications with the respective primer pairs. Lanes 1 and 4: unexposed controls. Lanes 2 and 5: Exposed to 13,000 lux (13 klux) for 120 min. Lanes 3 and 6: Exposed to 13 klux for 120 min with a subsequent recovery period of 8 h in darkness. Panels on the right: Relative levels of the respective RNAs. Black bars: unexposed controls. Grey bars: relative RNA levels after exposure to 13 klux for 120 min. White bars: relative RNA levels after exposure to 13 klux for 120 min and a subsequent recovery period of 8 h in darkness. RNA levels of unexposed 129SV/Bl6 wildtype mice (shown in lane 1) were set as 1. Amplifications were done once (**C**, **F**), twice (**A**, **B**, **D**, **E**, **G**) or four times (**H**). The ranges of the two individual amplification values (**A**, **B**, **D**, **E**, **G**) are shown whenever the range exceeded a value of 0.2. Standard deviations (**H**; bars) are indicated. RNAs were isolated from one retina per timepoint and condition. Second independent experiments with other groups of mice are shown in Figure 2 and Figure 3.

Expression of c-fos *and* c-jun*:* Activation of c-Fos containing AP-1 is a prerequisite for the induction of the apoptotic program by excessive light [5,8,10]. Induction of the DNA binding activity of AP-1 only occurs in response to damaging light but not after exposure to physiological levels of light [5]. In contrast, c-fos gene expression is also induced by exposure to low levels of light. However, elevated *c-fos* mRNA levels persist only after exposure to damaging light. When the light pulse was of low intensity, *c-fos* RNA levels declined rapidly after the initial peak. Similar observations have been made during the exploitation of the diurnal expression of *c-fos*: when animals received a light pulse during the dark period, *c-fos* mRNA levels increased transiently for a period of 30 to 60 min [34].

Retinal *c-fos* mRNA expression increased even without illumination in our system (Figure 2A). Such a moderate increase in the *c-fos* mRNA levels could best be explained by stress induced gene expression as has also been observed in animals that were faced, for example, with novelty [35]. Our data of *c-fos* mRNA levels after handling of the animals, pupil dilation, and exposure to red light, support this hypothesis. In contrast to *c-fos*, *c-jun* mRNA varied slightly or not at all following low levels of illumination with white light. When exposed to damaging doses of light, however, *c-jun* mRNA levels were induced 3 to 5-fold and persisted at elevated levels for at least 20 h (Figure 2B). Induction of *c-fos* and *c-jun* gene expression preceded the light-induced increase of AP-1 DNA binding activity which peaks 6 h after the end of light exposure [5]. This suggests that newly made c-Fos and c-Jun proteins contribute to the increase of AP-1 activity. In *c-fos*$^{-/-}$mice, *c-jun* was slightly activated upon exposure to high levels of light (Figure 5B). However, activation did not persist as long as in wildtype animals suggesting that c-Fos containing AP-1 complexes might be involved in the transcriptional regulation of *c-jun* after light insult. This is supported by the finding that DNA binding of AP-1 is induced as early as 15 min after the onset of light exposure [5] but elevated *c-jun* mRNA levels could not be detected before 30 min of light exposure (Figure 2D).

Expression of pro- and anti-apoptotic genes: Apoptosis frequently involves modulation of the transcription of genes encoding proteins involved in the response to apoptotic stimuli. Expression of anti-apoptotic genes like bcl-2 and bcl-X_L, for example, have been found to be downregulated after induction of apoptosis by several stimuli in various cell systems [36-41]. This is in contrast to expression of apoptosis promoting genes like bax [42-44], bad [45], and the cysteine proteases (caspases), which may be upregulated following a pro-apoptotic insult [46-48]. Overexpression of bcl-2 has protective effects on retinal apoptosis induced by a variety of stimuli [17,18,20-23,49]. A major function of Bcl-2 is the regulation of cytochrome c release from mitochondria [50-52] which may induce the apoptotic execution cascade by activating caspase-3 [53,54] a central executioner in many apoptotic systems.

In light-induced photoreceptor degeneration, however, we were unable to detect an alteration in the expression of bcl-2 or caspase-3 (Figure 3 and Figure 5). Similarly, an induction of the enzymatic activity of caspase-3 or the cytoplasmic appearance of cytochrome c could not be detected after light exposure (unpublished results). Therefore, regulation of apoptosis by bcl-2 and/or caspase-3 might play a minor role in light-induced retinal degeneration. The lack of regulation of bcl-X_L, another protein thought to be involved in the control of the mitochondrial membrane integrity, and therefore of cytochrome c release [55] supports this hypothesis.

The role of Caspase-1: In the system of light-induced apoptosis of photoreceptors, *caspase-1* was the only gene that was differentially regulated by light exposure. The gene was strongly induced at 6 to 8 h after the end of illumination in two different mouse strains. At present, we do not know why activation of *caspase-1* was less strong in BALB/c mice than in 129SV/Bl6 mice. It might be possible, though, that the difference in gene activation reflects strain differences in the regulation of the apoptotic response to damaging light. Caspase-1 (ICE) is an enzyme that might be involved in neuronal cell death. Dorsal root ganglion neurons undergo apoptosis upon withdrawal of nerve growth factor. However, they are protected by the cytokine response modifier crmA, a serpin that specifically inhibits Caspase-1 [56]. Moreover, mice deficient for Caspase-1 [57,58] and mice expressing a dominant negative mutant of this protease [59] are more resistant to ischemic insult than control animals [60-62]. The main substrate of Caspase-1 is pro-interleukin-1 beta (IL-1β). Cleavage of pro-IL-1β results in the release of the active and pro-inflammatory form of IL-1β [63,64] which has been suggested to have pro-apoptotic effects [65]. A recent report suggests that Caspase-1 can also cleave the inhibitor of caspase-activated deoxyribonuclease (ICAD) leading to the activation of the endonuclease and to the fragmentation of genomic DNA [66]. In the vertebrate retina (rat), *caspase-1* expression has been predominantly found in the outer nuclear layer (ONL) [67]. Injection of a specific inhibitor of Caspase-1 decreased the number of apoptotic cells in the ONL in an ischemia-reperfusion model [67]. Increased Caspase-1 activity was also detected during retinal degeneration in RCS rats and inhibitors of Caspase-1 delayed this degenerative process at least partially [29]. Interestingly, light seems to accelerate retinal degeneration in RCS rats [68]. Furthermore, light exposure induced oxidative stress in cultured retinal cells and decreased the anti-apoptotic activity of the transcription factor nuclear factor-kappaB (NF-kappaB) in a Caspase-1 dependent manner [69]. The strong induction of *caspase-1* in our model of light-induced retinal degeneration suggests that Caspase-1 might be involved in mediation of apoptotic cell death of photoreceptors after light insult. It is of importance that Caspase-1 was induced only in wildtype mice that were exposed to damaging levels of light but not in mice exposed to physiological light levels or in mutant mice protected against light-induced apoptosis (*c-fos*$^{-/-}$). This shows that the induction was not due to the experimental procedure (handling, stress, etc.), and suggests that Caspase-1 might act downstream of c-Fos/AP-1 in the cascade of light-induced photoreceptor apoptosis.

In future experiments, we will test the specific roles of Caspase-1 and of IL-1β in our model of light-induced retinal

degeneration and we will test the hypothesis that Caspase-1 plays a central role in the degenerative process of inherited retinal degenerations that are enhanced by light.

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