Retinal degeneration modulates intracellular localization of CDC42 in photoreceptors

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Purpose: Rho GTPases such as RAS-related C3 botulinum substrate 1 (RAC1) and cell division cycle 42 homolog (S. cerevisiae; CDC42) have been linked to cellular processes including movement, development, and apoptosis. Recently, RAC1 has been shown to be a pro-apoptotic factor in the retina during light-induced photoreceptor degeneration. Here, we analyzed the role of CDC42 in the degenerating retina.

Methods: Photoreceptor degeneration was studied in a mouse model for autosomal dominant retinitis pigmentosa (VPP) with or without a rod-specific knockdown of Cdc42, as well as in wild-type and Cdc42 knockdown mice after light exposure. Gene and protein expression were analyzed by real-time PCR, western blotting, and immunofluorescence. Retinal morphology and function were assessed by light microscopy and electretinography, respectively.

Results: CDC42 accumulated in the perinuclear region of terminal deoxynucleotidyl transferase dUTP nick end labeling–negative photoreceptors during retinal degeneration induced by excessive light exposure and in the rd1, rd10, and VPP mouse models of retinitis pigmentosa. The knockdown of Cdc42 did not affect retinal morphology or function in the adult mice and did not influence photoreceptor apoptosis or molecular signaling during induced and inherited retinal degeneration.

Conclusions: Retinal degeneration induces the accumulation of CDC42 in the perinuclear region of photoreceptors. In contrast to RAC1, however, lack of CDC42 does not affect the progression of degeneration. CDC42 is also dispensable for normal morphology and function of adult rod photoreceptor cells. Received: May 25, 2011 Accepted: November 10, 2011

Retinitis pigmentosa (RP) and age-related macular degeneration are diseases that result in the loss of vision due to photoreceptor apoptosis [1,2]. To study mechanisms of photoreceptor death, several mouse models of RP have been developed. Exposure to white light is an inducible model in which the severity of degeneration depends on light intensity and duration of exposure [3]. In this model, photoreceptors die and are cleared from the subretinal space within a period of approximately 10 days. Mouse models of inherited retinal degeneration include retinal degeneration (rd1) [4], rd10 [5], VPP [6], and others [7]. Rd1 and rd10 mice carry a recessive nonsense or missense mutation, respectively, in the β-subunit of the cGMP phosphodiesterase gene. In rd1, this results in an early onset (postnatal day [P]10) and rapid photoreceptor degeneration, whereas in rd10 the degeneration has a later onset (P15) and a slower progression. The VPP mouse expresses a rhodopsin transgene encoding a mutant protein with three amino acid substitutions (V20G, P23H, P27L). Photoreceptor cell death in this mouse begins around P15 and progresses over several weeks.

Rho guanosine triphosphate (GTP)ases such as RAS-related C3 botulinum substrate 1 (Rac1) and cell division cycle 42 homolog (S. cerevisiae; Cdc42) are well known modulators of microtubule and actin structures [8]. Rho GTPases cycle between an inactive guanosine diphosphate–bound state and an active GTP-bound state [9]. Active Rho GTPases bind to a host of different effector proteins [10-13] to elicit a myriad of signaling responses involved in the regulation of cellular movement, adhesion, axon guidance, differentiation, and apoptosis [13-17]. Despite the importance of Rho GTPases in many physiologic and pathophysiologic processes, only little is known about their roles in the eye.

Although few in number, there have been some studies on CDC42 documenting a variety of ocular functions. For example, CDC42 has been shown to be important for wound-healing processes in the corneal endothelium [18]. In addition, CDC42 was suggested to be involved in lens pit invagination during eye morphogenesis [19] and—based on the spatial and temporal expression pattern—in retinal development [20]. Despite these studies, there is a lack of understanding of the function of CDC42 in the mature retina. RAC1, however, has recently been implicated in photoreceptor degeneration as a...
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**METHODS**

**Animals and light exposure:** All procedures were conducted in accordance with the guidelines published by the Institute for Laboratory Animal Research and with the regulations of the Veterinary Authority of Zurich. Cdc42 floxed mice (Cdc42flox/ﬂox) [23] were a generous gift from Dr. Joao Relvas (Institute of Cell Biology, ETH Zurich, Zurich, Switzerland). The 129S6/SvEvTac mice were purchased from Taconics (Eiby, Denmark). Mice with Cre recombinase expression controlled by the rod opsin promoter (LMOPC1, which will be called opsin-Cre from now on) [24] were provided by Dr. Yun Le (University of Oklahoma, Oklahoma City, OK). The mouse model for autosomal dominant RP (called VPP from Yun Le (University of Oklahoma, Oklahoma City, OK). The be called opsin-Cre from now on) [24] were provided by Dr. Muna Naash (University of Oklahoma, Oklahoma City, OK). The late onset of Cre recombinase expression postnatally allowed a normal retinal development, permitting the use of the Cdc42flox/ﬂox;opsin-Cre to study the role of CDC42 in adult rod photoreceptor cells.

Genotypes were determined using genomic DNA from ear biopsies, and the following conventional PCR conditions: initial denaturation (95 °C, 5 min); 35 cycles of denaturation (95 °C, 45 s), annealing (60 °C, 45 s), and elongation (72 °C, 45 s); and final extension (72 °C, 10 min). For the detection of the floxed Cdc42 allele, primers (forward: 5′-TTC TTC ATC GCC ATC TTC CAG CAG G-3′) specific for the floxed allele, primers (forward: 5′-TTC TTC ATC GCC ATC TTC CAG CAG G-3′) specific for the floxed allele; reverse 5′-CTA GCC ATC TTC CAG CAG G-3′, reverse 5′-TGC TGT GTG TGG CAT TTG CTG C-3′) spanning both loxP sites were used. The amplicon of the floxed allele was 1.5 kb long, whereas amplification of the wild-type allele yielded a 1.3 kb fragment. Excision of the floxed sequence by the Cre recombinase resulted in an amplification product of 0.9 kb. For the detection of the Cre transgene, primers (forward: 5′-AGG TGT AGA GAA GGC ACT TAG C-3′, reverse 5′-CTA ATC GCC ATC CCC CAG CAG G-3′) specific for Cre were used. PCR products were run on a 1% agarose gel for size detection.

**RNA preparation and semiquantitative RT–PCR:** Retinas were removed through a slit in the cornea and immediately frozen in liquid nitrogen. Total RNA was prepared with an RNA isolation kit (RNaseasy; Qiagen, Hilden, Germany) including a DNase treatment to remove residual genomic DNA. Identical amounts of RNA were used for reverse transcription using oligo(dT) and M-MLV reverse transcriptase (Promega, Madison, WI). Gene expression was analyzed by real-time PCR using specific primer pairs (Table 2) spanning an intronic region of the respective gene, a polymerase ready mix (LightCycler 480 SYBR Green I Master Mix; Roche Diagnostics, Indianapolis, IN), and a

| Animal model | Gene | Mutation / Transgene | Time course of degeneration | Model for disease | Origin | Reference |
|--------------|------|-----------------------|----------------------------|------------------|--------|----------|
| Rd1          | Pde6b| Nonsense              | PND8–21                    | arRP             | Harlan, Horst, The Netherlands | [4]      |
| Rd10         | Pde6b| Missense              | PND16–60                   | arRP             | Jackson Laboratory, Bar Harbor, ME | [5]      |
| VPP          | Rho  | Tg(V20G,P23H,P27L)    | PND20–250                  | adRP             | Dr. Muna Naash | [6]      |

**Abbreviations:** Pde6β: Phosphodiesterase 6β; Rho: Rhodopsin; adRP: autosomal dominant retinitis pigmentosa, arRP: autosomal recessive retinitis pigmentosa, Rd: retinal degeneration, PND: postnatal day, Tg: transgene.
thermocycler (LightCycler Roche Diagnostics). Signals were normalized to Actb and relative expression was calculated by the comparative threshold cycle (ΔΔCT) method using a control sample for calibration [27].

**Laser capture microdissection:** Mouse eyes were enucleated and immediately frozen in tissue-freezing medium using a methylbutanol bath cooled by liquid nitrogen. Retinal sections (20 µm) were cut, fixed (5 min acetone), air-dried (5 min), and dehydrated (30 s in 100% ethanol, 5 min in xylol). Microdissection of the retinal nuclear layers was performed with an Arcturus XT Laser capture device (Molecular Devices, Silicon Valley, CA). RNA was isolated using the Arcturus RNA isolation kit (Molecular Devices), and residual genomic DNA removed by a DNase treatment. cDNA synthesis was performed as described in the previous section. Gene expression was determined by conventional PCR (40 cycles) or semiquantitative PCR using the primer pairs listed in Table 2. Amplified fragments were run on a nondenaturing polyacrylamide gel and detected after staining with ethidium bromide.

**Light microscopy and spider diagram:** Enucleated eyes were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) overnight at 4 °C. The cornea and lens were removed and the superior and inferior retina of each eye was prepared, washed in cacodylate buffer, incubated in osmium tetroxide for 1 h, dehydrated in a series of increasing ethanol concentrations, and embedded in Epon 812. Semithin cross-sections (500 nm) were prepared from the ventral central retina, the most affected region in our light-damage model [3]. Sections were counterstained with toluidine blue and analyzed by light microscopy.

The thickness of the photoreceptor nuclear layer was measured using the Adobe Photoshop CS3 ruler tool at 250, 500, 1,000, 1,500, 1,750, 2,000, and 2,250 µm distances from the optic nerve head in both the dorsal and ventral directions. Results of n=3 retinas were plotted as a spider diagram.

**Immunofluorescence:** Eyes were enucleated and fixed in 4% (wt/vol) paraformaldehyde in 0.1 M phosphate buffered saline (PBS; pH 7.4) overnight. After removing the cornea and lens, eyecups were postfixed in 4% paraformaldehyde for an additional 2 h before being immersed in 30% sucrose in PBS at 4 °C overnight. The eyes were embedded in tissue-freezing medium (Leica Microsystems Nussloch GmbH, Nussloch, Germany) and frozen in a 2-methylbutane bath cooled by liquid nitrogen. Retinal sections (12 µm) were cut, placed on slides and incubated with a blocking solution (3% normal goat serum, 0.3% Triton X-100 in 0.1M PBS) for 1 h at room temperature. After three washes with PBS, slides were incubated with the appropriate secondary antibody coupled to Cy3 or Cy2 for 1 h at room temperature, washed, counterstained with 4′,6-diamidino-2-phenylindole (DAPI), and mounted with antifade medium (10% Mowiol 4–88; vol/vol; Calbiochem, San Diego, CA), in 100 mM Tris (pH 8.5), 25% glycerol (wt/vol), and 0.1% 1,4-diazabicyclo (2.2.2) octane.

**TUNEL assay and cell death detection:** Detection of photoreceptor cell death in retinal sections was done using the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) method according to the manufacturer’s recommendations (Roche Diagnostics, Rotkreuz, Switzerland). Immunofluorescent signals were analyzed with a digital microscope (Axiovision; Carl Zeiss Meditec, Inc., Dublin, CA).

Apoptotic cell death was quantified 36 h after light exposure by measuring the release of free nucleosomes in isolated retinas using the cell death detection kit (Roche Diagnostics, Dublin, CA).
results

Accumulation of CDC42 in the perinuclear region of TUNEL-negative photoreceptors during retinal degeneration: Immunoﬂuorescent stainings of CDC42 on retinal sections showed that CDC42 was ubiquitously expressed in the retina. This was supported by analysis of Cdc42 mRNA (mRNA) expression in individual retinal layers separated by laser capture microdissection (Figure 1E). Localization of CDC42 in the inner retinal layers remained unchanged after light exposure (Figure 1A) and in the genetic models of photoreceptor degeneration studied (not shown). However, after induction of photoreceptor degeneration by light, CDC42 was additionally detected in distinct cell bodies in the outer nuclear layer (ONL; Figure 1A). Closer inspection of CDC42 localization by confocal microscopy revealed that CDC42 accumulated in the perinuclear region of photoreceptors (Figure 1B). Perinuclear accumulation of CDC42 in the ONL of degenerating retinas was an early and transient event, peaking around 14 h after light exposure. At this time point, Cdc42 showed a tendency of increased gene expression (Figure 1D). A similar CDC42 staining pattern was observed in all tested inherited mouse models (rd1, rd10, and VPP) of retinal degeneration (Figure 1C). This suggested that the perinuclear accumulation of CDC42 in individual cells of the ONL is a common mechanism in retinas with degenerating photoreceptors, and is independent of the toxic stimulus (light, mutation) that induced cell death.

Cotaining for CDC42 and apoptotic cells using the TUNEL assay showed that perinuclear CDC42 staining appeared around 6 h after light exposure and was strongest around 14 h (Figure 2). It decreased rapidly thereafter as the number of TUNEL-positive cells rose (Figure 2). At the peak of apoptosis, around 36 h after light exposure [3], perinuclear CDC42 staining was no longer detectable in photoreceptors (data not shown). Importantly, CDC42 accumulated in the

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Western blot analysis: Retinas were homogenized in 0.1 M Tris/HCl (pH 8.0) and protein content was analyzed using Bradford reagent. Equivalent amounts of proteins were resolved by electrophoresis on sodium dodecyl sulfate–polyacrylamide gels and transferred to nitrocellulose membranes. Membranes were blocked in 5% milk (Bio-Rad, Hercules, CA) in TBST (10 mM Tris/HCl [pH 8.0], 150 mM NaCl, and 0.05% Tween-20) for 1 h at room temperature before they were incubated overnight at 4 °C in 5% milk (in TBST) containing the respective primary antibody (Table 3). Detection was with horseradish peroxidase (HRP)-conjugated secondary antibodies and proteins were visualized using the Renaissance Western Blot Detection Kit (PerkinElmer Life Sciences, Boston, MA).

Electroretinography: Electroretinograms (ERGs) were recorded from both eyes simultaneously, as previously described [28,29]. Briefly, mice were dark-adapted overnight and anesthetized the next day with ketamine (66.7 mg/kg) and xylazine (11.7 mg/kg). Pupils were dilated before performing single flash ERG recordings under dark-adapted (scotopic) conditions. Light adaptation was accomplished with a background illumination of 30 cd/m² starting 10 min before photopic recording. Single white-flash stimulus intensity ranged from −3 to 1.5 log cd*s/m² under scotopic and from −2 to 1.5 log cd*s/m² under photopic conditions, divided into 10 and 8 steps, respectively. Ten responses were averaged with an interstimulus interval of either 5 s or 17 s (for 0, 0.5, 1, and 1.5 log cd*s/m²).

Statistical analysis: Statistical analyses were performed using Prism4 software. All data are the mean ± standard deviation (SD) of three animals per group. Statistical differences of means were calculated using ANOVA (ANOVA) followed by a Bonferroni post-hoc test. A p value of less than 0.05 was considered significant.

Table 3. Antibodies for immunoblotting and immunofluorescence

| Antigen | Host | Dilution | Catalog number | Company |
|---------|------|----------|----------------|---------|
| CDC42   | Rabbit | 1:500    | Sc-87          | Santa Cruz Biotechnology |
| RHO     | Mouse  | 1:100    |                | Gift from Dr. Hicks |
| GNAT1   | Rabbit | 1:500    | Sc-389         | Santa Cruz Biotechnology |
| OPN1SW  | Goat   | 1:500    | Sc-14363       | Santa Cruz Biotechnology |
| JAK2    | Rabbit | 1:500    | #44-604        | Invitrogen, Basel, Switzerland |
| p-JAK2  | Rabbit | 1:250    | #44-426        | Invitrogen |
| STAT1   | Rabbit | 1:1000   | #9172          | Cell signaling technology, Beverly MA |
| p-STAT1 | Rabbit | 1:1000   | #9171          | Cell signaling technology |
| STAT3   | Rabbit | 1:1000   | #9132          | Cell signaling technology |
| p-STAT3 | Rabbit | 1:500    | #9131          | Cell signaling technology |
| AKT     | Rabbit | 1:2500   | #9272          | Cell signaling technology |
| p-AKT   | Rabbit | 1:1000   | #9271          | Cell signaling technology |
| ACTB    | Mouse  | 1:5000   | #5441          | Sigma, St. Louis, MO |
perinuclear region exclusively in TUNEL-negative photoreceptors, suggesting that CDC42 may support survival of cells or, alternatively, may prepare photoreceptors to die.

Rod photoreceptor–specific knockdown of Cdc42: To address the role of CDC42 in photoreceptor survival or death directly, we generated Cdc42<sup>flox/flox</sup>;opsin-Cre mice that lack functional CDC42 specifically in rods. Although the expression of Cre recombinase has been described as being somewhat patchy in opsin-cre mice, expression is specific to rods and affects about 75% of photoreceptors [24,30]. By 12 weeks of age, Cre-mediated excision of Cdc42 genomic sequences was analyzed by amplifying genomic DNA isolated from retinal tissue. A 0.9 kb fragment was detected in Cdc42 knockdown (Cdc42<sup>flox/flox</sup>;opsin-Cre) but not in control Cdc42<sup>flox/flox</sup> mice, indicating successful excision of the floxed exon 2 of Cdc42 in the presence of Cre recombinase (Figure 3A). Since Cre recombinase was specifically expressed in rods, other retinal cells still carried the full-length floxed Cdc42 gene. Thus, PCR amplification of total retinal genomic DNA isolated from Cdc42<sup>flox/flox</sup>;opsin-Cre mice resulted in two bands representing the knockdown (0.9 kb) and floxed exon 2 (1.5 kb) alleles of Cdc42, respectively. A successful knockdown of Cdc42 in the ONL was also determined by the relative quantification of Cdc42 mRNA levels in the individual retinal layers after laser capture microdissection. Expression of Cdc42 was reduced by 60% in the ONL of Cdc42 knockdown mice relative to control mice (Figure 3B). Thus, we estimate that about 60% of the floxed Cdc42 alleles have been deleted in the ONL. Given that only about roughly 75% of rods express the CRE recombinase [24] we expect that 25% to 30% of rods are wild type for Cdc42 (Cdc42<sup>+/+</sup>). Of
the remaining rods, 40% to 50% have to be full knockouts (Cdc42<sup>−/−</sup>), whereas the remaining 20% to 30% may be Cdc42<sup>+/−</sup>. The reason for the slightly elevated levels of Cdc42 mRNA in the inner nuclear layer (INL) and the ganglion cell layer (GCL) of knockdown mice is not known, but may potentially include some reactions compensating for the absence of Cdc42 in the ONL. The reduced expression of Cdc42 in the ONL of knockdown mice was also reflected in a reduced number of photoreceptors with a perinuclear localization of CDC42 in the degenerating retina (Figure 3C). Together, these results suggested an effective CDC42 knockdown at 12 weeks of age. All subsequent experiments were performed on animals at this age, unless otherwise stated.

Rod-specific ablation of CDC42 does not affect retinal morphology and function: Recent studies have shown that CDC42 is involved in photoreceptor morphogenesis and polarity in Drosophila [31,32]. To test whether rod-specific ablation of CDC42 has an effect in the mouse retina, we analyzed tissue morphology, the expression of photoreceptor markers, and the retinal function in knockdown animals. The overall retinal morphology and photoreceptor structure of Cdc42 knockdown mice was maintained relative to controls (Figure 4A). Similarly, ablating CDC42 did not change expression levels of markers for rod and cone photoreceptors (Figure 4B). Furthermore, the intracellular localization pattern of rhodopsin, rod transducin, and short-wavelength (SWL) cone opsin was normal in Cdc42 knockdowns and indistinguishable from control littermates (Figure 4C). In particular, no mislocalization of these proteins was observed, suggesting that protein transport through the cilium was not affected by the lack of CDC42 (Figure 4C). Normal rod (and retinal) physiology in Cdc42 knockdown mice was also supported by normal scotopic and photopic ERG responses to light stimuli (Figure 4D). Together, these results suggest that deficiency of CDC42 in adult mouse rods does not influence photoreceptor physiology or function.

Rod-specific knock-down of CDC42 does not affect progression of photoreceptor degeneration: RAC1, another classical member of the small Rho GTPases, has recently been shown to have a pro-apoptotic role in light-induced retinal degeneration [21]. The perinuclear localization of CDC42 in
Degeneration of photoreceptors activates a LIF-controlled protective signaling cascade in the retina [33]. This signaling involves induced expression of endothelin 2 (Edn2) and fibroblast growth factor 2 (Fgf2), as well as activation of JAK2, STAT1, STAT3, and AKT by phosphorylation in the early degeneration phase after light exposure (Figure 6A). Activation of JAK2, STAT1, STAT3, and AKT by phosphorylation was not grossly affected by the lack of CDC42 (Figure 6B). Together, these results suggest that lack of CDC42 in photoreceptors does not strongly affect the regulation of the LIF-controlled survival pathway and corroborates our conclusion of a nonessential role of CDC42 in photoreceptor degeneration.

DISCUSSION

The small GTPase RAC1 was recently shown to be a pro-apoptotic factor in the model of light-induced retinal degeneration [21]. Here, we show that CDC42, another member of the small GTPase family, does not play a major role in photoreceptor death, even though CDC42 specifically localized to the perinuclear region of some photoreceptor cells during induced and inherited retinal degeneration. Although CDC42 is involved in the maintenance of cellular polarity [19, 39] and vesicular trafficking [40], both of which may be important for correct protein localization, ablation of CDC42 did not affect the expression and localization of rod and cone markers.

Rod-specific ablation of CDC42 does not affect the induction of endogenous survival pathways after light exposure:

Degeneration of photoreceptors activates a LIF-controlled protective signaling cascade in the retina [33]. This signaling involves induced expression of endothelin 2 (Edn2) and fibroblast growth factor 2 (Fgf2), as well as activation of JAK2, STAT1, and STAT3 proteins through phosphorylation [33-36]. Since it has been shown in other systems that CDC42 can participate in the endothelin [37] and LIF signaling pathways [38], we tested whether the ablation of CDC42 in rods might affect the molecular response during retinal degeneration. Semi-quantitative analysis of gene expression after light exposure showed a similar fold induction over the respective basal levels in control and Cdc42 knockdown mice (Figure 6A). However, basal expression of Edn2 and Fgf2 was elevated in retinas of untreated knockdown mice, as well as in the early degeneration phase after light exposure (Figure 6A). Activation of JAK2, STAT1, STAT3, and AKT by phosphorylation was not grossly affected by the lack of CDC42 (Figure 6B). Together, these results suggest that lack of CDC42 in photoreceptors does not strongly affect the regulation of the LIF-controlled survival pathway and corroborates our conclusion of a nonessential role of CDC42 in photoreceptor degeneration.
nonessential function in mature rods or other small GTPases like the four CDC42-like GTPases might compensate for the lack of CDC42, as suggested by others [41].

CDC42 is not only expressed in photoreceptors but in all layers of the adult retina (Figure 1) [20,42]. In retinal ganglion cells, CDC42 may have modulatory functions and be involved in neurite outgrowth and growth cone dynamics [43,44]. The function of CDC42 in the INL has not been addressed, but the strong immunolabeling in the inner plexiform layer (IPL) and INL may point to a modulatory function in normal neuron physiology rather than neuroprotection. To resolve the function of CDC42 in the inner retina, additional cell-type-specific knockdowns may be needed and analyzed.

Laser scanning confocal microscopy showed that CDC42 localized around the nucleus of some photoreceptors in the degenerating retina (Figure 1 and data not shown).
Figure 5. Ablation of cell division cycle 42 homolog (S. cerevisiae; CDC42) does not affect progression of photoreceptor degeneration. A: Retinal morphologies of Cdc42 conditional knockdown (cKD) and control mice before (dark control) or at 24 h and 10 days after light exposure are shown. B: Photoreceptor apoptosis was quantified in Cdc42 knockdown (black bars) and wild-type controls (white bars) 36 h after light exposure. Shown are results from n=4 retinas per time point and genotype. Statistical analysis showed no significant difference in apoptosis between wild-type and knockdown mice (Student t-test). C: Retinal morphologies of Cdc42\texttextsc{flox/flox}/opsin-Cre; VPP (cKD VPP) and Cdc42\texttextsc{flox/flox};VPP (control VPP) mice were examined at 4 weeks (weeks), 12 weeks and 20 weeks of age. Shown are representative sections of n=3. D: Thickness of the outer nuclear layer (ONL) of Cdc42\texttextsc{flox/flox}/opsin-cre;VPP (cKD) and Cdc42\texttextsc{flox/flox};VPP (ctrl) mice at 4, 12, and 20 weeks of age is shown in a spider diagram. Measurements from morphological sections of 3 mice per genotype and age are shown. Retinal thickness of wild-type and knockdown mice were comparable at all time points and locations (ANOVA [ANOVA] followed by bonferroni post-hoc test). OPL: outer plexiform layer, INL: inner nuclear layer, IPL: inner plexiform layer, GCL: ganglion cell layer, IS: photoreceptor inner segment, OS: photoreceptor outer segment. Scale bars: 50 mm.
A: Twelve-week-old Cdc42 conditional knockdown (cKD; black box) and control (white box) mice were or were not (dark control; DC) exposed to 13 klux of white light for 2 h, and retinal tissue was isolated at different time points after light exposure as indicated (0–120 h). Relative mRNA levels of indicated genes were compared to the respective levels of non-exposed control mice (DC), which were set to ‘1’. Shown are mean values±SD of 3 independent mice. Statistical differences of means were calculated using ANOVA (ANOVA) followed by a bonferroni post-hoc test. * p<0.05; ** p<0.01; *** p<0.001.

B: Total retinal extracts from Cdc42 cKD and wild type controls (control) at indicated time points after light exposure (as in A) were immunoblotted. Shown are representative blots of n=3.

Figure 6. Ablation of cell division cycle 42 homolog (S.cerevisiae; CDC42) does not affect endogenous signaling pathways after light exposure.
localization was an early and transient event detected exclusively in TUNEL-negative cells. However, it seems unlikely that this accumulation predisposes cells to survive, since in the rd1 mouse for example, all photoreceptors eventually die despite the detection of perinuclear CDC42 localization early during the degenerative process.

Perinuclear localization of CDC42 has also been found in mammalian cells in vitro during serum-dependent processes [45] and in migrating cortical neurons during development [46]. It has also been reported that CDC42 may be involved in the subcellular distribution of its effector proteins including the mixed lineage kinase 3 [47] and the p21 activated kinase 5 (PAK5) [48]. Interestingly, members of both the mixed lineage kinase and PAK family of proteins have been connected to cell death signaling [49,50]. Altered Pak5 nucleocytoplasmic shuttling, for example, changed the sensitivity of neuroblastoma cells and neural stem cells to apoptosis [48]. However, we did not observe altered intracellular distribution of PAK5 in the degenerating retina (data not shown), suggesting that a CDC42-dependent PAK5 translocation mechanism similar to neuroblastoma may not be involved in photoreceptor apoptosis. Thus, the functional consequences of the perinuclear accumulation of CDC42 in photoreceptors of the degenerating retina need to be further studied.

The perinuclear localization of CDC42 in TUNEL-negative photoreceptor cells during retinal degeneration seems to be a general reaction of the retina to degeneration. This localization points to a prominent role of CDC42 in the retinal response to photoreceptor injury. Several studies have indicated an influence of CDC42 on apoptosis and/or survival through a variety of pathways in different systems [22,51, 52]. Many of these publications also include data on RAC1 and suggest overlapping roles of the two GTPases [53,54]. Such an overlap or redundancy can also be suspected for the maintenance of the integrity of normal rod photoreceptors in the adult mouse retina. Separate ablation of RAC1 [21] and CDC42 (this work) did not affect the structure and function of these sensory cells. During photoreceptor degeneration, however, RAC1 and CDC42 may have distinct roles. First, CDC42 localized in TUNEL-negative (Figure 2), whereas RAC1 colocalized with TUNEL-positive cells [55]. Second, the rod-specific knockdown of CDC42 did not prevent photoreceptor loss in the light-induced or inherited model of retinal degeneration—in contrast to the rod-specific ablation of RAC1, which protected rods after excessive light exposure [21]. This protection was attributed to reduced oxidative stress through inhibition of NADPH oxidase [21]. These differences are remarkable and add to the growing list of cellular settings where CDC42 and RAC1 exhibit functional differences.

Our gene expression data showed that light exposure caused a similar fold induction (over basal levels) of genes involved in the LIF-controlled survival pathway in both wild-type and Cdc42 knockdowns. Similar observations were made for the RAC1 knockdowns, where it was found that the expression of members of LIF pathway was also unaltered [21]. However, the CDC42 knockdown resulted in an increased basal expression of Gfap and Edn2. Recently, the binding of endothelins to endothelin receptor type beta (EDNRB) and EDNRA was shown to activate astrocytes near the optic nerve head [56]. Indeed, Cdc42 knockout mice may have an increased astrocytic network in the GCL (data not shown). Astrocyte activation was also reported after Cdc42 ablation in cortical progenitors during development [57]. The relevance of these observations needs to be elucidated, and it needs to be established how the lack of CDC42 may stimulate Edn2 expression.

In summary, we showed that CDC42 accumulates in the perinuclear region of photoreceptors during retinal degeneration. However, the susceptibility of photoreceptors to degeneration and progression of cell death were not affected by the Cdc42 knockdown. Therefore, the precise role of CDC42 in photoreceptors remains to be identified but it may include a modulatory function of the cellular physiology to adapt to stress situations. Our results are especially of relevance when choosing a particular member of the family of small GTPases as a potential target for therapeutic interventions in retinal degenerative diseases.

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