Suppressing thyroid hormone signaling preserves cone photoreceptors in mouse models of retinal degeneration

Hongwei Ma[a], Arjun Thapa[a], Lynsie Morris[a], T. Michael Redmond[b], Wolfgang Baehr[c], and Xi-Qin Ding[a,1]

[a]Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104; [b]Laboratory of Retinal Cell and Molecular Biology, National Eye Institute, Bethesda, MD 20892; and [c]John A. Moran Eye Center, University of Utah, Salt Lake City, UT 84132

Edited by Jeremy Nathans, Johns Hopkins University, Baltimore, MD, and approved January 24, 2014 (received for review September 9, 2013)

Cone phototransduction and survival of cones in the human macula is essential for color vision and for visual acuity. Progressive cone degeneration in age-related macular degeneration, Stargardt disease, and recessive cone dystrophies is a major cause of blindness. Thyroid hormone (TH) signaling, which regulates cell proliferation, differentiation, and apoptosis, plays a central role in cone opsin expression and patterning in the retina. Here, we investigated whether TH signaling affects cone viability in inherited retinal degeneration mouse models. Retinol isomerase RPE65-deficient mice [a model of Leber congenital amaurosis (LCA) with rapid cone loss] and cone photoreceptor function loss type 1 mice (severe recessive achromatopsia) were used to determine whether suppressing TH signaling with antithyroid treatment reduces cone death. Further, cone cyclic nucleotide-gated channel B subunit-deficient mice (moderate achromatopsia) and guanylate cyclase 2e-deficient mice (LCA with slower cone loss) were used to determine whether triiodothyronine (T3) treatment (stimulating TH signaling) causes deterioration of cones. We found that cone density in retinol isomerase RPE65-deficient and cone photoreceptor function loss type 1 mice increased about sixfold following antithyroid treatment. Cone density in cone cyclic nucleotide-gated channel B subunit-deficient and guanylate cyclase 2e-deficient mice decreased about 40% following T3 treatment. The effect of TH signaling on cone viability appears to be independent of its regulation on cone opsin expression. This work demonstrates that suppressing TH signaling in retina dystrophy mouse models is protective of cones, providing insights into cone preservation and therapeutic interventions.

Rod and cone photoreceptors degenerate under a variety of pathological conditions, including a wide array of hereditary retinal diseases, such as retinitis pigmentosa, macular degeneration, and cone–rod dystrophies. Defects in a large number of genes are linked to inherited retinal degenerative disorders (www.sph.uth.tmc.edu/RetNet/disease.htm), including those encoding enzymes involved in the recycling of 11-*cis* retinal in the retinal pigment epithelium (RPE), retinoid isomerase (RPE65), and lecithin retinol acyltransferase (LRAT), and the phototransduction-associated proteins (opsins, subunits of transducin, cGMP phosphodiesterase PDE6, guanylate cyclase, and cyclic nucleotide-gated channel). There are currently no treatments for human retinal dystrophies. Despite a high genetic heterogeneity, the degenerating photoreceptors show common cellular disorder features, including oxidative damage (1, 2), endoplasmic reticulum stress (3, 4), and apoptosis (5, 6).

Thyroid hormone (TH) signaling regulates cell proliferation, differentiation, and apoptosis. The role of TH signaling in retina regarding its regulation of cone opsin expression and patterning has been well documented (7, 8). Most mammals possess dichromatic color vision that is mediated by two opsins with peak sensitivities to medium-long (M, green) and short (S, blue) wavelengths of light (9, 10). In mouse, M- and S-opsins are expressed in opposing gradients such that varying amounts of both opsins are coexpressed in cones in midretinal regions, whereas M-opsin predominates in dorsal (superior) regions and S-opsin predominates in ventral (inferior) regions (10, 11) (Fig. S1). During development and in the adult postmitotic retina, TH signaling via its receptor type β2 (TRβ2) suppresses expression of S-opsin, induces expression of M-opsin, and promotes the dorsal–ventral opsin patterning (7, 8). Importantly, TH signaling has been associated with cone viability. Triiodothyronine (T3) treatment was shown to cause cone death in mice and this effect was reversed by deletion of TRβ2 gene (12). Excessive TH signaling was also shown to induce auditory defects and cochlear degeneration in mice (13). TH signaling has been associated with apoptosis of a variety of human cell lines, including lymphocytes (14), breast cancer cells (15), HeLa cells (16), and pituitary tumor cells (17), and TH signaling has been well documented in apoptotic tissue remodeling during anuran metamorphosis (18, 19). To determine whether TH signaling affects cone viability in inherited retinal degeneration, we investigated cone death/survival in retinal degeneration mouse models following TH signaling suppression and stimulation. Retinol isomerase RPE65-deficient (*Rpe65*$^{-/-}$) (a model of Leber congenital amaurosis, LCA) (20, 21) and cone photoreceptor function loss type 1 (*cpfl1*) mice (PDE6C mutation, a model of achromatopsia) (22), displaying fast and severe cone degeneration, were used to determine whether suppressing TH signaling with antithyroid treatment reduces cone degeneration. Cone cyclic nucleotide-gated channel B subunit-deficient (*Cngb3*$^{-/-}$) (a model of achromatopsia) (23) and guanylate cyclase 2e-deficient (*Gucy2e*$^{-/-}$) (another model of LCA) mice (24), displaying relatively slow progressive and moderate cone degeneration, were used to determine whether stimulating TH signaling (with T3

Significance

Photoreceptors degenerate in a wide array of hereditary retinal diseases and age-related macular degeneration. There is currently no treatment available for retinal degenerations. While outnumbered roughly 20:1 by rods in the human retina, it is the cones that mediate color vision and visual acuity, and their survival is critical for vision. In this communication, we investigate whether thyroid hormone (TH) signaling affects cone viability in retinal degeneration mouse models. TH signaling is known to be important for cone pigment expression and patterning, but excess TH signaling causes death of cones. We demonstrate that suppressing TH signaling in mouse models of cone–rod dystrophy preserves cones. This finding may lead to a novel and substantially different approach for retinal degeneration management.

Author contributions: H.M. and X.-Q.D. designed research; H.M., A.T., and L.M. performed research; H.M., A.T., and L.M. analyzed data; and H.M., T.M.R., W.B., and X.-Q.D. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence should be addressed. E-mail: xi-qin-ding@ouhsc.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1317041111/-/DCSupplemental.

treatment) deteriorates cones. We report here that cone survival was greatly improved in *Rpe65*$^{-/-}$ and *cpfl1* mice following TH signaling suppression, whereas cone degeneration was significantly increased in *Cngb3*$^{-/-}$ and *Gucy2e*$^{-/-}$ mice following TH signaling stimulation, demonstrating a protective role of suppressing TH signaling in cones.

Results

Suppressing TH Signaling Preserves Cones in Rpe65$^{-/-}$ Mice. *Rpe65*$^{-/-}$, a severe cone degeneration model, displays rapid ventral and central cone loss, whereas dorsal cones are preserved relatively well (20, 21). Our cone density evaluation by peanut agglutinin (PNA) and cone arrestin (CAR) labeling in *Rpe65*$^{-/-}$ mice showed a degeneration pattern similar to that reported previously (20, 25), i.e., the ventral and central retina shows early onset, fast cone degeneration (about 10% of the wild-type level remained at postnatal day 30, P30), whereas the peripheral dorsal retina degenerated more slowly (about 50% of the wild-type level remained at P30) (Fig. 1 *A* and *B*). *Rpe65*$^{-/-}$ mice received antithyroid drug (methimazole and sodium perchlorate monohydrate) treatment for 30 d, beginning on P1. The antithyroid treatment reduced serum T3 levels by about 30% in the treated mice, compared with untreated controls, when measured on the last day of the treatment (Table S1). This treatment significantly increased cone density. Cone density evaluation showed that the number of PNA-labeled cones in the ventral and dorsal retinas in antithyroid-treated *Rpe65*$^{-/-}$ mice increased about 6- and 1.3-fold, respectively, compared with untreated controls (Fig. 1 *A* and *B*). Similarly, CAR-labeled cones in the ventral retinas increased about 4-fold. The increased cone density in antithyroid-treated *Rpe65*$^{-/-}$ mice was also shown by PNA and CAR labeling on retinal cross-sections (Fig. S2). These results demonstrate a protective role of suppressing TH signaling in *Rpe65*$^{-/-}$ cones.

The effect of TH signaling on cone opsin expression has been well characterized (7, 8). We examined M- and S-cone density by labeling M- and S-opsin with specific antibodies. In agreement with reported information in wild-type mice, M-opsin–labeled cones (M cones) were significantly reduced in *Rpe65*$^{-/-}$ mice following antithyroid treatment, whereas S-opsin–labeled cones (S cones) were greatly increased, compared with untreated controls (Fig. 1 *A* and *B* and Fig. S2). Cone preservation in *Rpe65*$^{-/-}$ mice following TH signaling suppression was also evaluated by examining the expression levels of cone specific proteins. CAR and cone transducin α-subunit [guanine nucleotide-binding protein G(t) subunit alpha-2 (GNAT2)] expression levels in antithyroid-treated *Rpe65*$^{-/-}$ mice increased by 30–40%, compared with untreated

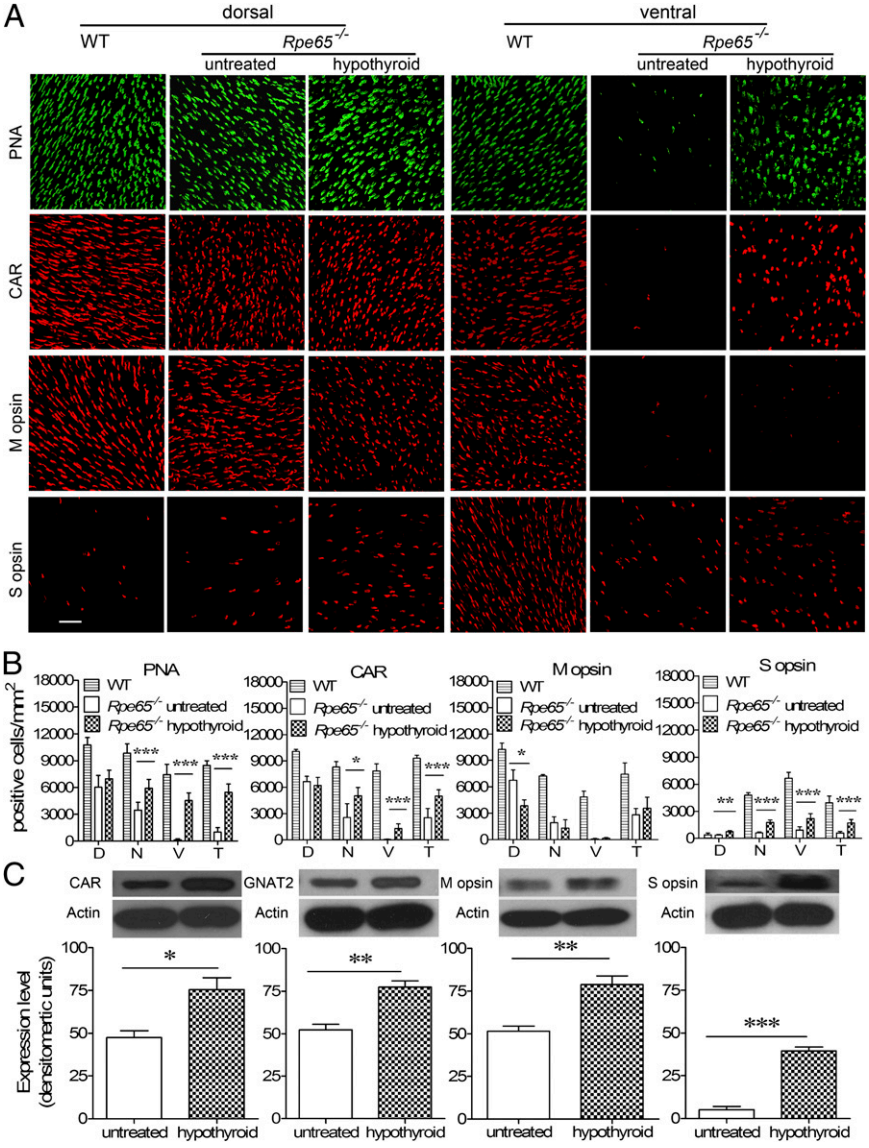

Fig. 1. Suppressing TH signaling preserves cones in *Rpe65*$^{-/-}$ mice. *Rpe65*$^{-/-}$ mice received antithyroid treatment for 30 d, beginning on P1. At the end of the treatment, cone density was evaluated by immunofluorescence labeling on retinal whole mounts, and cone-specific protein expression was evaluated by Western blotting. (*A*) Representative confocal images of immunofluorescence labeling of PNA, CAR, M-opsin, and S-opsin in hypothyroid and untreated *Rpe65*$^{-/-}$ mice and wild-type (WT) mice. (Scale bar, 10 μm.) (*B*) Correlating quantitative analysis of the immunofluorescence labeling. (*C*) Shown are representative images of the Western blot detection of CAR, GNAT2, M-opsin, and S-opsin, and the correlating quantifications. Data are represented as mean ± SEM of three to four assays using eyes/retinas from four mice. Unpaired Student *t* test was used to determine significance between hypothyroid and untreated *Rpe65*$^{-/-}$ mice (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$).

controls (Fig. 1*C*). Similar to S-opsin labeling of cones, S-opsin expression levels were increased about 5-fold. By quantitative (q)RT-PCR, the mRNA levels of S-opsin and M-opsin in antithyroid-treated *Rpe65*$^{-/-}$ mice increased about 3.8-fold and decreased by about 20%, respectively (Fig. S3). This finding is consistent with the previously demonstrated effect of TH signaling on cone opsin expression (7, 8).

Suppressing TH Signaling Preserves Cones in Cpfl1 Mice. *Cpfl1*, a naturally occurring cone degeneration mouse line, is another fast and severe cone degeneration model (26, 27). *Cpfl1* mice received antithyroid drug (methimazole and sodium perchlorate monohydrate) treatment for 30 d, beginning on P1. The antithyroid treatment reduced serum T3 levels in the treated mice by about 50%, compared with untreated controls, when measured on the last day of the treatment (Table S1). This treatment significantly improved cone survival. Fig. 2 shows representative images of PNA, GNAT2, M-opsin, and S-opsin labeling of retinal cross sections (Fig. 2*A*) and their quantifications (Fig. 2*B*) in P30 antithyroid-treated and untreated *cpfl1* mice. Cone density in *cpfl1* mice was about 50% of the wild-type level. Following antithyroid treatment, cone density was increased to about 70–80% of the wild-type level (Fig. 2 *A* and *B*). Similar to the observations in *Rpe65*$^{-/-}$ mice, M cones were significantly reduced, whereas S cones were greatly increased (about a 0.7- to 5.0-fold increase, depending on the retinal areas, with the maximal increase observed in the dorsal retina) (Fig. 2 and Fig. S4). qRT-PCR analysis showed a 3.8-fold increase in S-opsin mRNA levels and a 50% decrease of M-opsin mRNA level in *cpfl1* mice after antithyroid treatment (Fig. S5).

To determine whether suppressing TH signaling affects postmitotic cone survival, we treated adult *cpfl1* mice with antithyroid drugs, beginning on P25 and continuing for 30 d. Cone density analysis revealed that PNA- and CAR-labeled cones on retinal sections prepared from treated *cpfl1* mice increased about 30–40%, compared with age-matched, untreated controls (Fig. 3). The improved cone survival was also confirmed by PNA labeling on retinal whole mounts (Fig. S6). Similar to the observation obtained in mice receiving treatment at an early age (Fig. 2 and Fig. S4), M cones were significantly reduced, whereas S cones were greatly increased (Fig. 3). These results indicate that suppressing TH signaling after retina development is completed, still preserves cones.

Stimulating TH Signaling Deteriorates Cones in Cngb3$^{-/-}$ Mice. We used *Cngb3*$^{-/-}$ mice, which show a slow progressive and moderate cone degeneration phenotype (23) to determine the effect of stimulating TH signaling with T3. *Cngb3*$^{-/-}$ mice received T3 treatment (0.1 μg/g body weight, s.c. injection, once each day) for 30 d, beginning on P1. The treatment increased serum T3 level in the treated mice by about eightfold, compared with untreated controls, when measured around 24 h after the last injection (Table S1). Following T3 treatment, cone density in *Cngb3*$^{-/-}$ mice was significantly reduced. PNA-labeled cones in the T3-treated mice were reduced by about 30%, and CAR-labeled cones by about 40%, compared with untreated *Cngb3*$^{-/-}$ mice (Fig. 4 and Fig. S7). These results demonstrate that stimulating TH signaling deteriorates cones. In agreement with the findings in wild-type mice (12), M- and S-opsin–labeled cones in T3-treated *Cngb3*$^{-/-}$ mice were greatly reduced, compared with untreated controls (Fig. 4 and Fig. S7).

Stimulating TH Signaling Deteriorates Cones in Gucy2e$^{-/-}$ Mice. The *Gucy2e*$^{-/-}$ mouse line, a model with relatively slow progressive and moderate cone degeneration (24), was also used to determine the effect of stimulating TH signaling on cone survival. *Gucy2e*$^{-/-}$ mice received T3 treatment (0.1 μg/g body weight, s.c. injection, once each day) for 30 d, beginning on P1. The treatment increased serum T3 level in the treated mice by about sixfold, compared with untreated controls, when measured around

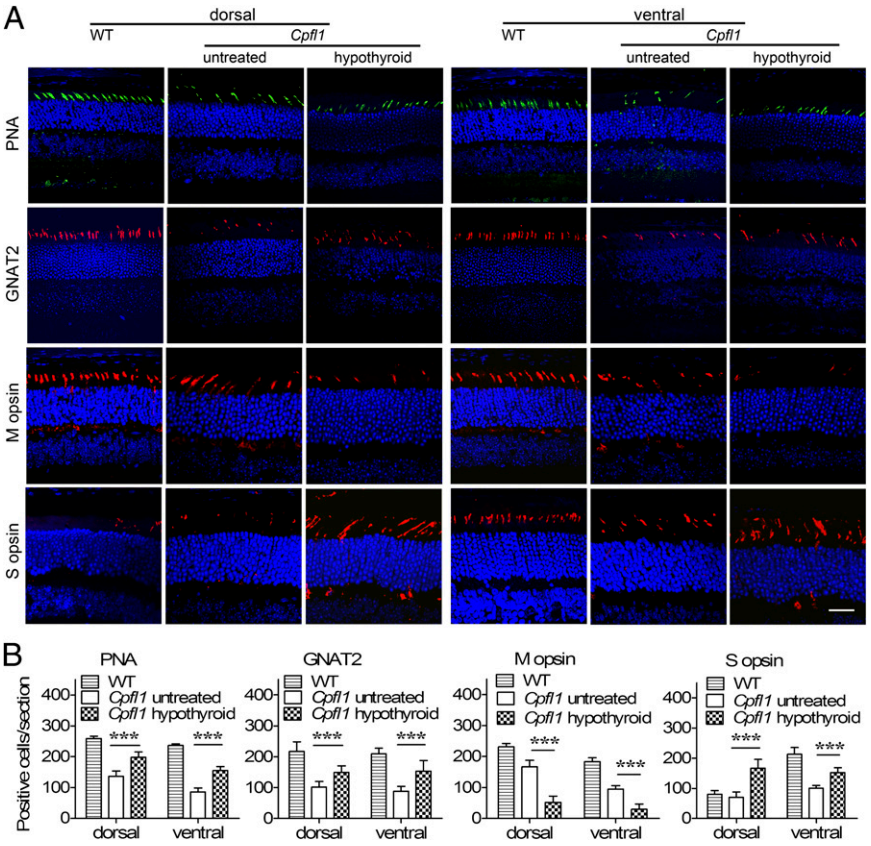

Fig. 2. Suppressing TH signaling preserves cones in *cpfl1* mice. *Cpfl1* mice received antithyroid treatment for 30 d, beginning on P1. At the end of the treatment, cone density was evaluated by immunofluorescence labeling on retinal cross-sections. (*A*) Representative confocal images of immunofluorescence labeling of PNA, GNAT2, M-opsin, and S-opsin in hypothyroid and untreated *cpfl1* mice and wild-type (WT) mice. (Scale bar, 50 μm.) (*B*) Correlating quantitative analysis of the immunofluorescence labeling. Data are represented as mean ± SEM of four assays using eyes from three to four mice. Unpaired Student *t* test was used to determine significance between hypothyroid and untreated *cpfl1* mice (***$P < 0.001$).

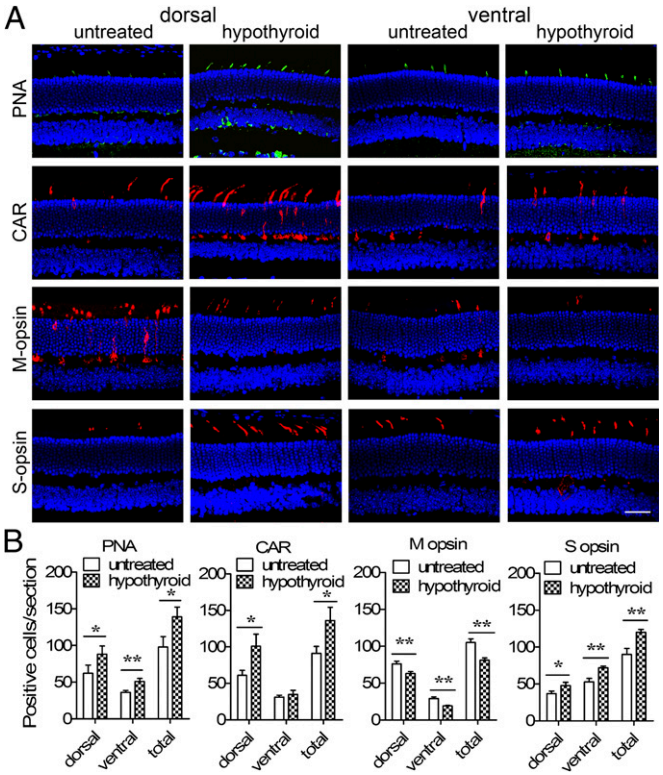

Fig. 3. Suppressing TH signaling preserves cones in *cpfl1* mice with treatment starting after weaning. *Cpfl1* mice received antithyroid treatment for 30 d, beginning on P25. At the end of the treatment, cone density was evaluated by immunofluorescence labeling on retinal sections. (*A*) Representative confocal images of immunofluorescence labeling of PNA, CAR, M-opsin, and S-opsin in hypothyroid and untreated mice. (Scale bar, 50 μm.) (*B*) Correlating quantitative analysis of the immunofluorescence labeling. Data are represented as mean ± SEM of three assays using eyes from four mice. Unpaired Student *t* test was used to determine significance between hypothyroid and untreated *cpfl1* mice (**P* < 0.05 and ***P* < 0.01).

24 h after the last injection (Table S1). Similar to the observation in $Cngb3^{-/-}$ mice, stimulating TH signaling significantly diminished cone density in $Gucy2e^{-/-}$ mice. Density of cones labeled by PNA, CAR, and cone opsin in T3-treated mice was reduced by about 30% compared with untreated controls (Fig. 5).

Effects of TH Signaling on Rod Photoreceptor Viability. To determine whether stimulating TH signaling affects rod survival, we examined retinal morphology and rod abundance in $Cngb3^{-/-}$ *and* $Gucy2e^{-/-}$ mice that received T3 treatment for 30 d. The evaluation was performed by directly counting the number of nuclei in the outer nuclear layer (ONL) and by measuring ONL thickness. These methods are reasonably accurate measurements of photoreceptor layer integrity (28) and are commonly used to assess photoreceptor cell damage. Both measurements yielded the same results, showing T3 treatment impaired ONL integrity/rod survival in our experimental animals. The average number of nuclei in the ONL in $Cngb3^{-/-}$ (Fig. 6*A*) and $Gucy2e^{-/-}$ (Fig. 6*B*) mice was reduced by about 30%, compared with their respective untreated controls. Similar results were obtained with measurement of ONL thickness (Fig. S8). In a separate experiment, we examined whether suppressing TH signaling has any negative effects on rod viability. The number of nuclei in the ONL and ONL thickness in antithyroid-treated *cpfl1* mice was evaluated, and the results showed no differences between treated and untreated *cpfl1* mice (Fig. 6*C* and Fig. S8). Thus, unlike the effect of increased TH signaling, suppressing TH signaling does not have negative effects on rod photoreceptor viability.

Discussion

As an essential growth regulator, TH plays a major role in cell proliferation, differentiation, and apoptosis. Using multiple mouse models, we demonstrate that suppressing TH signaling preserves cone photoreceptors in degenerating retinas. The protective effects were observed in both developing and postmitotic adult mice. Stimulating TH signaling, in contrast, promotes cone and rod photoreceptor death.

The effect of TH signaling on cone viability appears to be independent of its regulation of cone opsin expression. In the mouse retina, TH signaling plays a central role in cone opsin expression and dorsal–ventral patterning. It increases expression of M-opsin, suppresses expression of S-opsin, and promotes/maintains their dorsal–ventral gradient distribution (7, 8). The high expression level of S-opsin in the ventral retina has been associated with a more rapid degeneration in this region (compared with dorsal retina) that has been observed in retinal degeneration mouse models, including $Rpe65^{-/-}$ and $Lrat^{-/-}$ mice (29). It was shown that the rapid cone degeneration in $Rpe65^{-/-}$ and $Lrat^{-/-}$ mice is likely due to an absence of 11-*cis*-retinal, which causes aggregation of S-opsin and leads to apoptosis, and that the presence of a phenylalanine-rich region of S-opsin probably contributes to the protein aggregation (29).

This work shows that suppressing TH signaling in $Rpe65^{-/-}$ and *cpfl1* mice greatly reduced cone death, accompanied by increased expression of S-opsin and S cones and decreased M cones. Stimulating TH signaling in $Cngb3^{-/-}$ and $Gucy2e^{-/-}$ mice, in

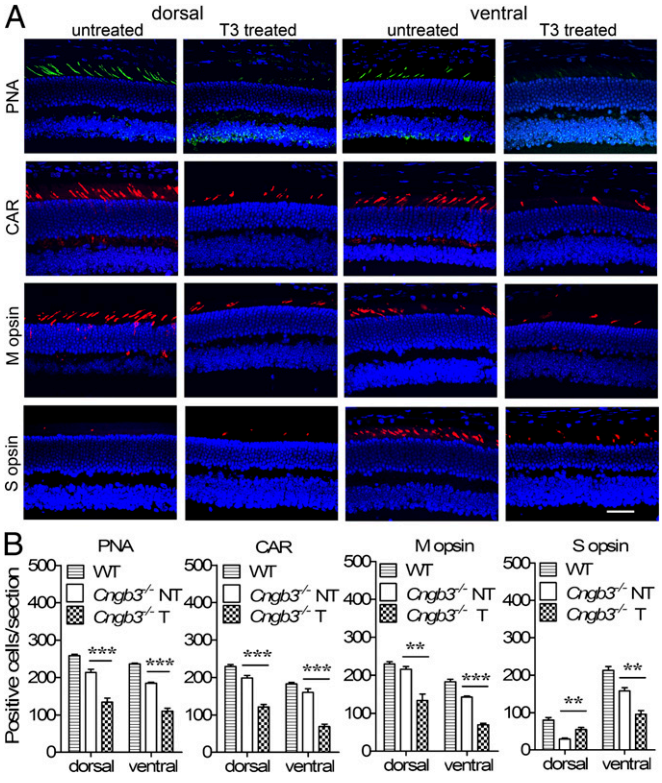

Fig. 4. Stimulating TH signaling deteriorates cones in $Cngb3^{-/-}$ mice. $Cngb3^{-/-}$ mice received T3 treatment for 30 d, beginning on P1. At the end of the treatment, cone density was evaluated by immunofluorescence labeling on retinal sections. (*A*) Representative confocal images of immunofluorescence labeling of PNA, CAR, M-opsin, and S-opsin in T3-treated and untreated $Cngb3^{-/-}$ mice. (Scale bar, 50 μm.) (*B*) Correlating quantitative analysis of the immunofluorescence labeling. NT, untreated; T, T3 treated. Data are represented as mean ± SEM of three to four assays using eyes from four mice. Unpaired Student *t* test was used to determine significance between T3-treated and untreated $Cngb3^{-/-}$ mice (***P* < 0.01 and ****P* < 0.001).

NEUROSCIENCE

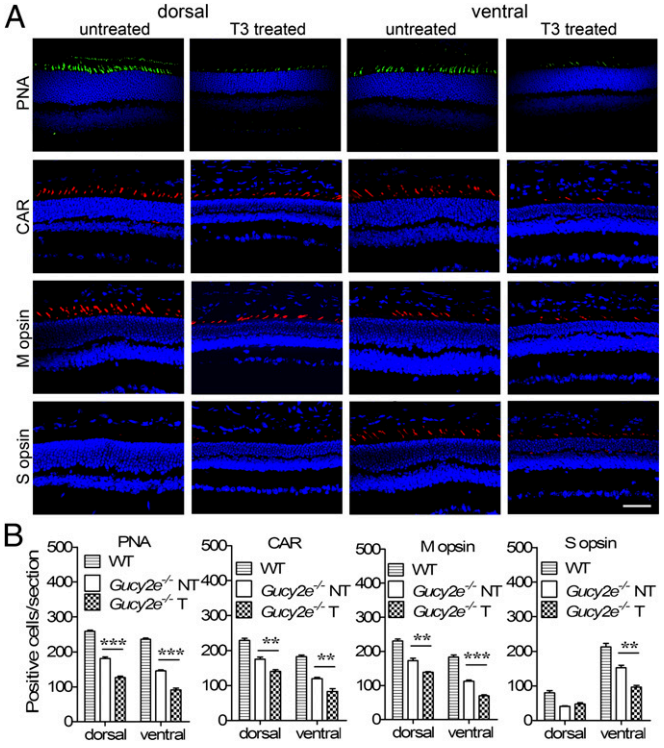

Fig. 5. Stimulating TH signaling deteriorates cones in *Gucy2e*$^{-/-}$ mice. *Gucy2e*$^{-/-}$ mice received T3 treatment for 30 d, beginning on P1. At the end of the treatment, cone density was evaluated by immunofluorescence labeling on retinal sections. (*A*) Representative confocal images of immunofluorescence labeling of PNA, CAR, M-opsin, and S-opsin in T3-treated and untreated *Gucy2e*$^{-/-}$ mice. (Scale bar, 50 μm.) (*B*) Correlating quantitative analysis of the immunofluorescence labeling. NT, untreated; T, T3 treated. Data are represented as mean ± SEM of three to four assays using eyes from four mice. Unpaired Student *t* test was used to determine significance between T3-treated and untreated *Gucy2e*$^{-/-}$ mice (**$P < 0.01$ and ***$P < 0.001$).

contrast, profoundly increased cone death, accompanied by reduced S-opsin expression level. Thus, TH signaling-mediated regulation of cone viability appears to be independent of TH regulation of cone opsin expression. This view is also supported by the following: (*i*) Stimulating TH signaling induces death of rods, which do not express cone opsin; (*ii*) high TH signaling causes auditory deficits and cochlear degeneration (13); and (*iii*) high TH signaling induces death of other cell types, including cancer cells (14–17), which do not express opsins.

Although TH signaling plays a major role in cone opsin expression and patterning and high TH signaling induces cone and rod death, suppressing TH signaling has no negative effect on cone and rod structure, function (as evaluated by electroretinogram analysis), or survival during development and in adult mice (7, 30, 31). This is in agreement with the observations in a human patient carrying TRβ2 mutations. This patient merely showed red/green color complications but rod morphology and rhodopsin expression level remained normal (32). In this study, we found no negative effect of antithyroid treatment on rod survival in our degenerating mouse models. The underlying mechanism in which high TH signaling becomes harmful for cones and rods remains to be determined, whereas low TH signaling is not detrimental, but protective.

TH signaling regulating cone viability is likely mediated via TRβ2, which, in the retina, is expressed only in cones (33, 34). T3-induced cone death did not occur in *Thrβ2*$^{-/-}$ mice, and cone degeneration caused by deficiency of type 3 iodothyronine deiodinase (DIO3, an enzyme that inactivates T3) was rescued in *Dio3*$^{-/-}$/*Thrβ2*$^{-/-}$ mice (12). It would be interesting to develop a *Rpe65*$^{-/-}$/*Thrβ2*$^{-/-}$ or a *cpfl1*/*Thrβ2*$^{-/-}$ double knockout mouse model to investigate whether TRβ2 is involved in the survival of cones following TH signaling suppression. Recently, the receptor-independent nongenomic mechanism, which does not include initial nuclear action of TH receptor or gene transcription, but involves the cell surface receptor and signal transduction pathway, has been implicated in TH signaling (35, 36). Thus, we cannot exclude the possibility that the receptor-independent nongenomic mechanism is also involved in TH signaling-regulated cone viability. Compared with our knowledge regarding the role of TH signaling and expression/function of TRβ2 in cones, we know little about the role of TH signaling in rods. How rods die following T3 treatment remains poorly understood. Although TRα is more universally expressed and its mRNA expression has been detected in the mouse eye (7), there is no firm evidence showing the role of TRα in mammalian photoreceptors. Thus, the mechanism of rod death following T3 treatment remains to be determined. Rod death might be associated with TRα, but it could equally likely involve the TRβ1 isoform (another product of the TRβ gene). In addition, we cannot exclude the possibility that the loss of

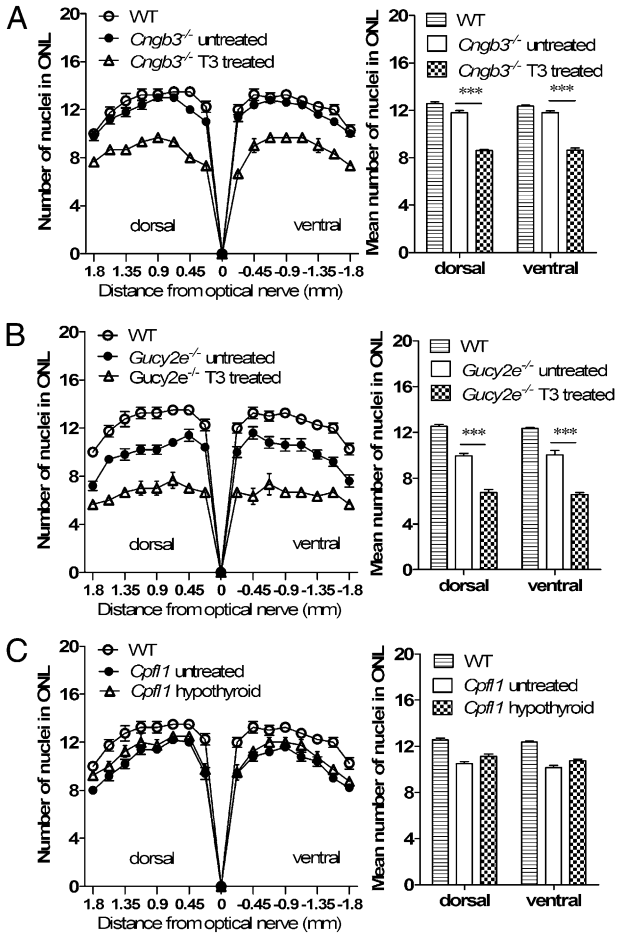

Fig. 6. Effects of TH signaling on rod photoreceptor viability. (*A* and *B*) Stimulating TH signaling causes rod degeneration in *Cngb3*$^{-/-}$ (*A*) and *Gucy2e*$^{-/-}$ (*B*) mice. Mice received T3 treatment for 30 d, beginning on P1. At the end of the treatment, retinal morphometric analysis was performed on retinal cross-sections. (*C*) Suppressing TH signaling does not affect rod viability in *cpfl1* mice. *Cpfl1* mice received antithyroid treatment for 30 d, beginning on P1. At the end of the treatment, retinal morphometric analysis was performed on retinal cross-sections. Shown are results of the nuclei count in the ONL (*Left*) and the mean numbers of nuclei in the ONL in the dorsal and ventral regions (*Right*). Data are represented as mean ± SEM of three to four assays using eyes from four mice. Unpaired Student *t* test was used to determine significance between treated and untreated mice (***$P < 0.001$).

rods following T3 treatment is indirect, and a result of primary cone loss.

Understanding the TH signaling regulation of photoreceptor viability provides insights into cone preservation and therapeutic interventions. As TRβ2 is expressed in cones only (in the retina), suppressing TH signaling locally (such as using a pharmacological agent or genetically knocking down TRβ2 or overexpressing DIO3) may represent a unique path to preserve cones in retinal degeneration. The impact of a local TH signaling inactivation has been observed in a variety of pathophysiological conditions (37). Indeed, a protective role of DIO3 in cochlear function and integrity has been demonstrated using *Dio3*$^{-/-}$ mice (13). Conversely, the findings that TH signaling promotes cone death might benefit the treatment of retinoblastoma, which has properties of a cone precursor tumor (38).

In summary, with multiple retinal degeneration mouse models, we demonstrate that TH signaling regulates photoreceptor viability in degenerating retinas. Suppressing TH signaling protects cones, whereas stimulating TH signaling has a negative effect on both cones and rods. The regulation by TH signaling of cone survival appears to be independent of its regulatory role in cone opsin expression. The findings of this study provide insights into cone preservation and therapeutic interventions.

Materials and Methods

Mice. The *Rpe65*$^{-/-}$ (21), *Cngb3*$^{-/-}$ (39), and *Gucy2e*$^{-/-}$ (24) mouse lines were generated as described previously. The *cpfl1* mouse line was obtained from The Jackson Laboratory and wild-type mice (C57BL/6) were purchased from Charles River Laboratories. All mice were maintained under cyclic light (12-h light–dark) conditions. Cage illumination was ∼7 foot candles during the light cycle. All animal maintenance and experiments were approved by the local Institutional Animal Care and Use Committee (Oklahoma City, OK) and conformed to the guidelines on the care and use of animals adopted by the Society for Neuroscience and the Association for Research in Vision and Ophthalmology (Rockville, MD).

Drug Treatments. For antithyroid treatment, mother *Rpe65*$^{-/-}$ and *cpfl1* mice were treated with methimazole (MMI) (Sigma-Aldrich, 0.05% wt/vol) and sodium perchlorate monohydrate (PM) (Sigma-Aldrich, 1.0% wt/vol) in drinking water, beginning on the day they delivered pups and the treatment (for both mother and pups) continued for 30 d. In a separate experiment, postweaning *cpfl1* mice received MMI and PM treatment for 30 d, beginning on P25. For T3 treatment, *Cngb3*$^{-/-}$ and *Gucy2e*$^{-/-}$ mice received T3 (Sigma-Aldrich, 0.1 μg/g body weight, s.c. injection, once each day) or vehicle (saline) for 30 d, beginning on P1. At the end of the treatments, blood samples were collected for measurements of serum T3 levels, and eyes and retinas were collected for evaluating cone density and cone-specific protein/mRNA expression. For T3-treated mice, blood samples were collected around 24 h after the last injection. Additional details are provided in *SI Materials and Methods*.

ACKNOWLEDGMENTS. We thank Drs. Cheryl Craft and Muna Naash for providing antibodies for M-opsin, cone arrestin, and S-opsin. This work was supported by grants from the National Center for Research Resources (P20RR017703), the National Eye Institute (P30EY12190, R01EY019490, and R01EY08123), and the Oklahoma Center for the Advancement of Science and Technology.

1. Shen J, et al. (2005) Oxidative damage is a potential cause of cone cell death in retinitis pigmentosa. *J Cell Physiol* 203(3):457–464.
2. Komeima K, Rogers BS, Lu L, Campochiaro PA (2006) Antioxidants reduce cone cell death in a model of retinitis pigmentosa. *Proc Natl Acad Sci USA* 103(30):11300–11305.
3. Gorbatyuk MS, et al. (2010) Restoration of visual function in P23H rhodopsin transgenic rats by gene delivery of BiP/Grp78. *Proc Natl Acad Sci USA* 107(13):5961–5966.
4. Yang LP, Wu LM, Guo XJ, Tso MO (2007) Activation of endoplasmic reticulum stress in degenerating photoreceptors of the rd1 mouse. *Invest Ophthalmol Vis Sci* 48(11): 5191–5198.
5. Dunaief JL, Dentchev T, Ying GS, Milam AH (2002) The role of apoptosis in age-related macular degeneration. *Arch Ophthalmol* 120(11):1435–1442.
6. Sanges D, Comitato A, Tammaro R, Marigo V (2006) Apoptosis in retinal degeneration involves cross-talk between apoptosis-inducing factor (AIF) and caspase-12 and is blocked by calpain inhibitors. *Proc Natl Acad Sci USA* 103(46):17366–17371.
7. Ng L, et al. (2001) A thyroid hormone receptor that is required for the development of green cone photoreceptors. *Nat Genet* 27(1):94–98.
8. Roberts MR, Srinivas M, Forrest D, Morreale de Escobar G, Reh TA (2006) Making the gradient: Thyroid hormone regulates cone opsin expression in the developing mouse retina. *Proc Natl Acad Sci USA* 103(16):6218–6223.
9. Mollon JD, Bowmaker JK (1992) The spatial arrangement of cones in the primate fovea. *Nature* 360(6405):677–679.
10. Nathans J (1999) The evolution and physiology of human color vision: Insights from molecular genetic studies of visual pigments. *Neuron* 24(2):299–312.
11. Szél A, Röhlich P, Mieziewska K, Aguirre G, van Veen T (1993) Spatial and temporal differences between the expression of short- and middle-wave sensitive cone pigments in the mouse retina: A developmental study. *J Comp Neurol* 331(4):564–577.
12. Ng L, et al. (2010) Type 3 deiodinase, a thyroid-hormone-inactivating enzyme, controls survival and maturation of cone photoreceptors. *J Neurosci* 30(9):3347–3357.
13. Ng L, et al. (2009) A protective role for type 3 deiodinase, a thyroid hormone-inactivating enzyme, in cochlear development and auditory function. *Endocrinology* 150(4):1952–1960.
14. Mihara S, et al. (1999) Effects of thyroid hormones on apoptotic cell death of human lymphocytes. *J Clin Endocrinol Metab* 84(4):1378–1385.
15. Sar P, Peter R, Rath B, Das Mohapatra A, Mishra SK (2011) 3, 3′5 Triiodo L thyronine induces apoptosis in human breast cancer MCF-7 cells, repressing SMP30 expression through negative thyroid response elements. *PLoS ONE* 6(6):e20861.
16. Yamada-Okabe T, Satoh Y, Yamada-Okabe H (2003) Thyroid hormone induces the expression of 4-1BB and activation of caspases in a thyroid hormone receptor-dependent manner. *Eur J Biochem* 270(14):3064–3073.
17. Chiloeches A, Sánchez-Pacheco A, Gil-Araujo B, Aranda A, Lasa M (2008) Thyroid hormone-mediated activation of the ERK/dual specificity phosphatase 1 pathway augments the apoptosis of GH4C1 cells by down-regulating nuclear factor-kappaB activity. *Mol Endocrinol* 22(11):2466–2480.
18. Shi YB, Fu L, Hsia SC, Tomita A, Buchholz D (2001) Thyroid hormone regulation of apoptotic tissue remodeling during anuran metamorphosis. *Cell Res* 11(4):245–252.
19. Buchholz DR, Tomita A, Fu L, Paul BD, Shi YB (2004) Transgenic analysis reveals that thyroid hormone receptor is sufficient to mediate the thyroid hormone signal in frog metamorphosis. *Mol Cell Biol* 24(20):9026–9037.
20. Znoiko SL, et al. (2005) Downregulation of cone-specific gene expression and degeneration of cone photoreceptors in the *Rpe65*$^{-/-}$ mouse at early ages. *Invest Ophthalmol Vis Sci* 46(4):1473–1479.
21. Redmond TM, et al. (1998) Rpe65 is necessary for production of 11-*cis*-vitamin A in the retinal visual cycle. *Nat Genet* 20(4):344–351.
22. Chang B, et al. (2009) A homologous genetic basis of the murine *cpfl1* mutant and human achromatopsia linked to mutations in the PDE6C gene. *Proc Natl Acad Sci USA* 106(46):19581–19586.
23. Xu J, Morris L, Fliesler SJ, Sherry DM, Ding XQ (2011) Early-onset, slow progression of cone photoreceptor dysfunction and degeneration in CNG channel subunit CNGB3 deficiency. *Invest Ophthalmol Vis Sci* 52(6):3557–3566.
24. Yang RB, et al. (1999) Disruption of a retinal guanylyl cyclase gene leads to cone-specific dystrophy and paradoxical rod behavior. *J Neurosci* 19(14):5889–5897.
25. Chen Y, Moiseyev G, Takahashi Y, Ma JX (2006) RPE65 gene delivery restores isomerohydrolase activity and prevents early cone loss in *Rpe65*$^{-/-}$ mice. *Invest Ophthalmol Vis Sci* 47(3):1177–1184.
26. Chang B, et al. (2002) Retinal degeneration mutants in the mouse. *Vision Res* 42(4): 517–525.
27. Schaeferhoff K, et al. (2010) Induction of STAT3-related genes in fast degenerating cone photoreceptors of *cpfl1* mice. *Cell Mol Life Sci* 67(18):3173–3186.
28. Michon JJ, Li ZL, Shioura N, Anderson RJ, Tso MO (1991) A comparative study of methods of photoreceptor morphometry. *Invest Ophthalmol Vis Sci* 32(2):280–284.
29. Zhang T, Zhang N, Baehr W, Fu Y (2011) Cone opsin determines the time course of cone photoreceptor degeneration in Leber congenital amaurosis. *Proc Natl Acad Sci USA* 108(21):8879–8884.
30. Lu A, et al. (2009) Retarded developmental expression and patterning of retinal cone opsins in hypothyroid mice. *Endocrinology* 150(3):1536–1544.
31. Glaschke A, Glösmann M, Peichl L (2010) Developmental changes of cone opsin expression but not retinal morphology in the hypothyroid Pax8 knockout mouse. *Invest Ophthalmol Vis Sci* 51(3):1719–1727.
32. Weiss AH, Kelly JP, Bisset D, Deeb SS (2012) Reduced L- and M- and increased S-cone functions in an infant with thyroid hormone resistance due to mutations in the THRβ2 gene. *Ophthalmic Genet* 33(4):187–195.
33. Applebury ML, et al. (2007) Transient expression of thyroid hormone nuclear receptor TRbeta2 sets S opsin patterning during cone photoreceptor genesis. *Dev Dyn* 236(5): 1203–1212.
34. Ng L, Ma M, Curran T, Forrest D (2009) Developmental expression of thyroid hormone receptor β2 protein in cone photoreceptors in the mouse. *Neuroreport* 20(6):627–631.
35. Cheng SY, Leonard JL, Davis PJ (2010) Molecular aspects of thyroid hormone actions. *Endocr Rev* 31(2):139–170.
36. Hiroi Y, et al. (2006) Rapid nongenomic actions of thyroid hormone. *Proc Natl Acad Sci USA* 103(38):14104–14109.
37. Dentice M, Salvatore D (2011) Deiodinases: The balance of thyroid hormone: Local impact of thyroid hormone inactivation. *J Endocrinol* 209(3):273–282.
38. Xu XL, et al. (2009) Retinoblastoma has properties of a cone precursor tumor and depends upon cone-specific MDM2 signaling. *Cell* 137(6):1018–1031.
39. Ding XQ, et al. (2009) Impaired cone function and cone degeneration resulting from CNGB3 deficiency: Down-regulation of CNGA3 biosynthesis as a potential mechanism. *Hum Mol Genet* 18(24):4770–4780.