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Suppressing Thyroid Hormone Signaling Preserves Cone Photoreceptors in Mouse Models of Retinal Degeneration

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Introduction

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.

Methods

Rpe65$^{-/-}$ mice and *cpfl1* mice were used to determine whether suppressing TH signaling with anti-thyroid treatment reduces cone death. *Cngb3*$^{-/-}$ mice (moderate achromatopsia) and *Gucy2e*$^{-/-}$ mice (LCA with slower cone loss) were used to determine whether triiodothyronine (T3) treatment (stimulating TH signaling) causes deterioration of cones.

Results

Table 1. Serum T3 levels (nM) in anti-thyroid- or T3-treated mice

Mouse lines	Untreated	Anti-thyroid treated	T3 treated
Cpfl1	0.76±0.09	0.39±0.02**	
Rpe65$^{-/-}$	0.71±0.03	0.51±0.02**	
Cngb3$^{-/-}$	0.46±0.03		3.85±0.55**
Gucy2e$^{-/-}$	0.62±0.04		3.43±0.48***

**$p < 0.01$, ***$p < 0.001$

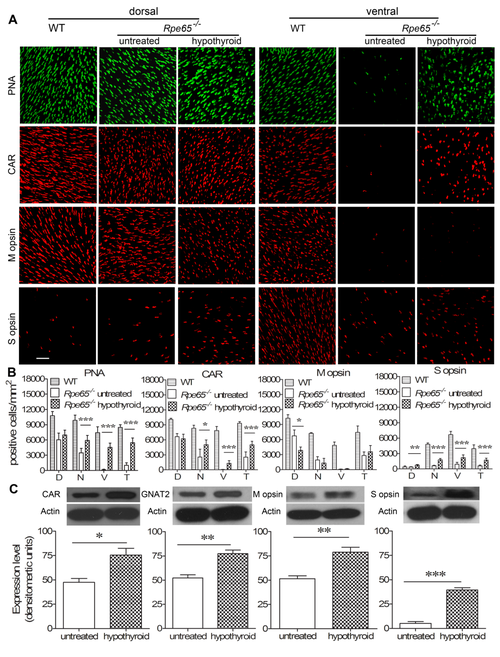

Figure 1. Suppressing TH signaling preserves cones in *Rpe65*$^{-/-}$ mice. *Rpe65*$^{-/-}$ mice received anti-thyroid treatment for 30 days, beginning on P1. At the end of the treatment, cone density was evaluated by immunofluorescence labeling on retinal whole mounts, and cone specific protein expression 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. (***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.

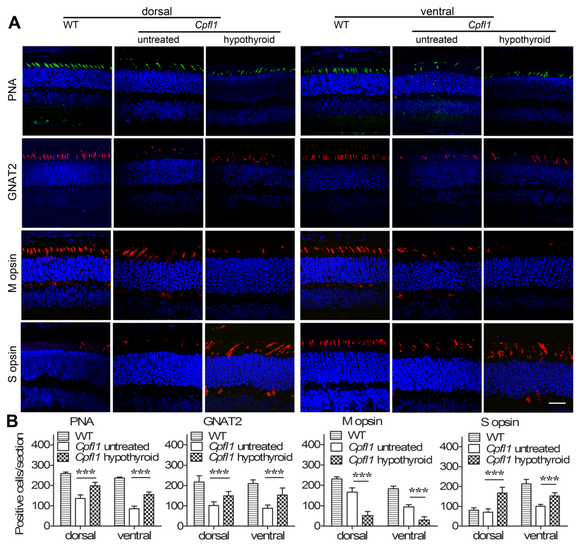

Figure 2. Suppressing TH signaling preserves cones in *cpfl1* mice. *Cpfl1* mice received anti-thyroid treatment for 30 days, beginning on P1. At the end of the treatment, cone density was evaluated by immunofluorescence labeling on retinal cross sections. (***A***) Representative confocal images of 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.

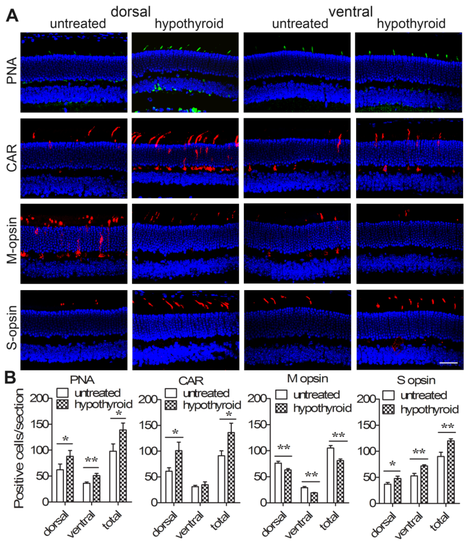

Figure 3. Suppressing TH signaling preserves cones in *cpfl1* mice with treatment starting after weaning. *Cpfl1* mice received anti-thyroid treatment for 30 days, beginning on P25. At the end of the treatment, cone density was evaluated by immunofluorescence labeling on retinal sections. (***A***) Representative 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.

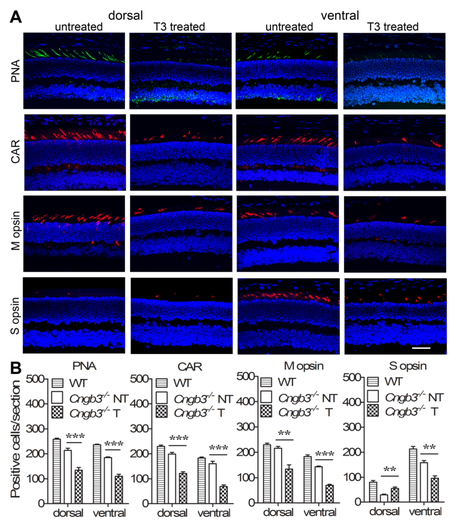

Figure 4. Stimulating TH signaling deteriorates cones in *Cngb3*$^{-/-}$ mice. *Cngb3*$^{-/-}$ mice received T3 treatment for 30 days, beginning on P1. At the end of the treatment, cone density was evaluated by immunofluorescence labeling on retinal sections. (***A***) Representative confocal images 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.

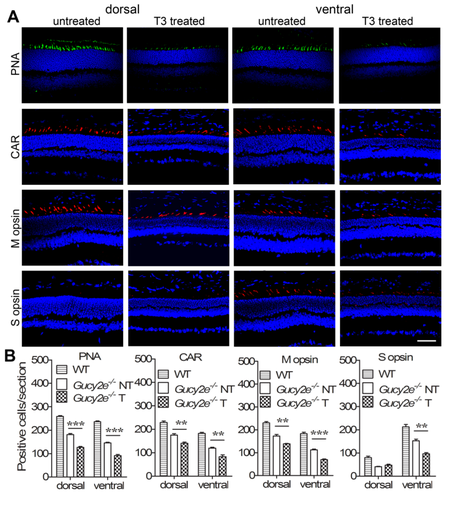

Figure 5. Stimulating TH signaling deteriorates cones in *Gucy2e*$^{-/-}$ mice. *Gucy2e*$^{-/-}$ mice received T3 treatment for 30 days, beginning on P1. At the end of the treatment, cone density was evaluated by immunofluorescence labeling on retinal sections. (***A***) Representative confocal images 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.

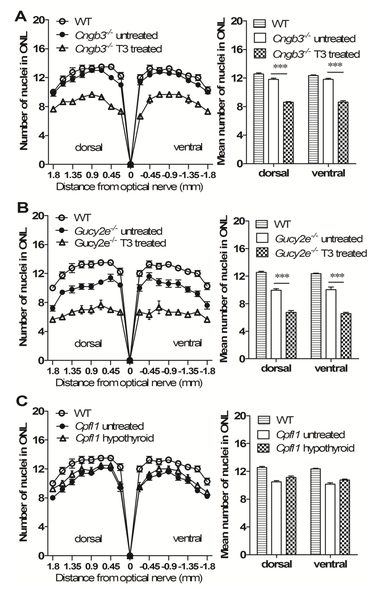

Figure 6. Effects of TH signaling on rod photoreceptor viability. (***A-B***) Stimulating TH signaling causes rod degeneration in *Cngb3*$^{-/-}$ (***A***) and *Gucy2e*$^{-/-}$ (***B***) mice. Mice received T3 treatment for 30 days, beginning on P1. At the end of the treatment, retinal morphometric analysis was performed on retinal cross sections. (***C***) Suppressing TH signaling does not affect rod viability in *cpfl1* mice. *Cpfl1* mice received anti-thyroid treatment for 30 days, 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 panels*) and the mean numbers of nuclei in the ONL in the dorsal and ventral regions (*right panels*). Data are represented as mean ± SEM of three to four assays using eyes from four mice.

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 new insights into cone preservation and therapeutic interventions.

Acknowledgements

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Disclosures

None