Thyroid Hormone Action Is Required for Normal Cone Opsin Expression during Mouse Retinal Development

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Purpose. The expression of S- and M-opsins in the murine retina is altered in different transgenic mouse models with mutations in the thyroid hormone receptor (TR)-β gene, demonstrating an important role of thyroid hormone (TH) in retinal development.

Methods. The spatial expression of S- and M-opsin was compared in congenital hypothyroidism and in two different TR mutant mouse models. One mouse model contains a ligand-binding mutation that abolishes TH binding and results in constitutive binding to nuclear corepressors. The second model contains a mutation that blocks binding of coactivators to the AF-2 domain without affecting TH binding.

Results. Hypothyroid newborn mice showed an increase in S-opsin expression that was completely independent of the genotype. Concerning M-opsin expression, hypothyroidism caused a significant decrease ($P < 0.01$) only in wild-type animals. When TRβ1 and -β2 were T3-binding defective, the pattern of opsin expression was similar to TRβ ablation, showing increased S-opsin expression in the dorsal retina and no expression of M-opsin in the entire retina. In an unexpected finding, immunostaining for both opsins was detected when both subtypes of TRβ were mutated in the helix 12 AF-2 domain.

Conclusions. The results show, for the first time, that the expression of S- and M-opsin is dependent on normal thyroid hormone levels during development. (*Invest Ophthalmol Vis Sci.* 2008;49:2039–2045) DOI:10.1167/iovs.07-0908

Thyroid hormone (TH) plays a central role in central nervous system development, including the proper development of the retina. Its role was demonstrated first in rat models in which congenital hypothyroidism reduces retinal thickness, volume, and cell number.[1,2] Nuclear TH action is mediated by different isoforms of the thyroid hormone receptor (TR), which are members of the nuclear receptor super family of ligand-modulated transcriptional factors.[3] Two genes express all known ligand-binding TR isoforms: TRα1, and TRβ1 to -β3. It is well accepted that thyroid hormone receptors regulate gene expression either as homodimers or, more frequently, as heterodimeric complexes with retinoid X receptors (RXR).[3]

All isoforms of TR are present in the developing and adult retina of rodents.[4,5] The presence of TRβ2 is known to be essential for normal development of the retina, especially for the attainment of color vision.[6] For example, it has been demonstrated in vitro that TH promotes differentiation of cone photoreceptors of rat and human progenitor cells.[7] The mouse retina contains a mixture of rod and cone photoreceptors and, similar to many other mammals, has two types of cone photopigments[8]: short-wavelength (S)–sensitive pigment, with a maximum absorption in the ultraviolet range, and middle-wavelength (M)–sensitive pigment.[9] Recently, it has been demonstrated that mice can make dichromatic color discrimination, and their visual systems, therefore, must be able to detect spectral absorption differences.[10]

In vivo studies of flounder have suggested that TRβ and TRα are expressed in the retina and that TRα is apparently responsible for TH-dependent cone differentiation.[11] The first studies in vivo, in which rodents were used, demonstrated that TRβ2 knockout (KO) animals do not express M-opsin, suggesting that it is essential for the expression of this opsin.[6] In addition to TRβ2 KO animals, other animal models with impaired TRβ function were studied, and they also do not express M-opsin.[12–14] TRα1 does not seem to be critical in retinal development in rodents, based on the paucity of published reports.[11]

The syndrome of human resistance to thyroid hormone (RTH) is characterized by elevated levels of thyroid hormone and TSH, as well as other symptoms,[15–17] typically due to TRβ mutations.[15,18] Visual defects in patients with RTH have been reported[15]; however, to date, we are not aware of any systematic study of visual function in these patients.

In vitro, studies have demonstrated that TR action is regulated by interactions with corepressor (CoR) and coactivator (CoA) molecules.[3] T3 binding to TR results in dissociation of CoRs and recruitment of CoAs to the AF-2 domain (helix 12), resulting in activation of the transcription. Given the importance of TRs for opsin gene expression, we decided to determine what additional functions of TRβ, besides ligand-binding, are necessary for attainment of normal visual function in the mouse. In this study, we compared S- and M-opsin expression and distribution in different transgenic mouse models. In one model (E457A), helix 12 of TRβ was disrupted by a mutation that blocks CoA binding without affecting T3-binding.[19] In a second model (Δ337T), the ligand-binding domain was mutated so that CoR bound constitutively to TRβ in the absence or the presence of T3. TR expressing the Δ337T is unable to bind T3. Finally, we evaluated S- and M-opsin expression in WT and TRβ2 KO mice. Furthermore, we studied a model of congeni-

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Supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Third World Academy of Sciences (TWAS), Fundação Carlos Chagas Filho de Amparo a Pesquisa do Estado do Rio de Janeiro (FAPERJ), and Coordenação de Aperfeiçoamento de Pessoal de nível superior (CAPES).

Submitted for publication July 17, 2007; revised December 12, 2007; accepted March 6, 2008.

Disclosure: **C.N. Pessôa**, None; **L.A. Santiago**, None; **D.A. Santiago**, None; **D.S. Machado**, None; **F.A.F. Rocha**, None; **D.F. Ventura**, None; **J.N. Hokoç**, None; **C.C. Pazos-Moura**, None; **F.E. Wondisford**, None; **P.F. Gardino**, None; **T.M. Ortiga-Carvalho**, None

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tally hypothyroidism to evaluate its effects on S- and M-opsin expression.

METHODS

The original research reported herein was performed according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. In addition, all procedures were approved by the commission of animal care of the Institute of Biophysics Carlos Chagas Filho of the Federal University of Rio de Janeiro, according to the NIH Guide for the Care and Use of Laboratory Animals.

Animal Groups

Our animals were divided in five groups. Two different transgenic mouse lines expressing mutant TRβ were used (TR$\beta\Delta$337T and TRβE457A). The Δ337T is a natural mutation found in a family (kindred S) with resistance to TH.[15] The E457A mutation is an artificial mutation described earlier.[20] Both mutations were introduced in the germ line of mice by homologous recombination.[19,21] All mice used in these studies were from the same mixed genetic background (C57BL6/129Svj), and littermate wild-type ($TR\beta^{wt/wt}$) control animals were used in all experiments. The genotype of the animals was determined as described earlier in details.[19,21] From these two mutants, we divided the animals into the following groups: heterozygous ($TR\beta^{WT/\Delta337T}$), homozygous ($TR\beta^{\Delta337T/\Delta337T}$) for the Δ337T mutation, and heterozygous ($TR\beta^{WT/E457A}$), homozygous ($TR\beta^{E457A/E457A}$) for the E457A mutation. We also used as a negative control the KO animal for the TRβ gene ($TR\beta2^{0/0}$). The mice were maintained in 12-hour light/12-hour dark cycles (6 AM–6 PM)

Congenital Hypothyroidism

Heterozygous females for the mutation Δ337T on TRβ were rendered hypothyroid immediately after mating. Hypothyroidism was induced by giving the animals water containing 0.1% methimazole (MMI; Sigma-Aldrich, St. Louis, MO). The treatment with MMI continued until the pups completed 8 weeks of age, when they were killed. We examined animals from three to four heterozygous females. Wild-type ($TR\beta^{wt/wt}$), heterozygous ($TR\beta^{WT/\Delta337T}$), and homozygous ($TR\beta^{\Delta337T/\Delta337T}$) from the same litters were compared.

Blood samples were obtained from the animals for hormone determinations by radio-immunoassay (RIA). Total T4 and TSH were measured in duplicate in the same assay as described in detail earlier.[22]

Tissue Fixation

At 8 weeks after birth, animals from all groups were killed by keeping them in a box with CO_2, followed by decapitation. Immediately after enucleation, the eyeballs from the eyes were fixed separately by immersion in 4% paraformaldehyde (PA) in 0.1 M phosphate buffer (PB ; pH 7.2) for 2 hours and then were rinsed with PB. Whole retina, free from pigmented epithelium, was rinsed extensively in PB and maintained in phosphate-buffered saline (PBS) until processed for immunohistochemistry.

Some of the eyeballs were hemisectioned with a razor blade, and the posterior eye cup with the retina was kept in the same fixative solution for 2 hours, followed by several rinses in PB, and then cryoprotected in sucrose (15% and 30%). After that, the tissues were mounted in OCT embedding medium (Sakura Finetek, Torrance, CA), frozen, and cryosectioned. Sections perpendicular to the vitreous surface (10–14 μm) were collected on gelatinized slides. Sections of the tissue from all genotypes, from different treatment conditions, were collected and processed on the same glass slide.

Immunohistochemical Procedure

Polyclonal antibodies against S- and M-opsins (Chemicon International, Temecula, CA) were used. Sections or flatmounted preparations were first immersed in PBS plus Triton-X 0.25%. Then, the tissue was incubated in bovine serum albumin (BSA) 5%, for 2 hours, followed by incubation with primary antibodies (1:1000 in PBS plus Triton X 0.25%) for 1 to 6 days (in flatmounted retinas). After three 10-minute rinses in PBS, the sections were incubated with biotinylated secondary antibody against rabbit IgG (Vector Laboratories, Peterborough, UK) diluted 1:200 for 2 hours. Further washes in PBS were made and then incubated with a complex of biotin-avidin (Vectastain Elite; Vector Laboratories) diluted 1:50 in PBS plus Triton X 0.25% for another 2 hours. Finally, sections or flatmounted retinas were rinsed in PBS three times for 10 minutes each and reacted with a kit for HRP (SG; Vector Laboratories) for 5 to 10 minutes. After interruption of the reaction and several rinses, the slides were mounted with glycerol (40% in PB). Control sections and control whole-retina preparations were incubated with PBS in the absence of primary antibody and processed as experimental retinas. No immunoreactivity was detected in control sections.

Cell Quantification

We used flatmounted retinas to quantify S- and M-opsins in the tissue. First, retinal maps were obtained in a photographic magnifier and used for density analysis. Second, retinal samples were obtained by using an ocular grid (area equivalent to 256 μm^2) and the number of opsin immunoreactive photoreceptors was directly counted under a light microscope by using differential interference contrast optics (Axioskop; Carl Zeiss Meditec, Oberkochen, Germany). Finally, samples were taken every 0.5 mm of the retinal map in a systematic way, so that the entire retinal area was represented. Retinal samples corresponded to 10% of the total retinal area. Cell density, expressed as number of cells per square millimeter, was obtained from at least three preparations for each animal type under different hormonal conditions.

Electroretinogram Measurements

Spectral measurements were made using the equipment and general procedures described earlier.[23,24] In brief, the stimuli were delivered from a two-beam optical system, the output of which was presented in Maxwellian view (circular field 57° in diameter). In these experiments, a single beam originating from a monochromator (38-86-79; Bausch & Lomb, Tampa, FL; and LTi 01-001; Amko, Tornesch, Germany) equipped with a 75-W xenon arc lamp was used. A circular 4.0-log-unit neutral-density wedge was used to adjust the intensity of the light. In the plane of the pupil the spectral output of the optical system was calibrated with a radiometer (IL 1700, with modelED033 photodetector; International Light) at all combinations of wavelength and position of the neutral-density wedge. The mice were anesthetized with an intramuscular injection of a mixture of xylazine hydrochloride (21 mg/kg) and ketamine hydrochloride (108 mg/kg), and the pupil was dilated by topical application of a mixture of atropine sulfate (0.04%). The mice were positioned in a head restraint and aligned with the optical system. Electroretinograms (ERGs) were recorded with a stainless-steel electrode contacting the corneal surface through a layer of 1% methylcellulose. Ground (Grass E5 disc electrode; Grass-Telefactor, West Warwick, RI) and reference electrodes (Grass-Telefactor) were placed on the forehead and external canthi, respectively. All recordings were obtained in mice with fully dilated pupils (1% cyclopentolate hydrochloride; Alcon, Fort Worth, TX).

The signal of the microelectrodes was amplified (Grass-Telefactor) with a band-pass set at 0.3 and 1000 Hz, monitored on an oscilloscope (TDS 210; Tektronix, Richardson, TX), and continuously digitized at a rate of 1 kHz by a computer equipped with a data-acquisition board (National Instruments, Austin, TX). Recordings were made in a room illuminated by ceiling-mounted fluorescent lamps (150 lux) with 10 minutes of light adaptation before cone ERGs were recorded.[25]

The amplitude of the *b*-wave was measured from the trough of the *a*-wave to the most positive peak of the response,[25] a 14 different wavelengths (340, 360, 380, 400, 420, 440, 460, 480, 500, 520, 540, 560, 580, and 600 nm) with the same number of quanta (4.6×10^{14} quanta/s/cm^2).

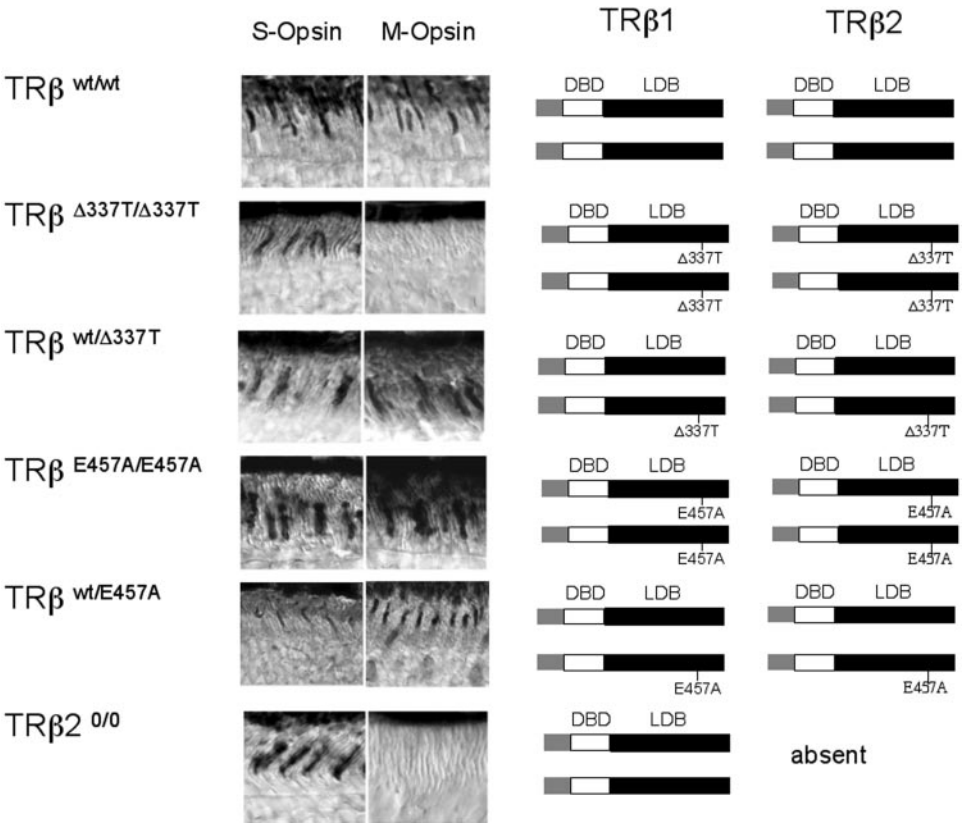

FIGURE 1. Immunohistochemical analysis of the retina in TR$\beta^{wt/wt}$, TR$\beta^{\Delta337T/\Delta337T}$, TR$\beta^{wt/\Delta337T}$, TR$\beta^{E457A/E457A}$, TR$\beta^{wt/E457A}$, and TR$\beta^{0/0}$ mice. Immunohistochemical staining for the S (*left*)- and M (*right*)-opsins in sections perpendicular to the vitreous surface.

Statistical Analysis

All data are reported as the mean ± SEM. One-way ANOVA followed by Student-Newman-Keuls multiple comparisons test was used for assessment of significance (Prism; GraphPad Software, Inc, San Diego, CA). Differences were considered to be significant at $P < 0.05$.

RESULTS

Effect of Different Mutations in the TRβ on M- and S-Opsin

Figure 1 shows different cone opsin expression in the retina of TR$\beta^{wt/wt}$, TR$\beta2^{0/0}$, and animals heterozygous and homozygous for the Δ337T (TR$\beta^{wt/\Delta337T}$, TR$\beta^{\Delta337T/\Delta337T}$) and E457A (TR$\beta^{wt/E457A}$, TR$\beta^{E457A/E457A}$) mutations in sections perpendicular to the vitreous surface. All genotypes expressed M- and S-opsin except TR$\beta2^{0/0}$ and TR$\beta^{\Delta337T/\Delta337T}$.

Δ337T Mutation in TRβ

The Δ337T mutation increased S-opsin expression but only in the dorsal region, where the mutation in homozygosis caused a 30% ($P < 0.01$) increase (see Fig. 3A, left).

In Figures 2 and 3, we confirm that wild-type animals presented a gradient of M-opsin expression and that more M-opsin immunostaining is seen in the dorsal region. In the TR$\beta^{wt/\Delta337T}$ animals this gradient was not observed. Actually, the presence of one mutated allele decreased M-opsin expression in the dorsal region 22% ($P < 0.01$) compared with WT levels (Fig. 3B), leading to the loss of the gradient. The alterations in M- and S-opsin expression were independent of TH levels as both TR$\beta^{wt/\Delta337T}$ and TR$\beta^{\Delta337T/\Delta337T}$ animals had elevated TH levels (Fig. 2). TR$\beta^{wt/\Delta337T}$ mice

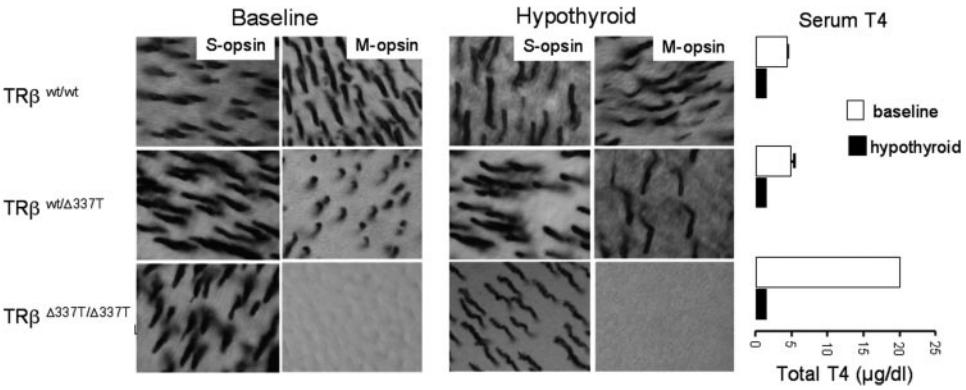

FIGURE 2. Immunohistochemical analysis of the retina in TR$\beta^{wt/wt}$, TR$\beta^{wt/\Delta337T}$, and TR$\beta^{\Delta337T/\Delta337T}$ mice at baseline and during hypothyroidism. Immunohistochemical staining for S (*left*)- and M (*right*)-opsins in flatmounted preparations. Serum total T4 of animals treated (hypothyroid) or not (baseline) with methimazole. Four to six animals were evaluated in each group. T4 data are reported as the mean ± SEM.

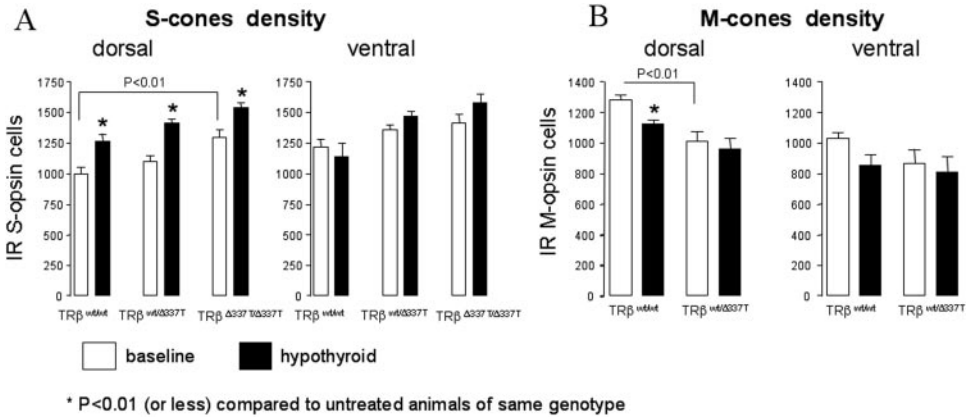

FIGURE 3. Selective loss of M-cone and altered S-cone distribution in TR$\beta^{wt/wt}$, TR$\beta^{wt/\Delta 337T}$, and TR$\beta^{\Delta 337T/\Delta 337T}$ mice at baseline and during hypothyroidism. The number of immunoreactive (IR) (**A**) S- and (**B**) M-opsin cells is shown in ventral and dorsal regions of the retina of animals treated (hypothyroid) or not (baseline) with methimazole. Four to six animals were evaluated in each group. Data are reported as the mean ± SEM. *$P < 0.05$ versus the same genotype.

expressed less M-opsin than did WT animals and the M/S ratio in the total retina was lower (Table 1).

Effect of Congenital Hypothyroidism on M- and S-Opsin Expression

At the moment of death, all animals treated with MMI, independent of the genotype, had undetectable levels of total T4 (<2 ng/dL, no differences between the genotypes, Fig. 2) and elevated TSH levels (data not shown), confirming the hypothyroid state of the mice. These congenital hypothyroid animals showed significant differences in S- and M-opsin expression in the dorsal region of the retina, with a 26% increase ($P < 0.01$) in S-opsin expression associated with a 13% decrease ($P < 0.01$) in M-opsin expression (Fig. 3).

S-opsin immunostaining in the dorsal portion of the retina was dependent on thyroid hormone levels (Fig. 3A). Congenital hypothyroidism caused an increase in S-opsin expression (20%–30%, $P < 0.01$) in the dorsal region in all genotypes (Figs. 2, 3A). However, we did not observe any difference in the ventral region (Fig. 2, 3A). Hypothyroidism was sufficient to decrease the M/S ratio (in total retina) in TR$\beta^{wt/wt}$ animals, but did not affect the already-reduced ratio of TR$\beta^{wt/\Delta 337T}$ (Table 1).

E457A Mutation in TRβ

The presence of the E457A mutation, caused no difference in S-opsin expression independent of the retina area evaluated (Fig. 4). The quantification of M-opsin immunoreactive cells (Fig. 4, right) revealed that TR$\beta^{E457A/E457A}$ retina showed a decrease (~30%) in both the dorsal and ventral halves when compared to TR$\beta^{wt/wt}$ and TR$\beta^{wt/E457A}$ retina.

Electroretinograms

To confirm that alterations in expression of M- and S-opsin cause alteration in function, we analyzed TR$\beta^{wt/wt}$, TR$\beta^{wt/\Delta 337T}$ and TR$\beta^{\Delta 337T/\Delta 337T}$ by spectral sensitivity measurements from ERGs (Fig. 5).

The spectral sensitivity function exhibited the expected bimodal profile, with two peaks, one around 360 nm and another around 500 nm, indicating that these animals are maximally sensitive to short-wavelength stimuli in the UV range and to middle-wavelength stimuli in the green spectral region. These peaks correspond to the maximum spectral absorption of the S- and M-opsin described in mice.

The averaged spectral sensitivities obtained from ERGs of TR$\beta^{\Delta 337T/\Delta 337T}$ animals showed ERG responses only to the short wavelengths of the spectrum, as expected (Fig. 5). No M-cone activity was detected in these spectral sensitivity functions. TR$\beta^{WT/\Delta 337T}$ animals showed responses to the shorter wavelengths of the spectrum, lower than in TR$\beta^{wt/wt}$, even though S-cones were present in the same number (Figs. 2, 3).

DISCUSSION

Essentially all tissues contain TRs.[3,14] TRβ1 and TRα1 isoforms are widely and abundantly expressed; however, the TRβ2 isoform is expressed only in the pituitary, hypothalamus, inner ear, and retina[3] and is essential for gene regulation within pituitary, inner ear, and retina.[26,27]

TRβ2 is expressed in the outer nuclear layer of the retina and regulates the expression of S- and M-opsin in the cone photoreceptors as shown for the first time by Forrest et al.[26] in TRβ2 KO mice. In this study, we compared opsin expression in two TRβ mutant mouse models. Both express a mutant TRβ that is capable of forming either homo- and heterodimers with RXR and to bind to DNA. As shown previously by our group, both models display impairment in negative regulation and have elevated levels of T3, T4, and TSH.[19,21] The main difference in function between these two mutant receptors is related to their ability to bind T3, CoRs, and CoAs. On genes with a positive TH response element and in the absence of T3, TR associates with CoR proteins assembling into a complex that inhibits gene transcription. In the presence of T3, this CoR complex dissociates and is replaced by a CoA protein that then

TABLE 1. Ratio between M- and S-opsin from Total Retina in TR$\beta^{wt/wt}$ and TR$\beta^{wt/\Delta 337T}$ Mice at Baseline and during MMI-Induced Hypothyroidism

	TR$\beta^{wt/wt}$ Not Treated	TR$\beta^{wt/wt}$ Treated with MMI	TR$\beta^{wt/\Delta 337T}$ Not Treated	TR$\beta^{wt/\Delta 337T}$ Treated with MMI
M/S ratio	0.90	0.80	0.60	0.58
Difference from TR$\beta^{wt/wt}$		$P < 0.05$	$P < 0.01$	$P < 0.01$

M/S-opsin ratio in TR$\beta^{wt/wt}$ and TR$\beta^{wt/\Delta 337T}$ mice treated or not treated with MMI. Data are derived from the number of immunoreactive (IR) S-opsin cells divided by the number of M-opsin cells in total retina of animals treated (hypothyroid) or not treated (baseline) with MMI. Four to six animals were evaluated in each group.

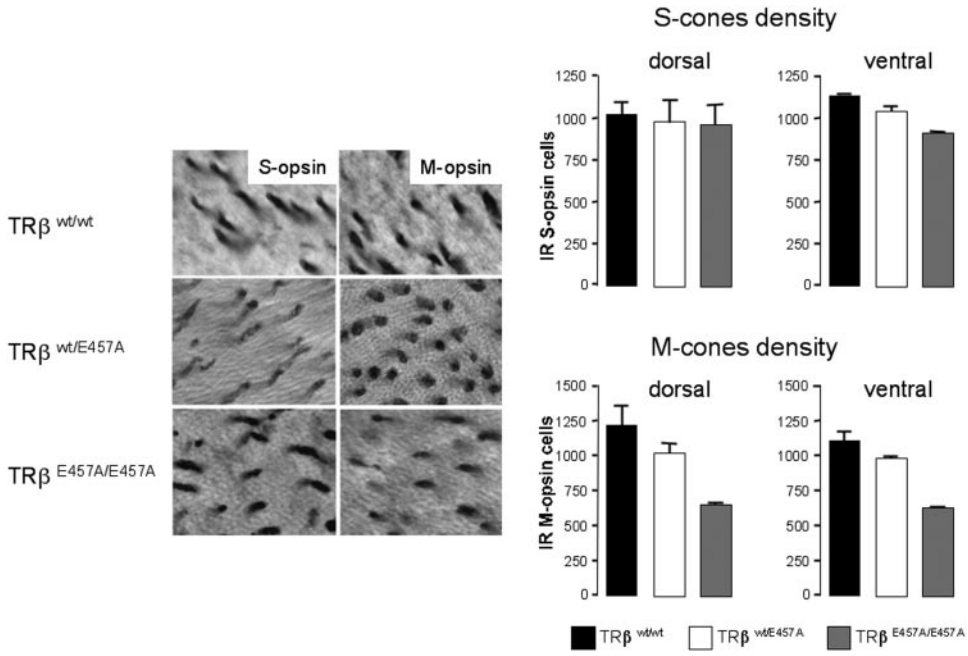

FIGURE 4. Immunohistochemical analysis of the retina in TR$\beta^{wt/wt}$, TR$\beta^{wt/E457A}$, and TR$\beta^{E457A/E457A}$ mice at baseline. Immunohistochemical staining for S- and M-opsin and the number of immunoreactive (IR) (**A**) S- and (**B**) M-opsin cells in ventral and dorsal regions of the retina. Two to three animals were evaluated in each group. Data are reported as the mean ± SEM.

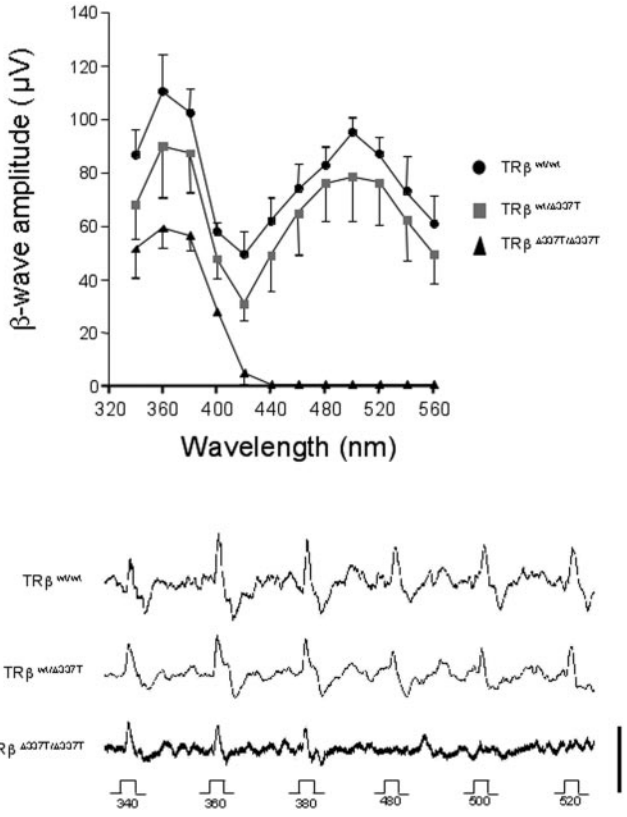

FIGURE 5. *Top*: Photoreceptor functionality of TR$\beta^{wt/wt}$, TR$\beta^{wt/\Delta337T}$, and TR$\beta^{\Delta337T/\Delta337T}$ mice. *Bottom*: ERG in 2-month-old animals. Three to five animals were evaluated in each group. Data are reported as the mean ± SEM.

activates transcription at the promoter.[3] The TRβ Δ337T mutant is unable to bind T3 and constitutively binds to nuclear CoRs. When this mutant isoform binds to another functional nuclear receptor (such as TRβ, TRα, or RXR), it inhibits its function, characterizing a negative dominant effect.[28]

We have confirmed that T3 binding to TRβ is essential for normal S- and M-opsin expression, as well as the presence of the TRβ2, as shown before by others.[6,13,14] Homozygous Δ337T mice have no functional TRβ receptors and are unable to express M-opsin, similar to TRβ2 KO mice. Homozygous Δ337T animals have also shown alterations in S-opsin expression, but this effect was restricted to the dorsal retina (Fig. 3A). However, heterozygous Δ337T animals showed no significant difference from WT animals. Based on these results, we suggest that expression of one normal allele of TRβ is sufficient to direct normal expression of S-opsin to the dorsal retina, excluding a dominant negative effect of the mutated receptor in this case.

The ERG is a tool commonly used in the investigation of diseases related to loss of visual function.[29,30] In the present study, we used ERG to confirm function loss of M-cones in TR$\beta^{\Delta337T/\Delta337T}$ mice (Fig. 5). The failure to detect cone-related signals with middle-wavelength stimulation (approximately 500 nm) in TR$\beta^{\Delta337T/\Delta337T}$ animals supported strongly our morphologic data that indicated the absence of M-cones in this lineage. On the other hand, the amplitudes of the responses were similar between TR$\beta^{WT/WT}$ and TR$\beta^{WT/\Delta337T}$ mice in the middle-wavelength spectral region, even though there was a small but significant reduction in M-opsin in the dorsal retina.

There was a difference in the amplitudes of the responses among the three genotypes with short-wavelength stimulation (~360 nm). The responses of the TR$\beta^{WT/WT}$ and TR$\beta^{WT/\Delta337T}$ mice were larger than in TR$\beta^{\Delta337T/\Delta337T}$. A reasonable explanation is that visual opsins possess a second peak of spectral absorption called the β-band, which occurs, in the UV region; thus M-cones may respond to UV light when stimulated. The ERG recordings in the UV region are therefore a sum of the

responses of two peaks. Since the TR$\beta^{\Delta 337T/\Delta 337T}$ animals do not express M-cones, the responses that were obtained were from the UV-cones, which could explain the smaller amplitude of the response compared with that of the other two genotypes.

It is interesting that the increase in S-cone population in TR$\beta^{\Delta 337T/\Delta 337T}$ was not reflected in the ERG measurements. When Ng et al.[6] tested the participation of TRβ2 in the photoreceptor expression in mice, they also observed an increase in S-cones, and the ERG measurements in mutant and wild-type mice were similar to our results. The increased sensitivity to short-wavelength light seems to be related to another molecule involved in cone photoreceptor development, the photoreceptor cell–specific nuclear receptor (PNR).[31,32] Mutation in the PNR gene causes overexpression of S-cone opsin and enhanced S-cone syndrome (ESCS) retinal disease.[31,33]

Different from M-opsin expression, S-opsin expression of heterozygous Δ337T mutant mice showed no significant changes. Nevertheless, the reduced ratio of total M/S-opsin in heterozygous mice emphasizes the imbalance in opsin expression caused by the mutation. It is interesting that this ligand-binding mutation occurs naturally in families with RTH, and most of the patients with diagnosed RTH are heterozygous.[15] Visual alterations in patients with RTH have not been reported, although it is possible that those alterations exist but are not a major concern to the patient.

The E457A mutation maintains the T3-binding function of the TRβ, but, unlike the wild-type receptor, Co-As, with histone acetyl transferase activity are not recruited. This alteration leads to only a partial response to T3[19,20,34,35] and does not achieve a maximum T3 response. The immunodetection of M-opsin in TRβ(E457A) mice, in contrast to its total absence in mice with the ligand-binding mutation, suggests that CoA is recruited for M-opsin expression, but it reduces the amplitude of T3's effect on the cones' density.

In addition, the fact that the E457A mutation does not impair the ability of the retinal cells to express M, leads us to conclude that CoR recruitment in the absence of T3 and its dissociation in the presence of T3 must be central to controlling S- and M-opsin gene expression in the retina.

Effects of Congenital Hypothyroidism

Several genes regulated by thyroid hormone are also regulated by the ligand-independent effects of the receptor.[28] When this is true, the mouse phenotype is more severe during hypothyroidism than in mice lacking TR. However, not all genes respond in this way. Regarding opsin regulation, the lack of the receptor seems to be more dramatic than a decrease in TH levels.

In previous studies, animals were derived from pregnancies in which the pups were exposed to higher levels of TH than were normal animals, because of elevated maternal hormone levels. For example, heterozygous mothers in all previously investigated mouse models[6,13,21] would have had higher blood concentrations of T3, T4, and TSH than would normal mice, and this fact could confound the results. To rule out the possibility that the phenotype was due to a thyroid hormone excess that acts through TRα during early stages of development, we used an animal model of congenital hypothyroidism. Treating the pregnant females with MMI from mating to delivery ensured us that the pups were exposed to low levels of TH. The pups were continuously treated (without interruption) until they were euthanized in adult life (Fig. 2).

Roberts et al.[13] stated that S- and M-opsin were regulated by exogenous T3, because a lack of T3 binding by the mutant TRβ would be responsible for the phenotype.[13] However, if this were correct, we would expect that our wild-type animals with congenital hypothyroidism would show a phenotype similar to that of TRβ mutants (Figs. 1, 2 and Refs. 13, 14). However, different from TR$\beta^{\Delta 337T/\Delta 337T}$, WT pups born of hypothyroid females showed a small but significant decrease of M-opsin expression. This observation, together with our data obtained from E457A mice suggests that mice need minimum levels of TH for expression of M-opsin. In addition, that the M-opsin density in heterozygous mice does not respond to hypothyroidism supports the view of a central role of TRβ in mediating the effect of TH on M-opsin regulation.

The fact that thyroid hormone deficiency affected S-cones in mice with no expression of TRβ, which binds T3, suggests the involvement of TRα in T3-induced suppression of S-cones (Figs. 2, 3). Previous studies have suggested that T3 administered in vivo or in vitro does not affect S-cones in the absence of TRβ.[13] However, our study suggests that there is cooperation between the TRs in the regulation of S-cone density. It is possible that the basal expression of S-opsins in the euthyroid state is suppressed by T3 through TRα, although additional repression by T3 may be mediated by TRβ. This repression seems to be the case in thyroid hormone regulation of heart rate and TSH synthesis.[36–38] Several papers have shown that in these two situations there is a role for both isoforms of the TR. Our data from E457A animals support the idea that different isoforms of TR might be regulating opsin expression. It is important to investigate the subject further, to clarify the role of α and β receptors in S-cones density in mice retina.

From previous results and our new findings, it is clear that the presence of TRβ, together with its ability to bind to T3 and DNA, is essential for the presence of M-opsin in the retina and normal density of S-opsin. However, the AF-2 domain of TRβ is unnecessary for detection of M-opsin, based on results we obtained in E457A mice. We have also shown, for the first time, that congenital hypothyroidism changes M- and S-opsin expression in adult mice.

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