The orphan nuclear hormone receptor *ERR*β controls rod photoreceptor survival

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Mutation of rod photoreceptor-enriched transcription factors is a major cause of inherited blindness. We identified the orphan nuclear hormone receptor estrogen-related receptor β (*ERR*β) as selectively expressed in rod photoreceptors. Overexpression of *ERR*β induces expression of rod-specific genes in retinas of wild-type as well as *Nrl*$^{-/-}$ mice, which lack rod photoreceptors. Mutation of *ERR*β results in dysfunction and degeneration of rods, whereas inverse agonists of *ERR*β trigger rapid rod degeneration, which is rescued by constitutively active mutants of *ERR*β. *ERR*β coordinates expression of multiple genes that are rate-limiting regulators of ATP generation and consumption in photoreceptors. Furthermore, enhancing *ERR*β activity rescues photoreceptor defects that result from loss of the photoreceptor-specific transcription factor Crx. Our findings demonstrate that *ERR*β is a critical regulator of rod photoreceptor function and survival, and suggest that *ERR*β agonists may be useful in the treatment of certain retinal dystrophies.

Crx | ligand | neurodegeneration | retina | development

The vertebrate retina contains two major subtypes of photoreceptors—rods and cones. Clinically, dysfunction and death of rod photoreceptors are the primary causes of most forms of inherited photoreceptor dystrophy. A number of rod-expressed transcription factors (1–7) have been identified that are required for rod photoreceptor differentiation or survival. Biochemical analysis has indicated that these factors are present at the promoters of rod-specific genes in vivo and directly activate expression of rod-specific genes (1, 8, 9). Mutation of rod-enriched transcription factors in humans can lead to rod photoreceptor dystrophy (10, 11). Failure to express normal levels of rod-specific genes thus results in rod photoreceptor degeneration, and correction of this defect may have considerable value in treating inherited blindness.

Analysis of gene expression in developing and mature rod photoreceptors has indicated that other transcription factors also show highly rod-enriched expression (12–15). Among these is estrogen-related receptor β (*ERR*β), an orphan nuclear hormone receptor homologous to the classical estrogen receptor but which constitutively activates transcription in the absence of estradiol (16, 17). *ERR*β is specifically expressed in differentiating and mature mouse rod photoreceptors (12, 13), with significant mRNA levels also detected in the human retina (18). Deletion of a floxed allele of *ERR*β in the embryoid body using a Sox2-Cre line (hereafter referred to as *ERR*β$^{-/-}$) results in mice with a defect in inner ear development but no obvious retinal defects (19). Loss-of-function mutations of *ERR*β have been reported in inherited forms of human deafness (20).

Given the prominent expression of *ERR*β in retinal photoreceptors, we hypothesized that *ERR*β might also play an important role in rod photoreceptor function. We observed that genetic or pharmacological disruption of function of *ERR*β leads to rod photoreceptor degeneration. *ERR*β also directly regulates expression of rod-specific genes, with *ERR*β being sufficient to both drive activation of rod-specific genes in vivo and rescue rod photoreceptors of *Crx*$^{-/-}$ mice from degeneration. These data imply that synthetic agonists of *ERR*β may prove useful in treating photoreceptor degeneration resulting from mutation of rod-specific transcription factors.

Results

Expression of *ERR*β in Developing and Mature Retina. Our previous work demonstrated that *ERR*β mRNA was first seen in the early postnatal mouse retina, initially showing expression in cells resembling immature horizontal cells, and from the second week of life was expressed in retinal photoreceptors (12, 13). To further clarify the distribution of *ERR*β protein, we performed immunohistochemical analysis with markers of rod, cone, and horizontal cells. We found that *ERR*β was coexpressed with Lhx1, a marker of horizontal cells, throughout the first week of life. However, the horizontal cell expression of *ERR*β was drastically down-regulated after postnatal day (P) 10, consistent with the previously reported mRNA expression pattern (13) (Fig. 1*A*). Starting at P7, *ERR*β immunostaining is detected across the outer nuclear layer (ONL), consistent with a photoreceptor-specific expression pattern. Immunostaining with the anti-rhodopsin antibody Rho4D2 and anti-*ERR*β reveals that *ERR*β and rhodopsin are colocalized at P14 (Fig. S1*A*). Furthermore, *ERR*β and the cone-specific marker S-opsin (Fig. 1*B*) are not colocalized at any age examined. Finally, *ERR*β protein is absent from photoreceptors of P10.5 *Nrl*$^{-/-}$ mice, although still detected in developing horizontal cells, demonstrating that *ERR*β is specifically expressed in rod photoreceptors (Fig. S1*B*). Expression of *ERR*β is still detected in photoreceptors of mice lacking functional Crx and Nr2e3, however, which retain rod photoreceptors (Fig. S1*B*). We thus conclude that *ERR*β is selectively expressed in immature horizontal cells and in rod photoreceptors at intermediate and late stages of differentiation.

Two homologs of *ERR*β, *ERR*α and *ERR*γ, are also present in the retina. *ERR*α is broadly expressed in the mature retina, with somewhat more prominent expression in inner retina than in ONL, as previously reported by in situ hybridization analysis (13). *ERR*γ, on the other hand, is prominently expressed in cone photoreceptors and in a subset of inner retinal cells, with only weak expression detectable in rod photoreceptors (Fig. S1*C*).

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The authors declare no conflict of interest.

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Data deposition: Primary microarray data files have been deposited in the Gene Expression Omnibus (GEO) database (accession no. GSE21944).

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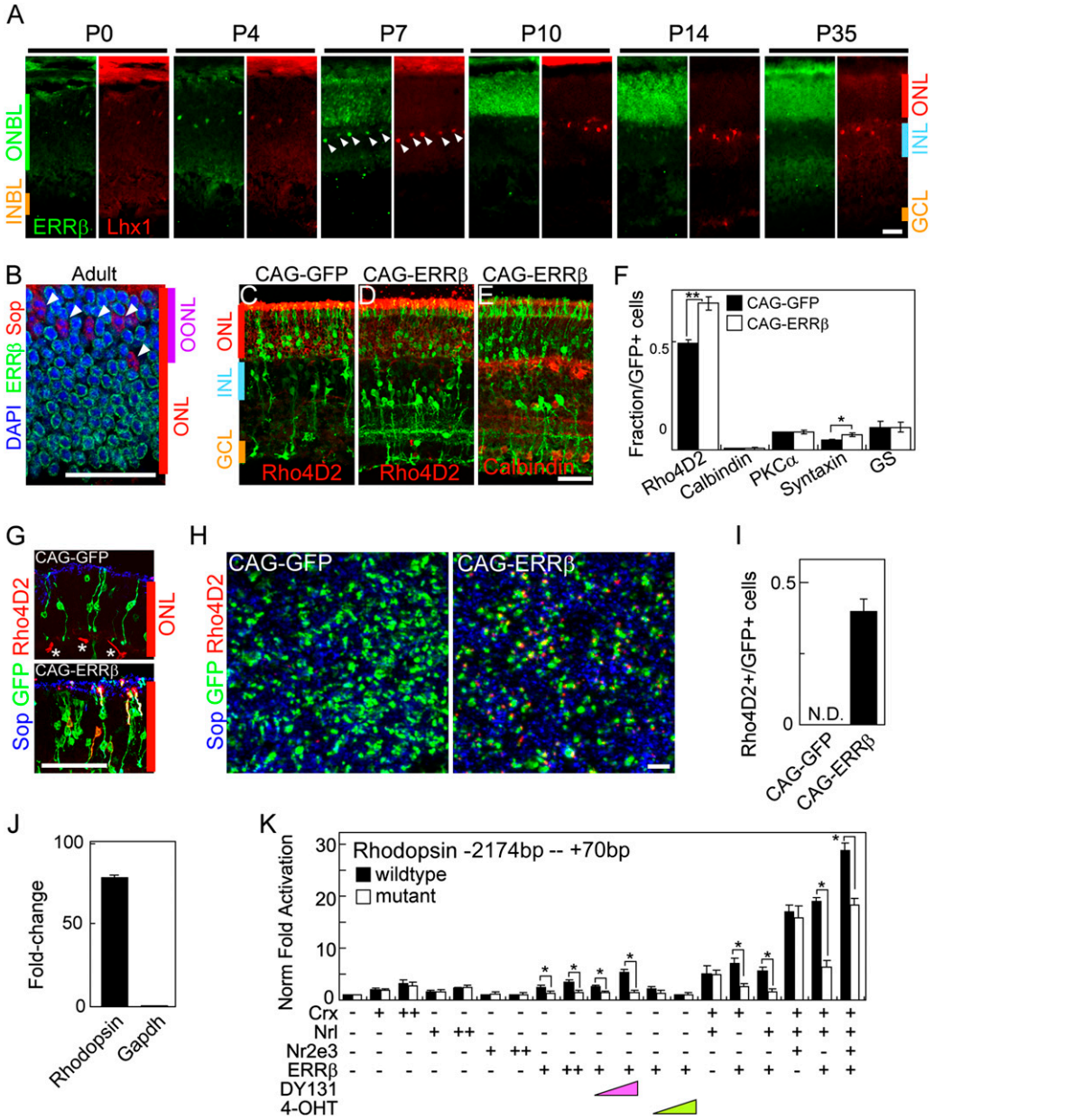

Fig. 1. *ERR*β is expressed in rod photoreceptors and promotes rod-specific gene expression. (*A*) Developmental expression of *ERR*β (green) coimmunostained with the horizontal cell marker Lhx1 (red) at P0, 4, 7, 10, 14, and 35. Expression overlaps in immature horizontal cells at P7 (white arrowheads). ONBL, outer neuroblastic layer; INBL, inner neuroblastic layer; ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. (*B*) Double immunostaining of *ERR*β (green) and S-opsin (red) with DAPI (blue) on a P40 retinal section, demonstrating nonoverlapping expression of the two markers. OONL, outer outer nuclear layer. (*C–E*) Section immunohistochemistry of P14 retinas electroporated in vivo at P0 with CAG-GFP or CAG-*ERR*β colabeled with Rho4D2 (*C* and *D*) or calbindin (*E*) antibodies. (*F*) Retinal cell-type composition of P14 retinas electroporated in vivo at P0 with CAG-GFP and CAG-*ERR*β estimated by dissociated immunohistochemistry using cell-specific markers (Rho4D2 for rods, calbindin for horizontal cells, PKCα for rod bipolar cells, syntaxin for amacrine cells, and glutamine synthetase for Muller glia). All data are represented as mean ± SD. $*P < 0.05$, $**P < 0.005$ by Student's *t* test ($n = 3$ electroporated retinas). (*G–J*) *ERR*β overexpression is sufficient to induce rhodopsin expression in photoreceptors of $Nrl^{-/-}$ mice. Section (*G*) and flat-mount (*H*) immunohistochemistry of P14 retinas electroporated in vivo at P0 with CAG-GFP and CAG-*ERR*β immunostained with S-opsin (blue) and Rho4D2 (red) antibodies. Asterisks indicate background staining of blood vessels. (*I*) Quantification of the fraction of Rho4D2/GFP double-positive photoreceptor cells from (*H*). All data are represented as mean ± SD ($n = 3$ electroporated retinas). (*J*) Quantification of rhodopsin and GAPDH mRNA in P7.5 $Nrl^{-/-}$ retinas electroporated with CAG-*ERR*β as measured by quantitative RT-PCR ($n = 4$ RNA preparations). Fold change in mRNA levels is expressed relative to the contralateral retina, which was not electroporated. Levels of mRNA are normalized relative to the fraction of electroporated cells (≈10% of the retinal surface, and 50% of photoreceptors within that area, were electroporated in the samples used). (*K*) *ERR*β directly regulates rhodopsin −2174 -- +70 bp promoter construct activity. Black and white bars represent luciferase levels relative to untransfected 293T cells expressed from wild-type and a mutant promoter construct, in which the *ERR*β-binding motif (AGGTCA) at the proximal promoter (-136 bp) has been mutated. Pink and green triangles represent dose treatment of DY131 and 4-OHT, respectively. + and ++ represent 15 and 30 ng, respectively. All data are represented as mean ± SD ($n = 3$ independent transfections). $*P < 0.05$ by Student's *t* test ($n = 3$ independent transfections, repeated in two separate trials). (Scale bars, 20 μm.)

Activation of Rhodopsin Expression by *ERR*β. To analyze the function of *ERR*β in the developing retina, we overexpressed full-length *ERR*β under the control of the ubiquitous CAG promoter as previously described (21, 22). Overexpression of *ERR*β increased the fraction of cells expressing rhodopsin relative to empty vector controls at P14 (Fig. 1 *C–F*), although no significant increase in the fraction of cells in the ONL was detected, implying that *ERR*β overexpression does not drive retinal precursors toward a rod photoreceptor fate. A modest increase in the fraction of cells expressing amacrine-specific markers and showing amacrine morphology was also observed (Fig. 1 *C–F*), although no increase in the fraction of cells expressing the horizontal cell marker calbindin was detected (Fig. 1 *E* and *F*).

Loss of function of the rod-specific transcription factor Nrl results in conversion of rod photoreceptors to short-wavelength cones (4), and provides an excellent model to determine whether *ERR*β is sufficient to induce expression of rod-specific genes. To investigate this, we overexpressed *ERR*β by electroporation of $Nrl^{-/-}$ retinas at P0.5. We saw robust induction of rhodopsin expression following CAG-*ERR*β electroporation at P14 as measured by both section and flat-mount immunohistochemistry and by quantitative RT-PCR (Fig. 1 *G–J*) reminiscent of the phenotype seen following transgenic overexpression of Nr2e3 in $Nrl^{-/-}$ mice (23). CAG-*ERR*β electroporation did not, however, result in repression of S-opsin synthesis, as is seen following Nr2e3 overexpression.

These data suggested that *ERR*β might directly activate expression of rod-specific genes, perhaps in conjunction with other known rod-enriched transcription factors. To address this question, we first analyzed whether *ERR*β directly activated rhodopsin expression by using a rhodopsin promoter luciferase construct (22). We observed a dose-dependent activation of luciferase expression by *ERR*β, whether alone or in combination with Crx, Nrl, and/or Nr2e3 (Fig. 1*K*). DY131, a selective agonist of *ERR*β (24), enhanced *ERR*β-dependent activation of luciferase expression. In contrast, 4-hydroxytamoxifen (4-OHT), an inverse agonist of *ERR*β, converted *ERR*β into a potent transcriptional repressor in a dose-dependent manner. Finally, mutation of a predicted estrogen-related receptor target sequence in the proximal rhodopsin promoter significantly reduced the *ERR*β-induced activation of luciferase expression. These data demonstrate that rhodopsin expression can be regulated by *ERR*β and that *ERR*β-dependent transcription of rhodopsin can be modulated by selective ligands of *ERR*β.

Regulation of Rod Photoreceptor Survival by *ERR*β. Although we did not observe any obvious morphological changes in P21 retinas of $ERR\beta^{-/-}$ mice, we investigated whether effects on rod photoreceptor function or survival were seen at later ages. We observed a slow degeneration of rod photoreceptors in mutant mice. Starting at 3 mo of age, a decrease in the length of rod outer segments (Fig. 2 *A*, *C*, and *F*) was detectable by hematoxylin and eosin (H&E) staining, with a significant decrease in the number of cell bodies in the outer nuclear layer detectable by 6 mo of age (Fig. 2 *A*, *B*, and *E*). By 24 mo of age, the great majority of rod photoreceptors were gone, although a subset of cone photoreceptors were preserved (Fig. 2 *B* and *C*). A significant decrease in the length of cone outer segments was observed in $ERR\beta^{-/-}$ mice by 18 mo of age (Fig. 2 *D* and *F*), although the total number of cone photoreceptor cells was not different between wild-type and mutant animals (Fig. 2*G*). No changes were observed in the number or structure of any inner retinal cell type (Fig. S2).

To determine whether photoreceptor function was lost, we measured the electroretinogram (ERG) of these mice at 10 and 24 mo of age. By 10 mo, a dramatic reduction in the scotopic *a* and *b* wave was observed, whereas the photopic *b* wave was not reduced (Fig. 2 *H* and *I*). The time to peak of the photopic *b* wave of $ERR\beta^{-/-}$ mice was also delayed, as previously reported in mice showing progressive rod dystrophy (25). At 24 mo of age, a severe deterioration of the scotopic response was seen, along with a secondary reduction in the photopic ERGs (Fig. S3). No abnormalities in either scotopic or photopic ERGs are observed at P33, in line with the normal cellular morphology observed at this age (Fig. 2*A* and Fig. S2). These data imply that *ERR*β expression is necessary for rod photoreceptor function and survival.

We next examined whether pharmacological inhibition of *ERR*β directly regulates rod photoreceptor survival. Diethylstilbestrol (DES) functions as an inverse agonist of *ERR*α, *ERR*β, and *ERR*γ (17), whereas 4-OHT is an inverse agonist for *ERR*β and *ERR*γ but not *ERR*α (16). Beginning at P14, we treated retinal explants with *ERR*β inverse agonists. By 2 d postexplanting, we observed selective rod photoreceptor degeneration in retinas treated with either 10 μM DES or 4-OHT, but not with 0.1% EtOH carrier, with prominent levels of TUNEL staining detected (Fig. 2 *J* and *K*). The increase in TUNEL staining was selective for rod photoreceptors, with no effect on cone or bipolar cell viability observed (Fig. S4). Surprisingly, an increase in TUNEL staining not only in photoreceptor cells but also in the inner retina of 4-OHT-treated retinas is observed in $ERR\beta^{-/-}$ retinas relative to wild-type animals (Fig. 2 *J* and *K*). Although the reason for this observation remains unclear, this may imply that the 4-OHT-induced selective cell death of rod photoreceptors is not being entirely mediated by *ERR*β, but also partially by *ERR*γ or an uncharacterized cellular target. The prominent cell death seen in mutant animals in cells of the inner retina, which do not express *ERR*β, also suggests that *ERR*β might regulate retinal cell survival through a partially non-cell-autonomous mechanism.

We next tested whether overexpression of a drug-insensitive, constitutively active *ERR*β could rescue rod degeneration observed following DES or 4-OHT treatment of retinal explants. Whereas overexpression of CAG-VP16 did not protect rod photoreceptors from degeneration, overexpression of the VP16 transcriptional activator domain fused to the DNA-binding domain of *ERR*β resulted in a significant level of protection of electroporated photoreceptors. Interestingly, this protection extended to nearby nonelectroporated regions. This suggests that *ERR*β might regulate either expression of a secreted neuroprotective factor or else allow removal of a diffusible toxic factor (Fig. S5 *A* and *B*).

Identification of *ERR*β Target Genes. Next, we used DNA microarray analysis to determine whether *ERR*β regulated expression of rod-specific genes in addition to rhodopsin. We observed 425 transcripts that were significantly down-regulated following loss of *ERR*β at P21, with 303 transcripts significantly up-regulated (fold change greater or less than 1.25×; $P < 0.05$). We found that down-regulated genes were significantly enriched for a known rod photoreceptor-enriched expression pattern ($P = 0.0004$). One of the most highly down-regulated genes was the transcription factor *Mef2c*, which is down-regulated in $Nrl^{-/-}$ mice (14, 15). A number of phototransduction-associated genes, such as rhodopsin, were also significantly reduced in expression, but several genes in this group stood out as particularly strongly down-regulated. Specifically, the guanylate cyclase activator proteins *Guca1a* and *Guca1b* and the sodium/potassium/calcium rod inner segment cation exchanger *Slc24a1* (Table S1) were greatly affected. In addition, we observed significant down-regulation of several genes involved in rate-limiting steps in glycolysis, including *Hk2*, *Pfkb2*, and *Pfkp* along with *Ppara*, a transcription factor known to directly regulate transcription of fatty acid-metabolizing enzymes (26). Each of these genes has previously been identified as a transcriptional target of *ERR*α and *ERR*γ in nonneuronal tissues (27), but a role for *ERR*β in regulation of metabolism has thus far not been reported. Quantitative real-time PCR analysis of a representative subset of such transcripts indicated that they were indeed significantly

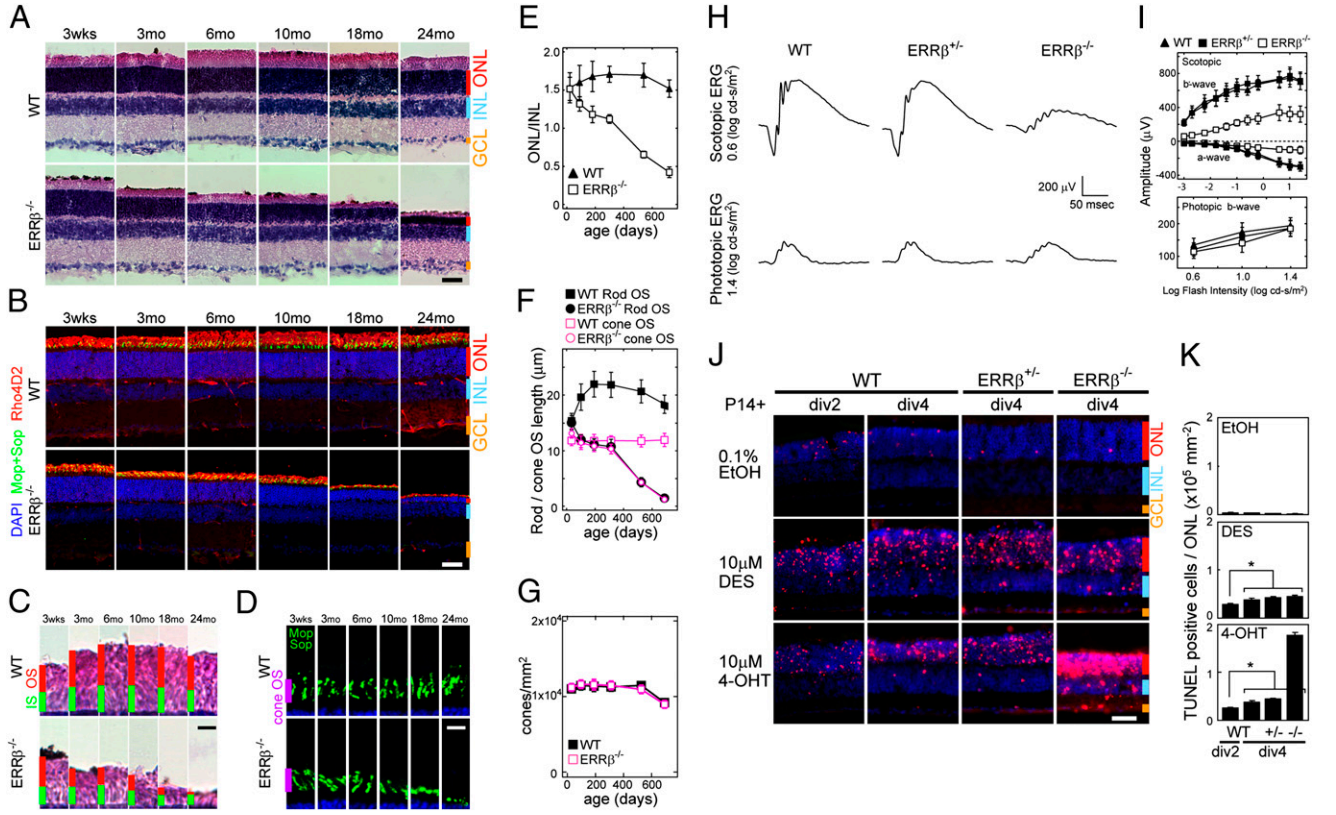

Fig. 2. *ERR*β controls rod photoreceptor survival. (*A* and *B*) H&E staining (*A*) and section immunohistochemistry (*B*) labeled with M-opsin and S-opsin (green) and Rho4D2 (red) antibodies of aged (P21, 3, 6, 10, 18, and 24 mo) mouse retinas. (*C* and *D*) High-power images of photoreceptor outer and inner segments (*C*) and cone outer segments (*D*) from *A* and *B*, respectively. (*E*) Quantification of ONL thickness normalized relative to the INL from *A*. (*F*) Quantification of rod and cone outer segment length from *C* and *D*, respectively. All data are represented as mean ± SD ($n = 20$ from three different individuals). (*G*) Density of cone photoreceptors estimated from *B*. All data are represented as mean ± SD ($n = 3$ retinas from different individuals). (*H*) Scotopic (rod) and photopic (cone) ERG responses from 10-mo-old wild-type, *ERR*β$^{+/-}$, and *ERR*β$^{-/-}$ mice. The amplitude of the scotopic ERG response of *ERR*β$^{-/-}$ mice was significantly reduced, whereas the wave forms of WT and *ERR*β$^{+/-}$ were similar. There were no significant differences in the amplitude of the photopic ERG response. (*I*) Scotopic *a*- and *b*-wave amplitudes of 11 flash intensities, and photopic *b*-wave amplitudes of 3 flash intensities. All data are represented as mean ± SD ($n = 4$ animals). (*J*) TUNEL staining (red) with DAPI (blue) of WT, *ERR*β$^{+/-}$, and *ERR*β$^{-/-}$ retinas that were harvested at P14 and explanted for 2 d in vitro (div 2) or 4 d in vitro (div 4) d exposed to 0.1% EtOH (carrier), 10 μM DES, and 10 μM 4-OHT. (*K*) The fraction of TUNEL-positive cells in ONL in *J* normalized by the area of ONL. All data are represented as mean ± SD ($n = 3$ independent explants). *$P < 0.05$ by Student's *t* test. [Scale bars, 20 μm (*A*, *B*, and *J*) and 10 μm (*C* and *D*).]

down-regulated in P21 *ERR*β$^{-/-}$ retinas (Fig. 3). The cellular expression pattern of a number of transcripts down-regulated in *ERR*β$^{-/-}$ retinas was examined using in situ hybridization. We determined that some were enriched in rod photoreceptors. Others, such as *Hk2*, are broadly expressed, but selectively down-regulated in the photoreceptor layer of P21 *ERR*β$^{-/-}$ mice (Fig. S6). See Dataset S1 for a full list of transcripts that are differentially expressed in the P21 *ERR*β$^{-/-}$ retina.

***ERR*β Activation Promotes Rod Photoreceptor Survival.** Finally, we investigated whether enhancing *ERR*β function could rescue defects caused by genetic disruption of other rod-expressed transcription factors. Because mutations in Crx are the most common disease-associated defect of this sort, we determined whether enhancing *ERR*β function through either overexpression or pharmacological manipulation could rescue defects in rod-specific gene expression and rod survival found in *Crx*$^{-/-}$ mice (28). Although *ERR*β expression is reduced in *Crx*$^{-/-}$ mice, it is still detectable in rod photoreceptors (Fig. S1*B*). Electroporation of CAG-*ERR*β in *Crx*$^{-/-}$ mice resulted in a substantial decrease in cell death in photoreceptor cells at P21, along with a restoration of rod-specific gene expression in electroporated cells (Fig. 4 *A* and *B*). In retinas electroporated with *ERR*β-CAG, the protection from apoptosis extended to nearby photoreceptors that were not GFP-positive, although induction of rhodopsin expression was confined to GFP-positive cells, reminiscent of the cell-non-autonomous cell protection observed in DES- and 4-OHT-treated retinas that were electroporated with *ERR*β-VP16. In addition, treatment of retinal explants obtained from *Crx*$^{-/-}$ mice with DY131, a selective agonist for *ERR*β/γ, resulted in a substantial decrease in the fraction of TUNEL-positive cells in the ONL, along with a significant increase in the expression of rhodopsin (Fig. 4 *C* and *D*). These data suggest that manipulation of *ERR*β activity may protect rod photoreceptors from dysfunction and death in mutants where expression of rod-specific transcripts is compromised.

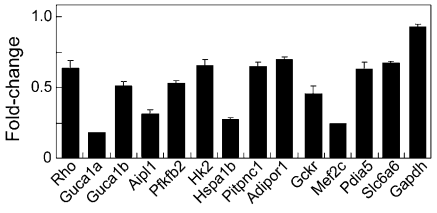

Fig. 3. Quantitative RT-PCR analysis of transcripts down-regulated in P21 *ERR*β$^{-/-}$ mice. RNA from whole dissected retinas was used, and the results were normalized to β-actin levels. Signal levels represent mRNA levels in mutant retinas normalized to those of age-matched wild-type retinas ($n = 3$ RNA preparations). Sequences for primers used for are shown in Table S2.

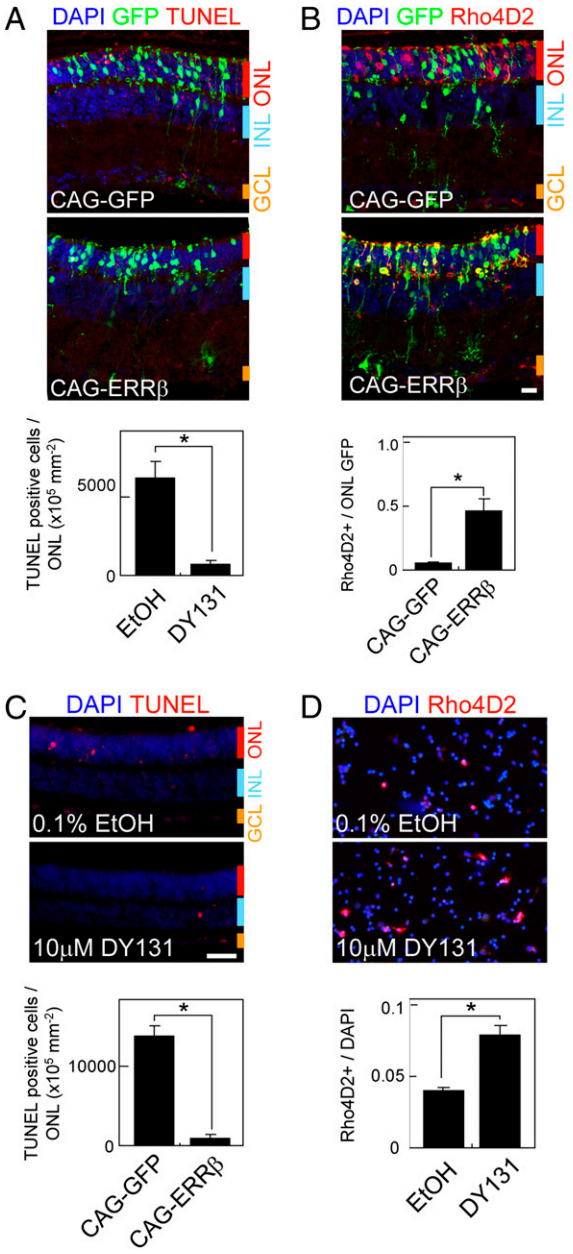

Fig. 4. *ERR*β overexpression and the *ERR*-specific agonist DY131 rescue defects in rod function in $Crx^{-/-}$ mice. (*A*) Retinal sections of P33 $Crx^{-/-}$ retinas electroporated in vivo at P0 with CAG-GFP and CAG-*ERR*β that were TUNEL-positive (*A*) and immunostained with Rho4D2 (*B*). The fractions of TUNEL-positive cells and Rho4D2-positive electroporated cells in ONL are shown in the lower panel. (*C*) TUNEL staining of P7 + 5 d in vitro (div5) $Crx^{-/-}$ retinal explants exposed to 0.1% EtOH (carrier) and 10 μM DY131. The fraction of TUNEL-positive cells in ONL was normalized by the area of ONL. All data are represented as mean ± SD (n = 3). (*D*) Dissociated cell immunohistochemistry using P7 + div5 $Crx^{-/-}$ retinal explants. The fraction of Rho4D2-positive cells is shown. All data are represented as mean ± SD (n = 3 retinas). *P < 0.05 by Student's t test. (Scale bars, 20 μm.)

Discussion

Our findings indicate that *ERR*β is a rod photoreceptor-specific transcription factor that regulates the expression of multiple rod-specific genes and is required for survival of this cell type. Targeted disruption of *ERR*β leads to a slow and selective degeneration of rod photoreceptors, preceded by loss of rod outer segments. Furthermore, pharmacological inhibition of *ERR*β/γ leads to a selective degeneration of rod photoreceptors, which can be rescued by overexpression of constitutively active *ERR*β. Selective down-regulation of a subset of rod-specific genes is also observed in mutant mice. A working model for the action of *ERR*β in regulation of rod photoreceptor-specific gene expression and rod survival based on these findings is shown in Fig. S7. *ERR*β—like Crx, Nrl, and Nr2e3—directly activates rhodopsin expression. Although *ERR*β expression is reduced in mice mutant for both Crx and Nrl, it is unclear whether these factors directly regulate *ERR*β transcription (14, 15). However, our finding that *ERR*β overexpression in $Nrl^{-/-}$ animals induces photoreceptor expression implies that *ERR*β can function independently of this gene to regulate rod-specific gene transcription. Like Crx, loss of function of *ERR*β leads to both dysfunction and degeneration of rod photoreceptors, although with substantially slower kinetics (28). Unlike in Nrl- and Nr2e3-deficient retinas, no ectopic activation of cone-specific genes is observed in $ERR\beta^{-/-}$ animals.

*ERR*β has been previously shown to regulate cell specification and survival at a number of different stages of development. This role in development is distinct from the role of its homologs *ERR*α and *ERR*γ, which act primarily to regulate cellular metabolism rather than differentiation (29). Targeted mutation of *ERR*β leads to defects in trophoblast cell differentiation and survival (30), which result in embryonic lethality. Epiblast-specific deletion of *ERR*β with Sox2-Cre leads to a defect in specification of endolymph-producing cells in the developing inner ear, resulting in deafness and vestibular defects (19). This defect is mirrored in humans carrying point mutants in the *ERR*β gene, who show early-onset hearing loss (20). Our findings extend the known functions of *ERR*β to the regulation of sensory neuron specification and survival, and further suggest that individuals lacking *ERR*β may turn out to suffer from late-onset rod photoreceptor dystrophy, a defect which may remain undetected owing to the relative youth of the patients investigated thus far.

The rod photoreceptor degeneration observed in $ERR\beta^{-/-}$ retinas manifests itself very slowly. It is not yet clear how *ERR*β loss of function leads to rod degeneration. Although expression of a number of genes that regulate phototransduction is reduced in $ERR\beta^{-/-}$ mice, the most dramatic reduction is seen for Guca1a and Guca1b, which are the rate-limiting regulators of cGMP production in photoreceptor outer segments, and Slc24a1, the rod inner segment sodium/potassium/calcium transporter. Inner segment cation transport and cGMP production are by far the largest users of free energy in rod photoreceptors (31). Selective defects are also seen in enzymes that regulate free energy generation in photoreceptors, notably enzymes regulating glycolysis and the pentose phosphate pathway, including Hk2, Pfkb2, and AldoA, each of which is photoreceptor-enriched and down-regulated in $Nrl^{-/-}$ mice (14, 15). Rod photoreceptors exhibit a high level of aerobic glycolysis, which acts to both regenerate cGMP in rod outer segments as well as generate high levels of NADPH via the pentose phosphate pathway for chromophore reduction. Selective defects in both functions have been proposed to lead to photoreceptor degeneration (32, 33). A general rescue of photoreceptor metabolism may be one reason why non-cell-autonomous protection of photoreceptors is observed in both DES- and 4-OHT-treated retinas overexpressing *ERR*β-VP16, and also in $Crx^{-/-}$ mice overexpressing *ERR*β (Fig. 4 *C* and *D*).

Our studies suggest that pharmacological manipulation of retinal *ERR*β activity may serve as a therapeutically useful tool for preventing rod photoreceptor degeneration in mutations that result in loss-of-function defects in transcription factors such as Crx, Nrl, and Nr2e3. These proteins regulate rod-specific gene expression and collectively comprise 3–5% of all identified forms of autosomal-dominant retinitis pigmentosa and 1–2% of Leber congenital amaurosis (34, 35). DY131, a selective agonist of *ERR*β and *ERR*γ (24), enhances expression of rod-specific genes and inhibits rod degeneration when applied to retinas of $Crx^{-/-}$ mice,

whereas overexpression of *ERR*β in photoreceptors of these mice achieves the same result. Selective ocular drug delivery is routinely used in treatment of a broad range of ophthalmic disorders, and takes advantage of the fact that the eye represents a relatively self-contained system that restricts the spread of drugs and resulting side effects on other organs. Our findings suggest that *ERR*β agonists may prove useful in treating select inherited forms of rod photoreceptor dystrophy.

Materials and Methods

Animals. Timed pregnant CD-1 and C57BL/6 mice were purchased from Charles River Breeding Laboratories and Jackson Laboratory, respectively. *ERR*$\beta^{-/-}$ mice were generated as previously described (19), with homozygous mutant mice generated by breeding to the Sox2-Cre line (36). All experimental procedures were preapproved by the Institutional Animal Care and Use Committee of Johns Hopkins University School of Medicine.

In Vivo Electroporation, Immunohistochemistry, and in Situ Hybridization. In vivo electroporation was performed at P0 as previously described (21). Protocols for section immunohistochemistry, dissociated cell immunohistochemistry, and in situ hybridization were performed essentially as previously described (13, 22). H&E staining solution (Sigma) was used according to the manufacturer's recommended protocol.

Electroretinogram. ERGs were recorded as previously described (37).

Retinal Explants and TUNEL Staining. P14 retinas were dissected and explanted as previously described (21). The culture media containing 0.1% EtOH (carrier), 10 μM DES (Sigma), 10 μM 4-OHT (Sigma), and 10 μM DY131 (Tocris Bioscience) were changed every 2 d.

Full experimental procedures for this study can be found in *SI Materials and Methods*.

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