Reprogramming of adult rod photoreceptors prevents retinal degeneration

Cynthia L. Montana[a], Alexander V. Kolesnikov[b], Susan Q. Shen[a], Connie A. Myers[a], Vladimir J. Kefalov[b], and Joseph C. Corbo[a,1]

Departments of [a]Pathology and Immunology and [b]Ophthalmology and Visual Sciences, Washington University School of Medicine, St. Louis, MO 63110

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A prime goal of regenerative medicine is to direct cell fates in a therapeutically useful manner. Retinitis pigmentosa is one of the most common degenerative diseases of the eye and is associated with early rod photoreceptor death followed by secondary cone degeneration. We hypothesized that converting adult rods into cones, via knockdown of the rod photoreceptor determinant *Nrl*, could make the cells resistant to the effects of mutations in rod-specific genes, thereby preventing secondary cone loss. To test this idea, we engineered a tamoxifen-inducible allele of *Nrl* to acutely inactivate the gene in adult rods. This manipulation resulted in reprogramming of rods into cells with a variety of cone-like molecular, histologic, and functional properties. Moreover, reprogramming of adult rods achieved cellular and functional rescue of retinal degeneration in a mouse model of retinitis pigmentosa. These findings suggest that elimination of Nrl in adult rods may represent a unique therapy for retinal degeneration.

transdifferentiation | rhodopsin | *rd1*

Heritable retinal degeneration is a common cause of visual impairment and blindness, affecting millions of people worldwide (1). Many research groups have focused on targeted gene therapy as a treatment for this disease (2, 3). However, retinal disease can be caused by mutations in any one of more than 200 genes (4), and the pathogenic mechanisms of various mutations differ greatly (5). Thus, there is a strong motivation to develop gene-independent therapies that would be more widely applicable (5, 6).

Retinitis pigmentosa is a subtype of retinal degeneration that might be particularly amenable to a gene-independent approach. Here, mutations in rod-enriched genes initiate a progressive sequence of rod cell death followed by cone loss (7). Cone dysfunction is particularly debilitating for patients, yet it appears to be secondary to rod death; studies in animal models suggest that collapse of the outer nuclear layer (ONL) during rod degeneration may generate an oxidative, nutrient-deficient environment that is toxic to cones (8, 9). In this case, preservation of rod cell bodies may be sufficient to forestall secondary cone death. For example, in certain mouse models, such as the *Gnat1*$^{-/-}$ mutant, there is severe rod dysfunction but no significant rod degeneration (10, 11). In such cases, cone function is entirely preserved.

In this study, we hypothesized that converting adult rods into cones could make the cells resistant to the effects of mutations in rod-specific genes, thereby preventing ONL collapse and secondary cone loss (Fig. 1*A*). Direct conversion of one differentiated cell type into another has been successful in numerous contexts; for example, investigators have converted pancreatic exocrine cells into β-cells (12), auditory endothelial cells into hair cells (13), and fibroblasts into neurons (14, 15). Although conversion of rods into cones would be expected to result in a loss of rod function and consequent night blindness (Fig. 1*A*), this disability is generally well tolerated by patients and might be considered an acceptable risk if coupled with significant cone rescue (16).

To transform rod photoreceptors into cones, we took advantage of the observation that the photoreceptor transcription factor Nrl acts as a cell fate switch during development: photoreceptor precursors that turn on *Nrl* become rods, whereas those that do not become cones (17, 18). We reasoned that acute inactivation of *Nrl* in adult rods might result in direct conversion of these cells into cones. Furthermore, a recent study demonstrated that retinas in which *Nrl* had been knocked out during development showed long-term survival of cone photoreceptors and preservation of the outer nuclear layer, after a transient initial phase of cell loss (19). This observation suggests that direct conversion of adult rods into cones could also lead to long-term survival of the transdifferentiated cells. To test this idea, we used a tamoxifen-inducible allele of *Nrl* to acutely inactivate the gene in adult mouse rods. This manipulation resulted in partial reprogramming of rods into cells with a variety of cone-like molecular, histologic, and functional properties. Importantly, this strategy succeeded in preventing rod cell death in the *Rho*$^{-/-}$ mouse model of retinitis pigmentosa, allowing the preservation of endogenous cone function. Thus, rod reprogramming may represent a unique therapeutic strategy for retinal disease caused by mutations in rod-enriched genes.

Results

Germ-Line *Nrl* KO Suppresses Retinal Degeneration in the *rd1* Mouse. We initially hypothesized that developmental reprogramming of rod precursors to cones, via germ-line *Nrl* KO, would protect photoreceptors from the deleterious effects of a mutation in a rod-specific gene. To test this idea, we created mice with mutations in both *Nrl* and *Pde6b*. *Pde6b* encodes a subunit of phosphodiesterase, a key component of the rod phototransduction cascade, and *Pde6b*$^{rd1/rd1}$ mice experience rapid degeneration of nearly all rods by the end of the second postnatal week (20) (Fig. 1*B*). Germ-line KO of *Nrl*, in contrast, results in a developmental reprogramming of rods into cones (17, 18) (Fig. 1 *A* and *B*). KO of *Nrl* in the *Pde6b*$^{rd1/rd1}$ background completely suppressed the degeneration phenotype (Fig. 1*B*), demonstrating that developmentally reprogrammed rods are resistant to degeneration.

Acute *Nrl* KO Reprograms Adult Rods into Cone-Like Cells. To determine whether acute *Nrl* loss is sufficient to abrogate retinal degeneration during adulthood, we next engineered a floxed allele of *Nrl* to enable conditional inactivation in the adult mouse (Fig. 2*A*). Replacement of WT *Nrl* alleles with floxed alleles did not affect normal retinal development or function (Fig. 2*B*; Fig. S1 *A–D*), and germ-line KO of the floxed *Nrl* allele produced a phenotype indistinguishable from the previously published germ-line *Nrl* KO (17, 21); there was a total loss of expression of rod-specific genes including *Rho* and a corresponding derepression of cone genes (Fig. 2*B*; Fig. S1 *A* and *B*). In addition, electroretinograms (ERGs) demonstrated loss of rod function and a corresponding gain of cone

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[1]To whom correspondence should be addressed. E-mail: jcorbo@pathology.wustl.edu.

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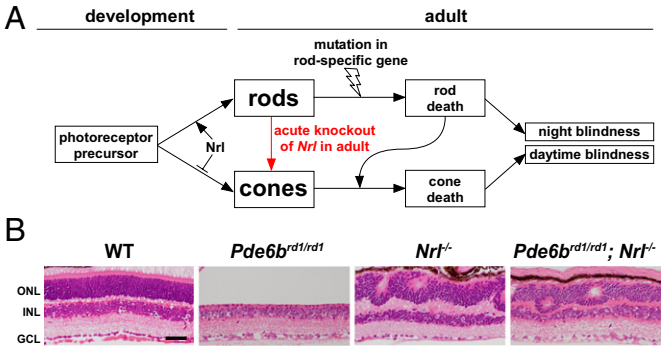

Fig. 1. Rod reprogramming therapy. (*A*) Hypothesis that the normal progression of photoreceptor degeneration in retinitis pigmentosa might be circumvented by reprogramming rods into cones via acute *Nrl* KO. (*B*) Mice homozygous for the mutant *rd1* allele of rod-specific *Pde6b* have lost nearly all rod photoreceptors by P36. Germ-line deletion of *Nrl* on the *Pde6b*$^{rd1/rd1}$ background suppresses the degeneration phenotype. Note that both the *Nrl*$^{-/-}$ and double mutants retinas display marked rosette formation in the ONL, a finding previously reported in the *Nrl*$^{-/-}$ retina (17). ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. (Scale bar, 50 μm.)

function consistent with functional conversion of rods into cones, as previously reported for the germ-line KO (18) (Fig. S1 *E* and *F*).

To directly test the hypothesis that acute *Nrl* knockdown in the adult mouse would result in reprogramming of rod photoreceptors into cones, we crossed the *Nrl* floxed mouse to a transgenic line carrying a tamoxifen-inducible Cre recombinase (22): CAG-Cre^{ERTM}. Both experimental (*Nrl*$^{f/f}$;CAG-Cre^{ERTM}) and control (*Nrl*$^{+/f}$;CAG-Cre^{ERTM}) mice were injected daily with 4-hydroxytamoxifen (4-OHT) from postnatal day 42 (P42) to 44, to induce acute *Nrl* inactivation, and the animals were evaluated 3 wk later (Fig. 3*A*), at which point the *Nrl* transcript and protein product were essentially undetectable in the experimental retinas (Fig. 3*B*; Fig. S2*A*). In situ hybridization (ISH) revealed that most rod genes were down-regulated, although some genes such as *Rho* had residual expression (Fig. 3*B*; Fig. S2*B*). Conversely, several cone genes including *Gnat2*, *Gnb3*, and *Pde6c* were derepressed, but the two cone opsins—*Opn1mw* and *Opn1sw*—were unaffected (Fig. 3*B*; Fig. S2*B*). Quantitative RT-PCR (qRT-PCR) was used to measure the relative expression levels of selected genes in acute *Nrl* KO retinas and controls (Fig. S2*C*). These analyses fully confirm the ISH results and demonstrate that the reprogrammed rods show a number of cone-like molecular features.

Next we performed electron microscopy to assess morphological changes induced by acute *Nrl* KO. Ultrastructurally, WT rod cell bodies typically have scant, organelle-free cytoplasm and small, round nuclei with a large mass of heterochromatin in the center of the nucleus, surrounded by a thin rim of euchromatin (23–25). Cones, in contrast, typically have more abundant juxtanuclear cytoplasm, often containing mitochondria, and larger, more ovoid nuclei with a higher ratio of euchromatin to heterochromatin. In the adult *Nrl* KO retina, many reprogrammed rods had larger nuclei, more frequent juxtanuclear mitochondria, and more abundant euchromatin than controls (Fig. 3*C*). Thus, the reprogrammed rods show some cone-like ultrastructural features.

To test whether the reprogrammed photoreceptors exhibit cone-like physiology, we performed two sets of functional studies. First, conventional ERGs recorded at P63 revealed that acute *Nrl* KO at P42–P44 causes a significant decrease in the retinal response to flashes under scotopic conditions, implying loss of rod function, although not to the same degree as embryonic *Nrl* KO (Fig. 3*D*; Fig. S2 *D* and *E*). In contrast, the reprogrammed retinas exhibited a significantly greater maximal photopic a-wave response compared with controls, suggesting enhanced cone function (Fig. 3*E*; Fig. S2 *D* and *E*). To directly observe the reduced rod function in acute *Nrl* KO retinas, we conducted transretinal ERG recordings of the photoreceptor light response in the presence of synaptic blockers (Fig. 3 *F* and *G*). Notably, dark-adapted reprogrammed rods showed a 35-fold desensitization and more rapid inactivation of their photoresponse compared with control cells, features reminiscent of cones (26) (Fig. 3*F*). Because normal cones, but not rods, can use the retinol form of visual chromophore to regenerate their photopigment after bleaching (27), we next tested whether the reprogrammed rods could use 9-*cis*-retinol (a commercially available analog of 11-*cis*-retinol) to regenerate their pigment in a retinal explant preparation (28). Following overnight dark adaptation, mouse retinas were dissected free of the retinal pigmented epithelium (RPE) under infrared illumination and placed in a recording chamber. Control retinas generated robust photoresponses to a series of light stimuli, whereas reprogrammed retinas produced lower maximal response amplitudes (Fig. 3*G*). However, when the photopigment was bleached after dissection and the retinas were allowed to dark adapt again in the presence of 9-*cis*-retinol, only photoreceptors from the reprogrammed retinas were able to completely recover their photoresponse and largely restore their sensitivity (Fig. 3*G*). Taken together, these findings are consistent with partial reprogramming of rods into cone-like cells in the acute *Nrl* KO, based on molecular, structural, and functional criteria.

Analysis of Potential Barriers to Complete Rod-to-Cone Reprogramming. Next, we sought to define the barriers to complete reprogramming of rods into cones. First, we hypothesized that the reprogramming process might simply require more time. However, even 2.5 and 6 mo following acute KO, rod and cone gene expression patterns were the same as those observed at 3 wk after KO (Fig. 3*B*; Figs. S2*B* and S3), and there was no additional gain of cone function as reflected by ERGs recorded 6 mo after acute *Nrl* KO (Fig. S4). Second, we hypothesized that persistence of the rod-specific transcription factor *Nr2e3*, even at low levels, might be sufficient to maintain the rod transcriptional program. *Nr2e3* acts downstream of *Nrl* in rods and is a well-characterized activator of rod genes and repressor of cone genes, including *Opn1sw* (24, 29). To test

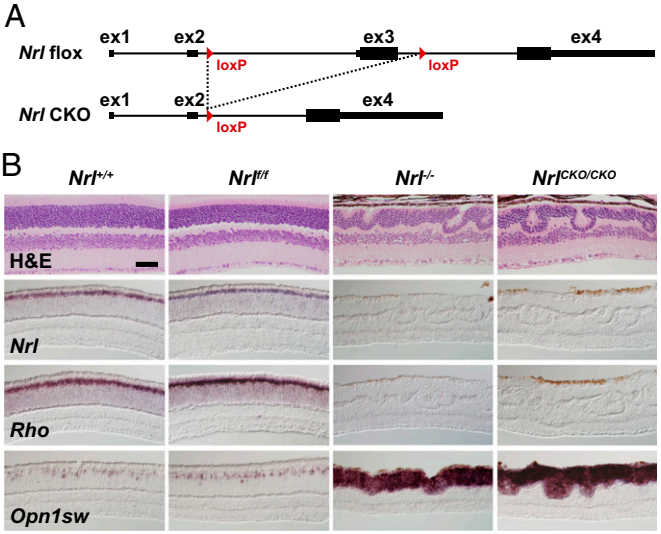

Fig. 2. Engineering a conditional allele of *Nrl*. (*A*) Schematic of the conditional *Nrl* allele; the first coding exon (ex3) is flanked by loxP sites. (*B*) *Nrl*$^{f/f}$ retinas are indistinguishable from WT retinas by H&E histology and ISH for rod (*Nrl* and *Rho*, rhodopsin) and cone (*Opn1sw*, short-wavelength cone opsin) transcripts. Germ-line recombination of the floxed *Nrl* alleles (*Nrl*$^{CKO/CKO}$) via Sox2-Cre recapitulates the *Nrl*$^{-/-}$ phenotype. (Scale bar, 50 μm.)

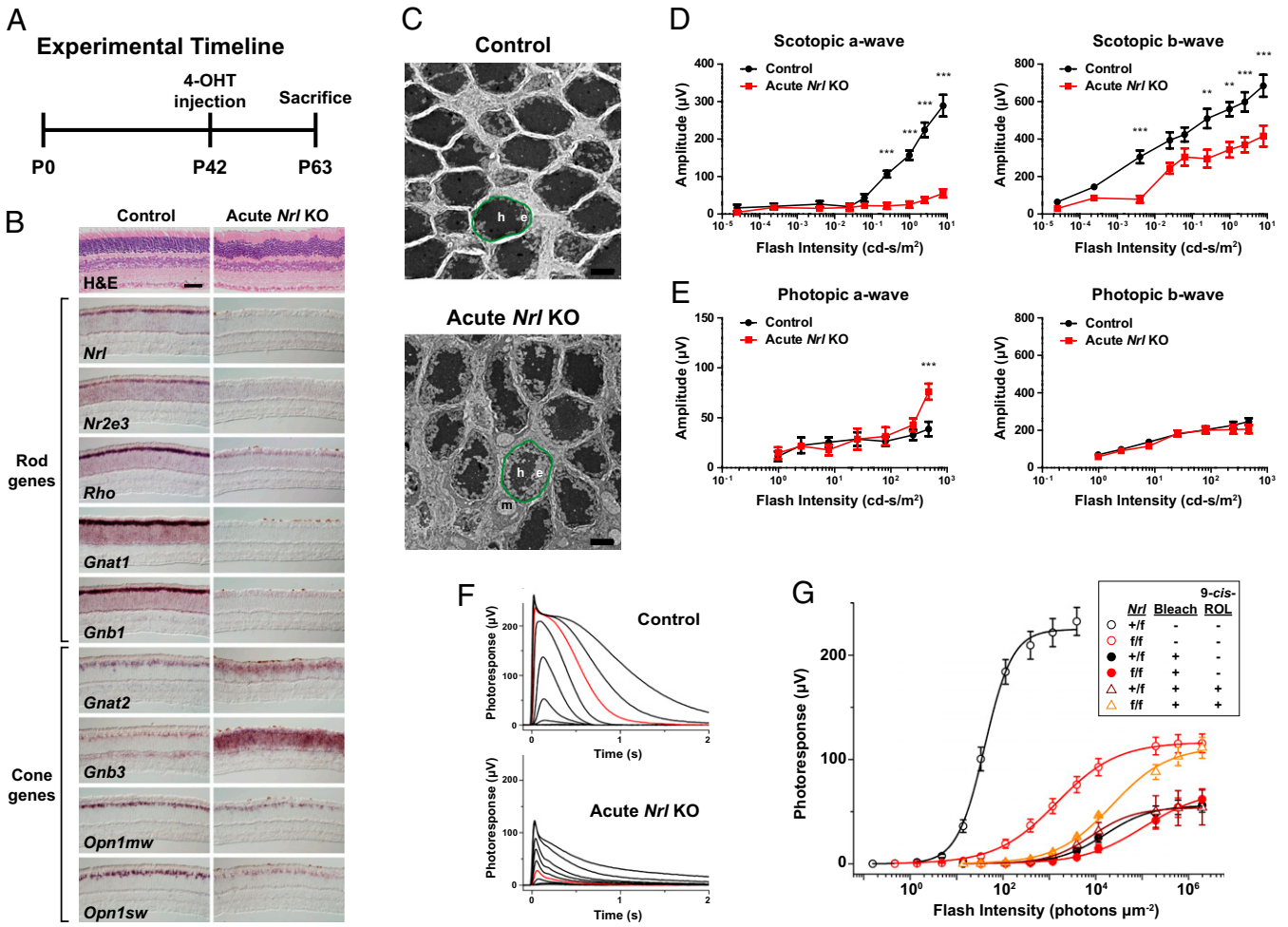

Fig. 3. Reprogramming of adult mouse rods into cone-like cells. (*A*) Timeline for the acute *Nrl* KO experiments. 4-OHT, 4-hydroxytamoxifen for Cre^{ERTM} induction. Unless otherwise specified, control is $Nrl^{+/f}$;Cre^{ERTM} + 4-OHT and acute *Nrl* KO is $Nrl^{f/f}$;Cre^{ERTM} + 4-OHT. (*B*) H&E and ISH staining for rod and cone transcripts. *Gnb1*, G protein β subunit 1 in rod transducin. Note that acute *Nrl* KO results in variable waviness of the ONL, but no frank rosette formation as observed in the germ-line KO. (*C*) Electron micrographs of photoreceptor nuclei in the ONL for control ($Nrl^{f/f}$;Cre^{ERTM} + vehicle) and acute *Nrl* KO ($Nrl^{f/f}$;Cre^{ERTM} + 4-OHT) mice. Sample control and reprogrammed nuclei are outlined in green; heterochromatin (h), euchromatin (e), and juxtanuclear mitochondria (m) are labeled. (Scale bar, 2 μm.) (*D* and *E*) Scotopic (*D*) and photopic (*E*) ERGs recorded from P63 control ($n = 10$) and acute *Nrl* KO mice ($n = 8$). *P* values for two-way ANOVA: $^{**}P < 0.01$, $^{***}P < 0.001$. Error bars are SEM. (*F*) Families of responses to increasing light intensities for dark-adapted, isolated control, and acute *Nrl* KO retinas. Red traces, photoresponses to a light intensity of 392 photons/μm^2 (505 nm). (*G*) Responses to 505-nm test flashes recorded from isolated retinas, lacking RPE, under various conditions ($n \geq 3$ animals per condition). Bleach, exposed to 520-nm light for 2 min followed by dark adaptation for 2.5 h; 9-*cis*-ROL, the 2.5-h dark adaptation was conducted in the presence of 130 μM 9-*cis*-retinol. Data were fitted with the Naka-Rushton hyperbolic function. Error bars are SEM.

this hypothesis, we performed acute *Nrl* KO on the $Nr2e3^{rd7/rd7}$ background, which lacks functional *Nr2e3* (30). However, even in the *Nr2e3*-null context, *Opn1sw* was not derepressed (Fig. S5), ruling out this possibility. Third, we hypothesized that persistence of an epigenetic modification, DNA methylation, might inhibit complete reprogramming. Accordingly, we examined the retinal DNA methylation pattern at the *Rho* and *Opn1sw* loci in four groups of animals at P63: WT (C57BL/6), $Nrl^{-/-}$, control ($Nrl^{+/f}$ recombined at P42), and acute *Nrl* KO ($Nrl^{f/f}$ recombined at P42) (Fig. 4). *Rho* is heavily transcribed in rod but not cone photoreceptors, and the locus is hypomethylated in the rod-enriched WT and control retinas, whereas it is heavily methylated in the cone-only $Nrl^{-/-}$ retina (Fig. 4*A*). Interestingly, acute *Nrl* KO failed to elicit an increase in methylation at the *Rho* locus. Conversely, *Opn1sw* is transcribed only in a subset of WT mouse cones (but in all of the photoreceptors of the $Nrl^{-/-}$ retina); the locus is highly methylated in WT and control retinas but hypomethylated in $Nrl^{-/-}$ retinas (Fig. 4*B*). Acute *Nrl* KO did not result in a decrease in methylation at the *Opn1sw* locus (Fig. 4*B*). These results indicate that *Nrl* KO during adulthood fails to reprogram DNA methylation patterns at key rod and cone gene loci, suggesting that persistent DNA methylation represents an epigenetic barrier to reprogramming.

Next, we sought to define the developmental window during which mouse rods become resistant to reprogramming into cones. Accordingly, we induced *Nrl* KO by 4-OHT injection at P0, P4, P8, P14, and P21 and then characterized the resulting retinas by ISH 3 wk after injection. We found that there was a progressive restriction of the capacity of rods to completely transdifferentiate into *Opn1sw*-expressing cones during the first postnatal week (Fig. S6*A*). At P0, transdifferentiation was complete; at P4, it was partial; and at P8, *Opn1sw* was not derepressed at all. We corroborated these changes by performing ISH for several additional rod and cone markers (Fig. S6*B*). It should be noted that at the P0 and P4 time points, recombination occurred only in the outer half of the ONL, and thus derepression of *Opn1sw* (and the corresponding decrease in rod gene expression) was restricted to this region (Fig. S6). We attribute this pattern of incomplete recombination to two

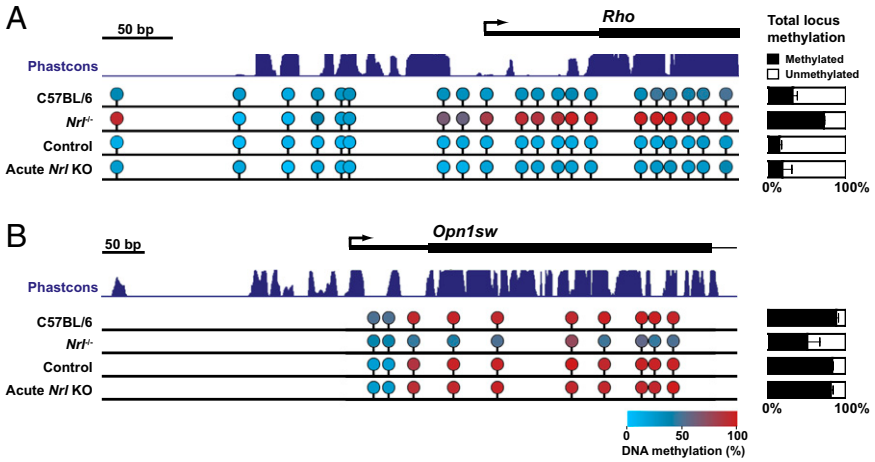

Fig. 4. Epigenetic barriers to reprogramming of rods into cones. Retinal DNA methylation status of the (*A*) *Rho* and (*B*) *Opn1sw* loci for four genotypes, C57BL/6 (WTe), $Nrl^{-/-}$, control ($Nrl^{+/f}$;Cre^{ERT2} + 4-OHT), and acute *Nrl* KO ($Nrl^{f/f}$;Cre^{ERT2} + 4-OHT). Each circle represents a single CpG (5′-CG-3′); shading indicates percentage methylation across ≥28 clones (from two biological replicates). Graph at right represents overall locus methylation; error bars are SD. Phastcons, vertebrate phylogenetic conservation track from UCSC Genome Browser.

factors: (*i*) incompleteness of the retinal vasculature at these developmental time points (31) (necessitating diffusion of 4-OHT from the choroidal vessels); and (*ii*) limitations on the dosing of 4-OHT in these small pups due to ethanol vehicle toxicity. Despite these caveats, the progressive restriction of transdifferentiation potential over the first postnatal week is clearly evident.

Rod Reprogramming Rescues Retinal Degeneration in the *Rho*-Null Mouse. The $Rho^{-/-}$ mouse is a well-established model of retinitis pigmentosa wherein an initial period of rod death is followed by secondary cone dysfunction and loss (32, 33) (Fig. 5*A*). To test the hypothesis that partial conversion of diseased rods into cones is sufficient to rescue retinal degeneration, we performed acute *Nrl* KO on the $Rho^{-/-}$ background. We induced acute *Nrl* KO by injecting *Nrl*-floxed $Rho^{-/-}$ mice with 4-OHT between P25 and P28, before the onset of the major rod death period (32). Evaluation of rescue was performed at P90, a time point when rod death is nearly complete and cone dysfunction is advanced (32, 33). We found that acute *Nrl* KO does indeed prevent photoreceptor cell death, with striking preservation of rod cell bodies and inner segments (Fig. 5 *B*–*D*). The reprogrammed $Rho^{-/-}$ retinas exhibited a gene expression pattern similar to that of reprogrammed WT retinas (Fig. S7*A*), and cone opsin expression was

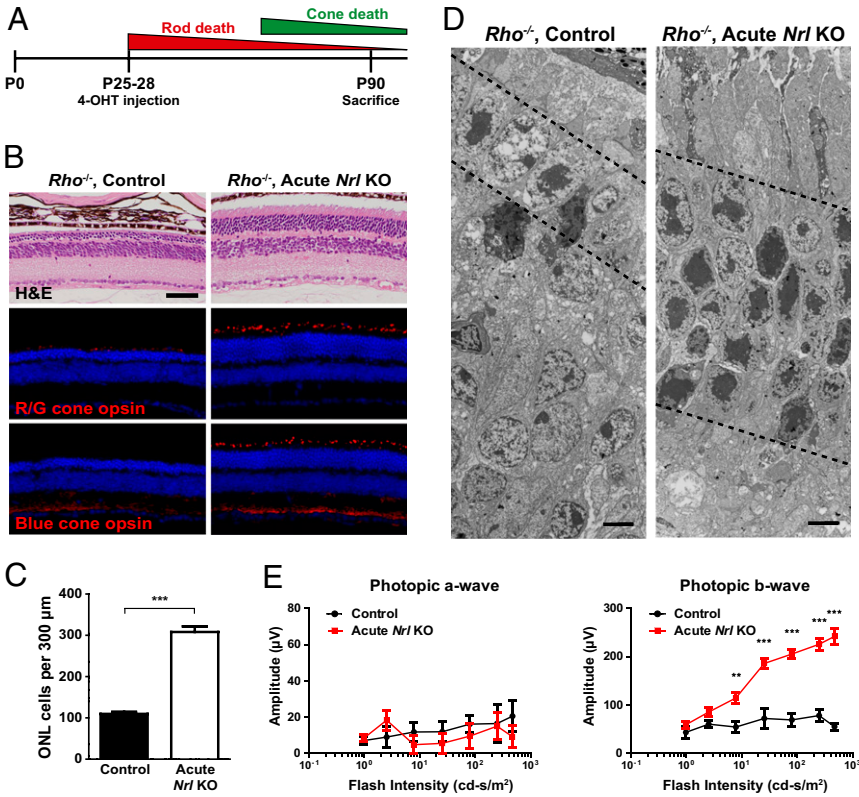

Fig. 5. Rod reprogramming prevents retinal degeneration. (*A*) Time course of photoreceptor degeneration in $Rho^{-/-}$ retinas (*Upper*) and timeline for acute *Nrl* KO experiments on the $Rho^{-/-}$ background (*Lower*). Control is $Rho^{-/-}$;$Nrl^{+/f}$;Cre^{ERTM} + 4-OHT and acute *Nrl* KO is $Rho^{-/-}$;$Nrl^{f/f}$;Cre^{ERTM} + 4-OHT. (*B*) H&E and antibody staining show increased preservation of the ONL and cone opsin expression in $Rho^{-/-}$ animals treated with acute *Nrl* KO relative to controls. (Scale bar, 50 μm.) (*C*) Quantification of ONL cells in 300-μm segments of retina (n = 3 animals per genotype). ***$P < 0.001$ by unpaired t test. Error bars are SD. (*D*) Electron micrograph montages of the ONL, indicated by dashed lines. (Scale bar, 4 μm.) (*E*) Photopic ERGs recorded from control (n = 10) and acute *Nrl* KO mice (n = 10) on the $Rho^{-/-}$ background. P values for two-way ANOVA: **$P < 0.01$, ***$P < 0.001$. Error bars are SEM.

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well preserved (Fig. 5*B*). Most importantly, the histologic and molecular rescue was accompanied by robust rescue of cone function; ERG analysis revealed an intact photopic b-wave over a wide range of flash intensities (Fig. 5*E*; Fig. S7*B*).

Discussion

In this study, we showed that acute knockdown of *Nrl* in the adult mouse retina causes partial reprogramming of rod photoreceptors into cones. The reprogrammed rods exhibit certain molecular, morphological, and functional characteristics of cones, with notable exceptions, including the lack of *Opn1sw* derepression and the relatively normal photopic ERG. Nevertheless, partial conversion of rod photoreceptors was sufficient to prevent photoreceptor cell death in the $Rho^{-/-}$ model of retinitis pigmentosa. This fact, coupled with the observation that reprogrammed rods can survive for at least 6 mo without significant degeneration, supports the notion that rod reprogramming may represent a potential treatment approach for rod-based diseases.

The role of Nrl in rod photoreceptor development has been well characterized (17, 18, 34, 35), and the present study has further demonstrated the functional importance of Nrl in the maintenance of the rod phenotype. We found that Nrl is essential for the sustained transcription of most rod-enriched genes in adult mice. Transcription of some genes, such as *Gnat1* and *Gnb1*, was completely abolished by acute *Nrl* KO, whereas others were down-regulated but not completely absent (e.g., *Rho*, *Pde6a*, *Pde6b,* and *Nr2e3*). On the other hand, certain cone genes such as *Gnb3*, *Gnat2*, and *Pde6c* were derepressed on acute *Nrl* ablation, whereas others like *Opn1sw* remained unchanged (Fig. 3*B*; Fig. S2 *B* and *C*). Thus, photoreceptor genes exhibit differing degrees of Nrl dependence in the adult retina.

What factors might account for this variability? First, Nrl may help establish epigenetic modifications at particular loci during photoreceptor differentiation, and these modifications may later preserve locus activation or repression independently of Nrl. We showed here that the DNA methylation patterns at two loci, *Rho* and *Opn1sw*, remain unchanged following acute *Nrl* KO (Fig. 4). Because DNA methylation is highly correlated with transcriptional activity (36), this may partly explain why *Rho* and *Opn1sw* transcript levels remain relatively unchanged. A second possibility is that Nrl may activate an unknown transcription factor "X" during development. X may then activate a subset of rod genes (e.g., *Rho* but not *Gnat1*) and repress a subset of cone genes (*Opn1sw* but not *Gnb3*). If expression of X becomes independent of Nrl during adulthood, via autoregulation for instance, then X may maintain its regulatory function even following acute *Nrl* KO. In this study, we investigated the rod-specific transcription factor Nr2e3 as a potential candidate for X. However, acute *Nrl* KO on the *Nr2e3* mutant background failed to elicit derepression of *Opn1sw* beyond what is normally observed in that mutant (Fig. S5). Thus, it is possible that another unknown transcription factor or factors downstream of Nrl may inhibit complete rod-to-cone transdifferentiation at the molecular level.

One interesting feature of the reprogrammed retinas is that the rod transducin α-subunit (*Gnat1*) appeared to be completely absent (Fig. 3*B*; Fig. S2*C*), yet the retinas still generated a modest scotopic ERG signal (Fig. 3*D*). *Gnat1* mutant mice, in contrast, completely lack a rod-driven photoresponse (11). This finding prompts speculation that derepressed cone transducin α-subunit (*Gnat2*) may be substituting for the rod subunit in reprogrammed rod photoreceptors. Indeed, a previous study demonstrated that the rod and cone transducin α-subunits are functionally interchangeable in photoreceptors, with comparable signaling properties (37).

Despite the incompleteness of rod-to-cone reprogramming, an interesting consequence of acute *Nrl* KO was the gain of cone-like physiological properties. Two of these features, the reduced scotopic ERG (Fig. 3*D*) and the 35-fold desensitization of reprogrammed photoreceptors compared with typical rods observed in transretinal recording (Fig. 3*F*), are likely due to a substantial decrease in the expression of rod-specific phototransduction components including rhodopsin, rod transducin, and rod phosphodiesterase. Additionally, isolated reprogrammed retinas exhibited faster inactivation of the photoresponse (Fig. 3*F*). Fast inactivation is a hallmark of cone phototransduction (38), and $Nrl^{-/-}$ cones exhibit this property as well (18, 39). In some species (e.g., carp and zebrafish) faster inactivation of phototransduction in cones relative to rods is achieved via a highly efficient, cone-specific opsin kinase, GRK7 (40, 41). Because mice express only a single opsin kinase, GRK1, in both rods and cones (42), the inactivation kinetics must also be modulated by other factors (39), such as the substantially higher expression of components of the transducin GAP complex in cones (43). Given the observed kinetics of photoresponse inactivation, these factors are likely preserved in both $Nrl^{-/-}$ cones and the rods reprogrammed by acute *Nrl* KO.

Another important gain-of-function phenotype in reprogrammed rods is their ability to use an 11-*cis*-retinol analog to regenerate their visual pigment. This capacity is normally restricted to cones (44). The ability of cones to oxidize 11-*cis*-retinol into 11-*cis*-retinal makes it possible for them to access the retinal visual cycle in which Müller glia convert all-*trans*-retinol into 11-*cis*-retinol via a series of enzymatic reactions, as demonstrated in the salamander, mouse, primate, and human (27, 45, 46). The fact that reprogrammed rods are able to regenerate pigment and dark-adapt by using 9-*cis*-retinol (an analog of 11-*cis*-retinol; Fig. 3*G*) implies that acute *Nrl* KO induces expression of a factor that is normally cone specific, possibly a retinol dehydrogenase, that can mediate conversion of 11-*cis*-retinol (or 9-*cis*-retinol) to the retinal form. Further experiments would be required to determine the identity of this enzyme.

Apart from providing insights into the plasticity and maintenance of rod photoreceptor identity, this study demonstrated that partial rod-to-cone reprogramming can forestall retinal degeneration in the $Rho^{-/-}$ model of retinitis pigmentosa (Fig. 5). At this time, the mechanism underlying the preservation of reprogrammed rod photoreceptors is unknown. Although these cells are not true cones, they exhibit sufficient down-regulation of rod-specific genes to resist the deleterious effects of a rod-specific mutation. Interestingly, some of the rod gene expression changes induced by acute *Nrl* KO are similar to those seen following treatment of the retina with ciliary neurotrophic factor (CNTF) (47). This observation suggests that these two manipulations, acute *Nrl* KO and CNTF delivery, may preserve rod photoreceptors via a related pathway. The neuroprotective mechanism of CNTF is unclear, but one hypothesis is that down-regulation of the rod phototransduction machinery reduces the metabolic stress on cells prone to degeneration (48). Down-regulation of rod genes in the acute *Nrl* KO may play a similar protective role.

It will be necessary to test acute *Nrl* KO in other models of retinitis pigmentosa, including models of autosomal dominant disease, to determine whether it can be used as a therapy for multiple forms of retinal degeneration. In addition, for this therapy to someday be applicable to humans, it will be necessary to implement *NRL* knockdown through a virus-based approach. The present treatment strategy might also be applicable to cone-based diseases. If it were possible to achieve complete reprogramming of rods into cones in the adult, the reprogrammed rods might serve as a localized in situ source of novel cones in diseases that preferentially afflict this cell type, such as age-related macular degeneration. This strategy would circumvent the considerable difficulties associated with conventional regenerative medicine approaches that require in vitro differentiation of cones from embryonic stem cells or induced pluripotent stem cells followed by engraftment into the patient.

Materials and Methods

Induction of Cre^{ERTM} activity was accomplished by i.p. injection of 4-hydroxytamoxifen as described in *SI Materials and Methods*. In situ hybridizations (49), ERGs (50), isolated retinal recordings (27), and qRT-PCR (51) were performed as described in previous publications, with modifications detailed in *SI Materials and Methods*. Oligonucleotides used in this study are listed in Tables S1 and S2. Bisulfite methylation analysis, electron microscopy, and Western blotting were performed on tissue isolated from animals at P63 as described in *SI Materials and Methods*.

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