Functional interchangeability of rod and cone transducin α-subunits

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Rod and cone photoreceptors use similar but distinct sets of phototransduction proteins to achieve different functional properties, suitable for their role as dim and bright light receptors, respectively. For example, rod and cone visual pigments couple to distinct variants of the heterotrimeric G protein transducin. However, the role of the structural differences between rod and cone transducin α subunits (Tα) in determining the functional differences between rods and cones is unknown. To address this question, we studied the translocation and signaling properties of rod Tα expressed in cones and cone Tα expressed in rods in three mouse strains: rod Tα knockout, cone Tα *GNAT2*^{cpfl3} mutant, and rod and cone Tα double mutant *rd17* mouse. Surprisingly, although the rod/cone Tα are only 79% identical, exogenously expressed rod or cone Tα localized and translocated identically to endogenous Tα in each photoreceptor type. Moreover, exogenously expressed rod or cone Tα rescued electroretinogram responses (ERGs) in mice lacking functional cone or rod Tα, respectively. Ex vivo transretinal ERG and single-cell recordings from *rd17* retinas treated with rod or cone Tα showed comparable rod sensitivity and response kinetics. These results demonstrate that cone Tα forms a functional heterotrimeric G protein complex in rods and that rod and cone Tα couple equally well to the rod phototransduction cascade. Thus, rod and cone transducin α-subunits are functionally interchangeable and their signaling properties do not contribute to the intrinsic light sensitivity differences between rods and cones. Additionally, the technology used here could be adapted for any such homologue swap desired.

photoreceptor | phototransduction | signaling property | translocation

Image-forming vision is mediated by two classes of photoreceptors with complementary functional properties. Rod photoreceptors have a high light sensitivity, which allows them to detect even a single photon and to function efficiently in dim light. In contrast, cone photoreceptors are 30–100-fold less sensitive, have more rapid light responses and function primarily in bright light (1). In both rods and cones, light detection takes place in the outer segment (OS) by homologous, but distinct phototransduction cascades. It has been suggested that differences in rod and cone light response characteristics arise primarily from differences in the amount or relative activities of their respective phototransduction elements (2).

By coupling a light-activated visual pigment to the effector enzyme phosphodiesterase (PDE), the G protein transducin plays a central role in phototransduction. Indeed, deletion of the α subunit (Tα) of either rod or cone transducin abolishes light responses in their respective photoreceptors (3, 4). Rod transducin consists of rod Tα, Gβ1, and Gγ1, whereas cone transducin is composed of cone Tα, Gβ3, and Gγ8. The mechanism of light detection and phototransduction is well understood at a molecular level in rods (5). In contrast, phototransduction in cones, although related, remains elusive largely because of the low percentage of cones (~3%) in the murine retina making biochemical and physiological studies of cones challenging. As a result, it is not known how the properties of rod and cone transducins affect the function of photoreceptors and whether differences between the two isoforms of transducin contribute to the functional differences between rods and cones. Furthermore, despite the important role of cones for our high resolution daytime vision, mechanisms of light adaptation that allow cones to reduce sensitivity in the presence of bright light are poorly understood. In rods, one mechanism believed to contribute to light adaptation occurs through translocation of transducin out of the OS in response to exposure to light (6). Rod Tα is primarily compartmentalized in the rod OS in the dark but redistributes into the inner segment (IS) and cell body after light exposure. However, under a light intensity sufficient to trigger rod Tα translocation (~600 lux), cone Tα remains in the cone OS (7). Although the different membrane affinities of rod and cone transducins have been shown to play a role in their distinct translocation properties (8), it is not known whether translocation depends exclusively on the rod or cone origin of the transducin or is also affected by other components of the phototransduction machinery.

To determine whether the structure of Tα contributes to these functional differences between rods and cones, we used AAV vector technology in combination with rod- or cone-preferred promoters to express and evaluate the function of rod Tα in cones and cone Tα in rods in strains of mice that lacked either rod Tα, cone Tα, or both.

Results

Light-Induced Translocation of Exogenous, Nonhomologous Rod and Cone Tα Parallels That of Endogenous Tα. We first documented the expression patterns of *rd17* retinas treated with AAV-CBA-rod Tα or AAV-CBA-cone Tα by immunostaining with rod or cone Tα-specific antibodies. The *rd17* mouse has a deletion in rod Tα and a homozygous *cpfl3* mutation in cone Tα and effectively lacks both proteins. The promiscuous CBA promoter drives transgene expression in both rods and cones (9). As expected, retinal whole-mount or section immunolabeling of untreated rd17 retinas showed no detectable rod Tα expression (Fig. S1*A* and Fig. 1*A Bottom*). Thus, there was no background signal due to endogenous rod Tα to interfere with detection of vector-derived rod Tα. In AAV-CBA-rod Tα-treated retinas, Tα expression was apparent, with more positive cells proximal to the

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Conflict of interest statement: W.W.H. and the University of Florida have a financial interest in the use of AAV therapies and own equity in a company (AGTC Inc.) that might, in the future, commercialize some aspects of this work.

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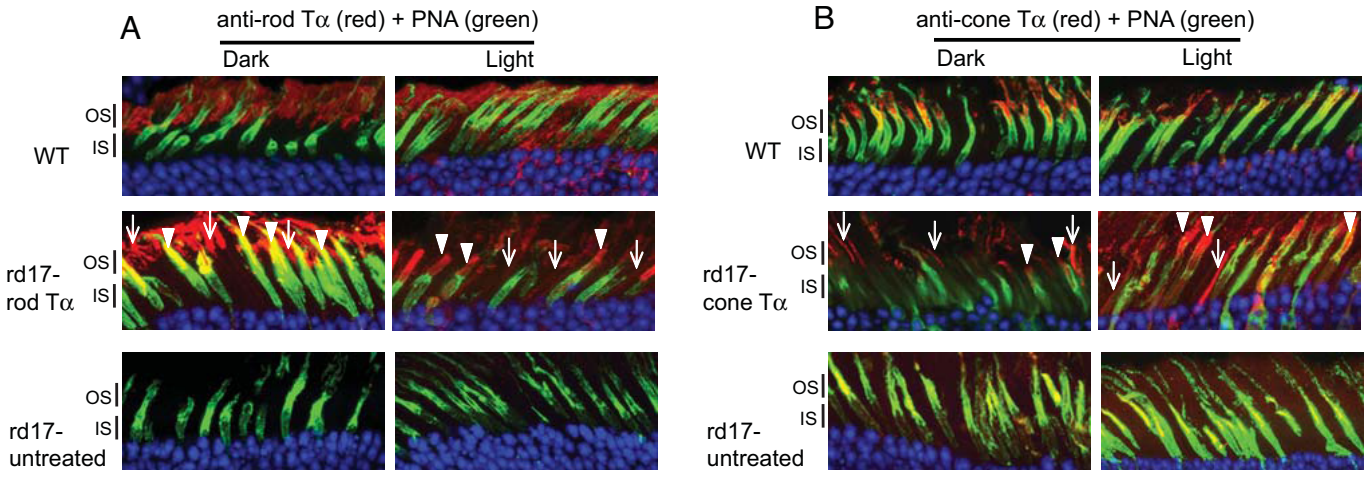

Fig. 1. Translocation of Tα in *rd17* retinas. AAV-expressed rod Tα and cone Tα was observed by immunostaining in both rods (arrows) and cones (arrow heads) in *rd17* retinas after treatment with AAV5-CBA-rod Tα (*A*) or AAV5-CBA-cone Tα (*B*) vectors. Cones were labeled with PNA (green); rod or cone Tα was labeled red by rod or cone-specific antibodies. (*A*) Localization of vector-expressed rod Tα in cones paralleled that of endogenous cone Tα. In the dark, AAV-expressed rod Tα was observed only in the OS in both rods and cones. Under light conditions, rod Tα was observed in both rod OS and IS, whereas rod Tα remained only in cone OS. (*B*) Localization of vector-expressed cone Tα in rods was similar to that of endogenous rod Tα. In the dark, cone Tα was observed only in OS of both rods and cones. Under light conditions, exogenously expressed cone Tα was redistributed into rod IS, whereas the majority of cone Tα remained localized to cone OS. (Scale bar, 20 μm.)

injection site than distal to it (Fig. S1*B*). For cone Tα, consistent with previous results (4), the *cpfl3* mutation in *rd17* mice resulted in reduced but detectable levels of cone Tα compared with wild-type retinas (Fig. S1*C* and Fig. 1*B Bottom*). This reduction in cone Tα expression in *rd17* mice combined with the naturally sparse expression of endogenous cone Tα in the rod-dominant mouse retina, also resulted in minimal staining interference. In AAV-CBA-cone Tα-treated retinas, expression was much more prominent, especially around the injection site, than in untreated *rd17* retinas (Fig. S1*D*). Western blot analysis with an antibody that recognizes both rod and cone Tα revealed that the total level of Tα was similar in AAV-CBA-rod Tα- and AAV-CBA-cone Tα-treated *rd17* eyes, at ~30% of total endogenous Tα levels seen in the wild-type retina (Fig. S2). The transducin levels in the untreated *rd17* retinas were ~20% of the wild-type, indicating that a mutated form of rod transducin may be detected by this antibody besides the cone *cpfl3* mutant by using the highly sensitive Odyssey Western blot procedure.

We then analyzed the translocation of rod Tα in cones and cone Tα in rods under various light conditions. In dark-adapted AAV-CBA-rod Tα-treated *rd17* mice, exogenous rod Tα was only observed in the rod OS (Fig. 1*A Middle Left*, arrows). Upon exposure to light just before harvesting the retinas, exogenous rod Tα was found in both the OS and IS of rods (Fig. 1 *Middle Right*, arrows) and had therefore translocated qualitatively normally. In *rd17* cones, exogenous rod Tα was observed primarily in the OS in both dark-adapted and light-adapted retinas (Fig. 1*A Middle*, arrow heads). Therefore, both rod Tα expressed in rods and rod Tα expressed in cones displayed the same light-mediated translocation behavior as endogenous rod Tα (Fig. 1*A Top*), or endogenous cone Tα (Fig. 1*B Top*) (Table S1).

Interestingly, the mutant *cpfl3* form of cone Tα expressed in *rd17* cones appeared to be mislocalized as it was found in both cone OS and IS under both dark- and light-adapted conditions (Fig. 1*B Bottom*). In *rd17* retinas treated with CBA-cone Tα, although exogenous cone Tα could not be distinguished from its endogenous mutant form by immunolabeling, cone Tα now localized primarily to the cone OS under both dark- and light-adapted conditions (Fig. 1*B Middle*, arrow heads). This behavior is as would be expected for wild-type cone Tα. The result is consistent with cone Tα expression being more robust in vector-treated than in untreated *rd17* retinas and suggests that cone Tα expressed from AAV predominates in *rd17* cones. In *rd17* rods treated with AAV-CBA-cone Tα, exogenous cone Tα was primarily localized to the rod OS in the dark but redistributed into the rod IS after light exposure (Fig. 1*B Middle*, arrows). Therefore, cone Tα expressed exogenously in rods displayed the same light-dependent translocation as endogenous rod Tα (Fig. 1*A Top*) (Table S1). The ratio of OS vs. IS vector-expressed rod or cone Tα in *rd17* rods under either dark- or light-adapted conditions was also comparable to that of endogenous rod Tα in wild-type retinas. We also found that there were no significant quantitative differences in light/dark induced translocation between rod and cone Tα when expressed in the same photoreceptor type (Fig. S3).

Exogenous Rod and Cone Tα Rescue in Vivo Rod and Cone ERG Responses Equivalently in Tα-Deficient Mice. To determine whether exogenous rod and cone Tα can rescue rod or cone function, we recorded full-field scotopic and photopic ERG responses from rod Tα deficient (Trα−/−), cone Tα-deficient (*GNAT2*^{cpfl3}), and *rd17* retinas treated with a rod targeting vector expressing cone Tα (AAV-mOP-cone Tα), a cone targeting vector expressing rod Tα (AAV-PR2.1-rod Tα) and rod/cone targeting vectors expressing either rod or cone Tα (AAV-CBA-rod or -cone Tα) (Table S1).

Trα−/− mice have no detectable rod Tα and consequently no rod-mediated ERG responses (3). Vector delivery of cone Tα to Trα−/− rods showed significant restoration of rod-driven ERG responses (Fig. S4*A*). The average rod-driven b-wave amplitude at a flash intensity of 0.01 cd.s.m^{-2} (candelas - seconds per meter squared) in treated eyes was 136 ± 76 μV (mean ± SD) whereas it was undetectable in fellow untreated eyes ($n = 6$; $P < 0.001$) (Fig. 2*A*). Thus, cone Tα was capable of restoring rod-like photopic ERG responses in rods.

It has been shown that cone-mediated ERG responses can be restored in *GNAT2*^{cpfl3} retinas treated with the cone targeting AAV-PR2.1-cone Tα vector (10). Here, we observed comparable levels of cone ERG restoration with a cone-targeted rod Tα vector (PR2.1-rod Tα) (Fig. S4*B*). Treated eyes showed significant cone-mediated ERG improvement (84 ± 15 μV at 10 cd.s.m^{-2}) compared with the undetectable levels in fellow

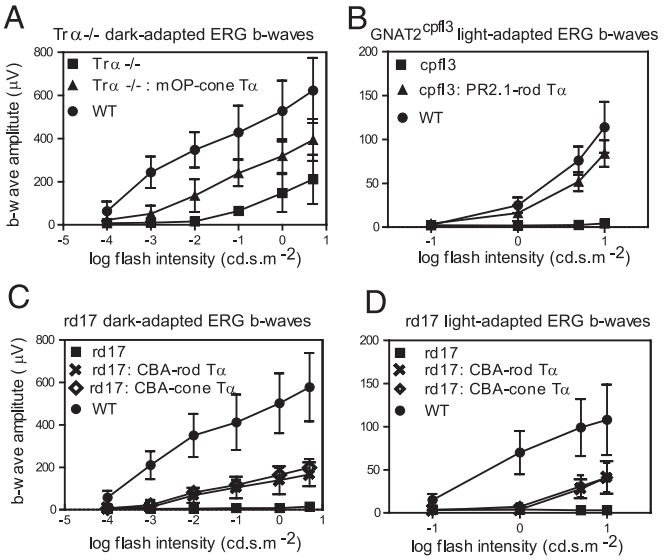

Fig. 2. Electrophysiological analysis of Trα−/−, *GNAT2*^{cpfl3}, and *rd17* mice after treatment with rod or cone Tα vectors. Each data point represents the mean ± SD of b-wave amplitudes recorded for each group at the indicated input flash intensity. (*A*) Comparison of dark-adapted ERG responses from wild-type, Trα−/−, and contralateral Trα−/− eyes treated with AAV5-mOP-cone Tα. The rod-driven b-wave (flash intensity at 0.01 cd.s.m^{-2}) missing in the untreated Trα−/− eye was partially restored after treatment with AAV5-mOP-cone Tα. Paired *t* test analysis showed that the b-wave amplitude at this intensity was significantly different between Trα−/− untreated eyes and fellow AAV5-mOP-cone Tα-treated eyes ($P < 0.001$). (*B*) Comparison of light-adapted cone-mediated ERGs in wild-type, *GNAT2*^{cpfl3} untreated and contralateral AAV5-PR2.1-rod Tα-treated *GNAT2*^{cpfl3}eyes. ERG responses were recorded after adaptation to a rod-saturating background light. Statistical analysis showed significant differences between untreated and fellow treated *GNAT2*^{cpfl3}eyes at flash intensities of 5 and 10 cd.s.m^{-2} ($P < 0.001$). (*C* and *D*) Both dark-adapted (*C*) and light-adapted (*D*) ERG b-waves were partially restored in *rd17* mice after treatment with either AAV5-CBA-rod Tα or AAV5-CBA-cone Tα vectors. Statistical analysis demonstrated significant differences between untreated and fellow vector-treated eyes for dark-adapted b-waves at 0.01, 0.1, 1, and 5 cd.s.m^{-2} ($P < 0.001$) and for light-adapted b-waves at 5 and 10 cd.s.m^{-2} ($P < 0.05$). No statistical difference in recovered b-wave amplitudes was found between AAV5-CBA-rod Tα and AAV5-CBA-cone Tα treated *rd17* eyes under either dark-adapted or light-adapted conditions. B-wave amplitudes at indicated flash intensities were compared by repeated-measures ANOVA, with the Bonferroni post hoc test for ANOVA ($P < 0.05$) used to compare means at individual flash intensities.

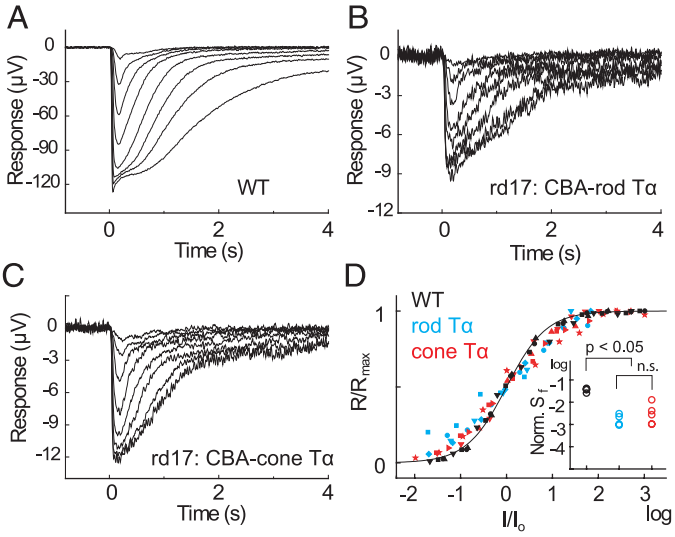

Fig. 3. Response families from ex vivo transretinal ERG recordings obtained from wild-type (*A*), rod Tα-treated (*B*), and cone Tα-treated (*C*) *rd17* retinas. Test flashes of incremental intensities, in 0.5 log steps, were delivered at time 0. The dimmest flashes delivered 7.6 (*A*) and 25 (*B* and *C*) photons μm^{-2}. (*D*) Intensity-response relations of individual retinas normalized for maximal response (R_{max}) and half-saturating flash intensity (I_o). Whereas the intensity-response relations in wild-type retinas (black symbols, $n = 4$) were well fit by Eq. **1** with $k = 1$ (solid line), those for rod Tα-treated (blue symbols, $n = 4$) and cone Tα-treated (red symbols, $n = 5$) retina were less steep than Eq. **1**. (*Inset*) Cumulative results of normalized sensitivity (S_f) from individual wild-type (black, $n = 4$), rod Tα-treated (blue, $n = 4$), and cone Tα-treated (red, $n = 5$) *rd17* retinas. Statistical analysis was carried out by the one-way ANOVA with the post hoc Bonferroni test.

untreated eyes ($n = 10, P < 0.001$) (Fig. 2*B*). Thus, rod Tα was capable of restoring cone-like ERG responses in cones.

Exogenous rod or cone Tα, driven by the CBA promoter, rescued both rod and cone-mediated ERG responses in *rd17* mice (Fig. S4 *C* and *D*). Although the rescue was only partial, the two transducin isoforms significantly improved both rod-mediated ERG ($n = 6, P < 0.001$) and cone-mediated ERG ($n = 6, P < 0.05$) responses compared with untreated fellow eyes (Fig. 2 *C* and *D*). Critically, there was no statistical difference in the abilities of rod and cone Tα to restore either rod-mediated ERG responses or cone-mediated ERG responses (both $P > 0.05$) when expressed in the ectopic photoreceptor cell type.

Exogenous Rod and Cone Tα Rescue Light Sensitivity in *rd17* Retinas. To further characterize the function of rods and cones expressing exogenous rod or cone Tα, we carried out transretinal ERG recordings from isolated AAV-CBA-rod or cone Tα-treated *rd17* retinas (Table S1). We isolated the photoreceptor component (a-wave) of transretinal ERG responses by pharmacologically blocking synaptic transmission (*SI Text*). This allowed us to measure maximum amplitudes and light sensitivities of the overall rod and cone response. Wild-type retinas produced robust ERG photoresponses with reproducible maximum amplitudes averaging 138 ± 14 μV (mean ± SEM, $n = 4$). Whereas dim flash responses were generated exclusively by the rods, brighter flashes elicited a mixed response containing slow (rod) and fast (cone) components (Fig. 3*A*). In contrast, *rd17* retinas from 3-week-old animals produced no detectable ERG responses, indicating complete loss of photosensitivity in both rods and cones. Vector treatment rescued photosensitivity in 10 of 11 rod Tα-transduced retinas and in 7 of 8 cone Tα-transduced retinas. However, the maximum response was less than 2 μV in 7 of 11 retinas expressing rod Tα and in 3 of 8 retinas expressing cone Tα. These results indicate incomplete photoreceptor transduction by the vector and are consistent with the retinal whole mounts immunostaining and Western blot analysis (Figs. S1 and S2). Excluding these, the maximum responses were 6.7 ± 1.6 μV ($n = 4$) and 11.6 ± 4.1 μV ($n = 5$) for rod Tα- and cone Tα-treated animals, respectively (Fig. 3 *B* and *C* and Table 1). Although the normalized flash sensitivity (photons^{-1} μm^2) was on average 3.5×10^{-2} for wild-type retinas ($n = 4$), it was significantly lower in the vector-treated *rd17* retinas, averaging 1.8×10^{-3} for rod Tα treatment ($n = 4$) and 4.3×10^{-3} for cone Tα treatment ($n = 5$) (Fig. 3*D Inset* and Table 1). Interestingly, the intensity-response relationships for both rod Tα- and cone Tα-treated retinas were shallower and wider than those of wild-type retinas (Fig. 3*D*). Together, these results demonstrate that both rod and cone Tα were able to rescue phototransduction in *rd17* retinas. This rescue, however, was only partial as neither maximal response nor sensitivity was restored to wild-type levels.

Exogenous Rod and Cone Tα Produce Comparable Light Responses in *rd17* Rods. We could not determine from transretinal recordings the relative efficiency of exogenous Tα to rescue rod vs. cone

Table 1. Transretinal ERG recordings parameters

	WT	*n*	rd17: CBA-rod Tα	*n*	rd17: CBA-cone Tα	*n*
I_o, photons μm^{-2}	74 ± 15	4	4000 ± 2500	4	860 ± 350	5
k	0.94 ± 0.06	4	0.59 ± 0.08*	4	0.72 ± 0.04	5
Norm. sensitivity, S_f, photon^{-1} μm^2	(3.5 ± 0.3) × 10^{-2}	4	(1.8 ± 0.5) × 10^{-3}*	4	(4.3 ± 2.2) × 10^{-3}*	5
Time-to-peak, ms	193 ± 10	4	238 ± 21	4	224 ± 45	5
Maximal response of a-wave, μV	138 ± 14	4	6.7 ± 1.6*	4	11.6 ± 4.1*	5

Mean ± SEM (*n*). I_o is the flash intensity producing half-maximal response. *k* is the Hill coefficient obtained from the fit with Eq. **1**. Normalized flash sensitivity (S_f) was determined as described in *SI Text*. One-way ANOVA with the post hoc Bonferroni test determined significant differences. *, $P < 0.05$ vs. WT. No significant difference was found between rod Tα and cone Tα.

function. To address this, we carried out single-cell recordings from both rod Tα- and cone Tα-treated rods (Fig. 4 *A–C* and Table S1). These experiments also allowed us to determine whether the limited rescue seen in vector-treated retinas was due to a small fraction of Tα-transduced photoreceptors or to partial restoration of function in a large number of individual photoreceptors. All wild-type rods (10 of 10 studied cells) responded to light stimulation, yielding an average normalized sensitivity of 4.8 × 10^{-2} photons^{-1} μm^2 (Table 2). In contrast, only 10% of *rd17* rods treated with rod Tα (11 of 108 cells in two animals) and 8.4% of cone Tα-treated *rd17* rods (17 of 202 cells in two animals) generated a detectable light response. We excluded from further analysis cells in which we did not obtain a complete family of responses or with maximal response to an unattenuated test flash (1.7 × 10^5 photons μm^{-2}) below 3 pA.

We found that the slope of intensity-response relations showed no significant difference between WT and Tα-treated rods (Fig. 4*D*). This indicates that the shallower slope in this relation for rod/cone Tα-treated retinas by transretinal ERG recording (Fig. 3*D*) was likely due to the variable expression of exogenous Tα from rod to rod, thus spreading the rod sensitivity over a wide range. Indeed, both rod Tα- and cone Tα-treated rods exhibited great variability in light sensitivity (Fig. 4*D Inset*). Compared with wild-type rods, the mean normalized flash sensitivity of *rd17* rods was 13-fold lower (3.8 × 10^{-3} photons^{-1} μm^2) for rod Tα-treated cells and 11-fold lower (4.4 × 10^{-3} photons^{-1} μm^2) for cone Tα-treated cells. Notably however, some rod/cone Tα-treated rods had light sensitivities comparable to that of wild-type rods, demonstrating that it was possible to achieve full rescue. Consistent with this notion, we also found that the maximal amplification constants of rod Tα-treated (8.8 s^{-2}) and cone Tα-treated (4.4 s^{-2}) *rd17* rods were comparable to the mean amplification constant of wild-type rods (12 s^{-2}).

In sum, we found no significant difference in sensitivity and amplification constant between rod Tα- and cone Tα-treated *rd17* rods (Fig. 4*D* and Table 2). In addition, we found no significant difference in the kinetics of dim-flash responses and in the dominant constant of recovery of rod Tα- and cone Tα-treated rods (Table 2). Thus, the signaling properties of rod and cone Tα subunits were comparable when either was expressed in *rd17* rods.

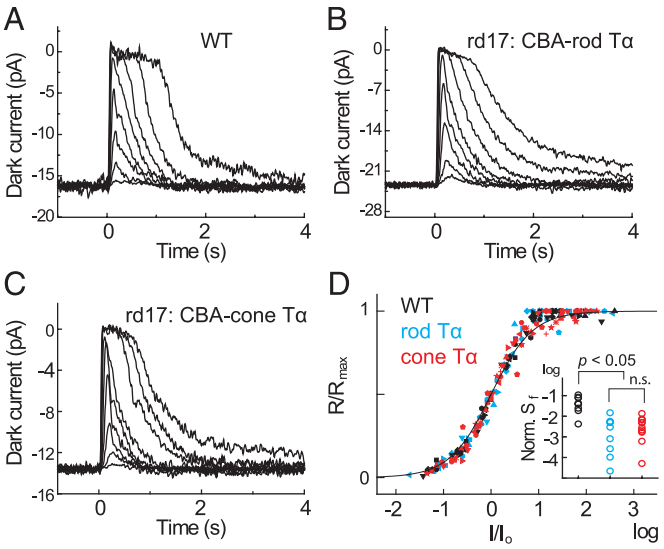

Fig. 4. Response families from single-cell recordings obtained from individual wild-type (*A*), rod Tα-treated (*B*), and cone Tα-treated (*C*) *rd17* rods. Test flashes of incremental intensities, in 0.5 log steps, were delivered at time 0. The dimmest flashes delivered 1.0 (*A*) and 4.4 (*B* and *C*) photons μm^{-2}. (*D*) Intensity-response relations of individual cells normalized for maximal response (R_{max}) and half-saturating flash intensity (I_o). Data from wild-type rods (black symbols, $n = 9$), rod Tα-treated *rd17* rods (blue symbols, $n = 9$), and cone Tα-treated rods *rd17* (red; $n = 12$) were all well fit by Eq. **1** with $k = 1$. (*Inset*) Cumulative results of normalized sensitivity (S_f) from individual wild-type (black, $n = 9$), rod Tα-treated (blue, $n = 9$), and cone Tα-treated (red, $n = 12$) rods. Statistical analysis was carried out by the one-way ANOVA with the post hoc Bonferroni test.

Exogenous Rod Tα Rescues Light Sensitivity in *rd17* Cones. The AAV-CBA vectors examined so far support expression in both rods and cones. To explore the function of rod Tα targeted preferentially to cones, we used the PR2.1 promoter to target rod Tα to cones (11). The saturating response of *rd17* retinas treated with AAV-PR2.1-rod Tα exhibited both slow (rod) and rapid (cone) components (Fig. 5*B*). The maximum response was approximately 60 μV with a threshold of 70 photons μm^{-2}, also consistent with robust rod rescue by the vector. The clear rod and cone components of AAV-PR2.1-rod Tα-treated retina suggest that although PR2.1 primarily targets cones, the vector was introduced into and rescued the function of some rods as well. We isolated the cone component from this mixed photoresponse by suppressing the rod component with an initial high intensity flash and then stimulating the retina with a second flash. The resultant response was comparable in kinetics to cone responses from wild-type cones (Fig. 5*B Inset*), thus confirming the cone rescue by PR2.1-rod Tα observed with full field cone ERG recordings (Fig. 2*B*).

In contrast, *rd17* retinas treated with AAV-PR2.1-cone Tα showed no fast, cone-like component in their bright flash responses (Fig. 5*A*). Thus, these photoresponses were most likely generated by rods. The low sensitivity and small (for rods) response amplitude could be explained by a small fraction of rods receiving limited levels of cone Tα. The failure of exogenous cone Tα to rescue cone function in *rd17* retina when using the PR2.1 promoter most likely reflects transduction efficiency limitations of this cone-targeted AAV vector delivery method.

Discussion

In this study, we expressed rod Tα in cones and cone Tα in rods to determine and compare the functional properties of these two transducin isoforms. Our results demonstrate that rod and cone

Table 2. Single-cell recordings parameters

	WT	*n*	rd17: CBA-rod Tα	*n*	rd17: CBA-cone Tα	*n*
Dark current, pA	17.2 ± 1.3	9	11.4 ± 1.9*	9	8.1 ± 1.1*	12
Norm. sensitivity, S_f, photon^{-1} μm^2	(4.8 ± 1.1) × 10^{-2}	9	(3.8 ± 1.5) × 10^{-3}*	9	(4.4 ± 1.1) × 10^{-3}*	12
Time-to-peak, ms	190 ± 6	9	247 ± 15	9	288 ± 20*	12
Amplification constant, s^{-2}	12.1 ± 0.8	9	1.5 ± 0.9*	9	1.6 ± 0.5*	12
Integration time, ms	489 ± 41	9	371 ± 13	9	476 ± 108	12
Recovery time constant, ms	380 ± 21	9	311 ± 13	9	392 ± 58	12
Dominant constant of recovery, ms	230 ± 19	9	223 ± 24	5	200 ± 15	7

Mean ± SEM (*n*). Normalized flash sensitivity (S_f) was determined as described in *SI Text*. Integration time was calculated by dividing the area of dim flash response by its amplitude. Recovery time constant was estimated by fitting the recovery phase of dim flash response with single exponential function. One-way ANOVA with the post hoc Bonferroni test determined significant differences. *, $P < 0.05$ vs. WT. No significant difference was found between rod Tα and cone Tα.

Tα subunits are functionally interchangeable. When expressed exogenously, both subunits exhibited intracellular localization patterns characteristic for each photoreceptor type under both dark- and light-adapted conditions. Thus, the distribution of exogenous cone Tα expressed in rods was similar to that of endogenous rod Tα. Similarly, exogenous rod Tα expressed in cones had a distribution similar to that of endogenous cone Tα. We also found that the two subunits could functionally substitute for each other and rescue full field or *ex vivo* ERG responses in rod Tα (Trα−/−), cone Tα (*GNAT2*^{cpfl3}) and rod/cone Tα (*rd17*) deficient mice. Finally, the sensitivity and response kinetics of *rd17* rods expressing rod or cone Tα, as measured from single-cell recordings, were essentially identical.

The interchangeability of Tα subunits without alteration in the rod and cone patterns of light-dependent Tα translocation clearly demonstrates that translocation is determined by components other than rod and cone Tα alone. This finding is consistent with previous experiments showing that both transiently transfected (12) and transgenic (8) cone Tα undergoes light-dependent translocation in rods similar to endogenous rod Tα. Our results also indicate that rod/cone Tα are not involved in controlling the light threshold for transducin translocation because exogenously expressed cone Tα and endogenous rod Tα translocate similarly under identical light conditions. In addition, exogenously expressed rod Tα remains in the cone OS under the same light environment as does endogenous cone Tα. It has been shown that the light threshold for normal rod transducin redistribution is related to the capacity of the GTPase-activating complex (GAC) to stimulate GTP hydrolysis and inactivate rod Tα (13). This threshold is shifted to either lower or higher light intensities in mutant mice lacking or overexpressing components of GAC (13, 14), respectively. Increased levels of activated rod Tα are apparently sufficient to trigger transducin translocation suggesting that downstream signaling events are not involved. Our results are consistent with the notion that the significantly higher threshold for transducin translocation in cones compared with rods is not a result of functional differences between rod and cone transducins, but is instead due to the inherently higher cone GAC activity unless cone $\beta_3\gamma_8$ subunits play a key role in Tα translocation (15).

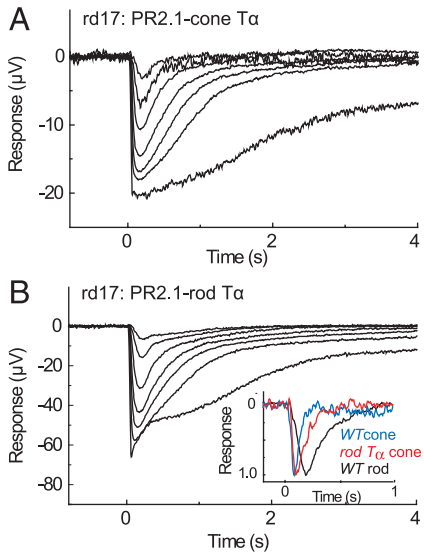

Fig. 5. Transretinal ERG response families from *rd17* retinas treated with AAV5-PR2.1-rod or cone Tα vector. (*A*) Although ERG responses could be restored in *rd17* retina by treating with AAV5-PR2.1-cone Tα, the resulting responses had rod-like kinetics and sensitivity, indicating rescue of rod function without detectable rescue of cone function. (*B*) Both rod (slow) and cone (fast) ERG responses could be restored in *rd17* retina by treating with AAV5-PR2.1-rod Tα, indicating rescue of both rod and cone function. Test flashes of incremental intensities, in 0.5 log steps, were delivered at time 0. In both *A* and *B*, the dimmest flash delivered 25 photons μm^{-2}. The largest response in each panel was generated by unattenuated white light. (*Inset*) Normalized cone dim flash responses from the retina in *B* (red trace) and from wild-type retina (blue trace) extracted by double-flash stimulation. For comparison, wild-type rod response (black trace) is also shown.

Our experiments also suggest that exogenously expressed rod or cone Tα can form functional heterotrimers with the endogenous βγ subunits of the opposite photoreceptor type. In rods, the resulting mixed heterotrimer is sequestered in the OS in darkness and is translocated to the IS after light activation. Similarly, vector-expressed rod Tα forms functional heterotrimers with endogenous cone β_3 and γ_8 subunits (G$\beta_3\gamma_8$), but this mixed heterotrimer remains within cone OS in the dark and after light exposure. Rosenzweig et al. (8) showed that the localization of transducin in photoreceptors is determined by its subunit dissociation status. In the dark, the transducin heterotrimer is predominantly localized in rod and cone OS, where it is associated with the OS disc membrane through lipid modification of the alpha and gamma subunits. Photoexcited rhodopsin activates rod transducin leading to disassociation of Tα from its G$\beta_1\gamma_1$ subunits (16, 17). In this state, the subunits have a lower membrane affinity and diffuse throughout all rod intracellular compartments. Our finding that $\beta_1\gamma_1$ subunits are partially mislocalized to the IS and cell body in dark-adapted *rd17* retinas is consistent with this model. However, to demonstrate that $\beta_1\gamma_1$ are correctly localized in the OS in the dark after Tα treatment proved experimentally difficult for two reasons: first, only a small fraction of rods were transduced and their expression levels were lower than for wild-type retinas; second, we were not able to carry out α and β_1 coimmunostaining because antibodies from two different species were not available to us. Nevertheless, the similarity in recovery time constant and integration time between rod Tα-treated and cone Tα-treated rods in *rd17* demonstrates that exogenous cone Tα must have formed functional

heterotrimers with $G\beta_1\gamma_1$ as stable as the normal rod $T\alpha\beta\gamma$ complex. Finally, the comparable dominant time constants of wild-type, rod Tα-treated, and cone Tα-treated rods indicate that the lifetime of activated transducin in rods is comparable independent of whether rod or cone Tα is present.

Our results suggest that rod Tα does not contribute to the unique rod photoresponse properties acquired during evolution. The comparable levels of restored sensitivity we observed in treated rods imply that rod and cone Tα couple equally well to the rod phototransduction cascade. Thus, the signaling properties of Tα do not appear to contribute to the intrinsic differences in light sensitivity between rods and cones. Our results are also consistent with previous studies in transgenic *Xenopus* (18) and in murine photoreceptors (19–21) indicating that rod and cone visual pigments can couple efficiently to the transducin of the opposing photoreceptor type. The unusual case of salamander green rods and blue cones that share the same visual pigment but use rod and cone transducins, respectively (22), is also consistent with that notion.

Finally, some rod/cone Tα-treated rods showed levels of light sensitivity similar to that of wild-type rods, demonstrating that full rescue in AAV-treated rods is possible, although not common. We note that the vector technology used here, which in the past has been generally used for the development of therapeutic applications, could be readily adapted for any such homologue swap desired.

Materials and Methods

AAV Vectors and Subretinal Injections. The rod Tα cDNA was subcloned under PR2.1 (11), and under the chicken β-actin promoter (CBA) (9) in AAV serotype 5 vectors (AAV5). The cone Tα cDNA was subcloned under the mouse opsin promoter (mOP) (23) and under the CBA promoter in AAV5 vectors. All mice were injected at 3 weeks of age in one eye (*SI Text*). One microliter of vector containing 10^9 vector genomes was injected in all cases, except that 1 μL of PR2.1-rod Tα and PR2.1-cone Tα contained 10^{11} vector genomes.

Electroretinograms. ERG analysis was performed 6 weeks after injection according to procedures reported in ref. 24.

Immunohistochemistry. *Rd17* and age-matched wild-type ALR mice were either dark adapted for 5 h or light adapted for 30 min by exposing to ~1,000 lux white light. The eyes were then immediately processed according to methods reported in ref. 24. Rod Tα- (anti-Gαt1, Santa Cruz) or cone Tα- (anti-Gαt2, Santa Cruz) specific antibody, and biotinylated Peanut agglutinin lectin (PNA) (Vector Laboratories) was used in the primary antibody diluant. The Alexa594-conjugated antibody (Invitrogen) and Fluorescein Avidin D (Vector Laboratories) was used in the secondary diluant.

Ex Vivo Electrophysiology. Single-cell recordings and transretinal ERG recordings were performed according to published methods (20, 25). Further details are described in the *SI Text*. Amplification constants were determined by fitting the equation of Pugh and Lamb (26) to the early rising phase of flash responses (effective collecting area; 0.5 μm^2). Dominant constants of recovery were obtained from a slope of the Pepperberg plot (27).

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1. Korenbrot JI, Rebrik TI (2002) Tuning outer segment Ca2+ homeostasis to phototransduction in rods and cones. *Adv Exp Med Biol* 514:179–203.
2. Kawamura S, Tachibanaki S (2008) Rod and cone photoreceptors: Molecular basis of the difference in their physiology. *Comp Biochem Physiol A Mol Integr Physiol* 150:369–377.
3. Calvert PD, et al. (2000) Phototransduction in transgenic mice after targeted deletion of the rod transducin alpha -subunit. *Proc Natl Acad Sci USA* 97:13913–13918.
4. Chang B, et al. (2006) Cone photoreceptor function loss-3, a novel mouse model of achromatopsia due to a mutation in Gnat2. *Invest Ophthalmol Vis Sci* 47:5017–5021.
5. Burns ME, Arshavsky VY (2005) Beyond counting photons: Trials and trends in vertebrate visual transduction. *Neuron* 48:387–401.
6. Calvert PD, Strissel KJ, Schiesser WE, Pugh EN, Jr, Arshavsky VY (2006) Light-driven translocation of signaling proteins in vertebrate photoreceptors. *Trends Cell Biol* 16:560–568.
7. Elias RV, Sezate SS, Cao W, McGinnis JF (2004) Temporal kinetics of the light/dark translocation and compartmentation of arrestin and alpha-transducin in mouse photoreceptor cells. *Mol Vis* 10:672–681.
8. Rosenzweig DH, et al. (2007) Subunit dissociation and diffusion determine the subcellular localization of rod and cone transducins. *J Neurosci* 27:5484–5494.
9. Gee Sanftner LH, Abel H, Hauswirth WW, Flannery JG (2001) Glial cell line derived neurotrophic factor delays photoreceptor degeneration in a transgenic rat model of retinitis pigmentosa. *Mol Ther* 4:622–629.
10. Alexander JJ, et al. (2007) Restoration of cone vision in a mouse model of achromatopsia. *Nat Med* 13:685–687.
11. Li Q, Timmers AM, Guy J, Pang J, Hauswirth WW (2008) Cone-specific expression using a human red opsin promoter in recombinant AAV. *Vision Res* 48:332–338.
12. Chen J, Wu M, Sezate SA, McGinnis JF (2007) Light threshold-controlled cone alpha-transducin translocation. *Invest Ophthalmol Vis Sci* 48:3350–3355.
13. Lobanova ES, et al. (2007) Transducin translocation in rods is triggered by saturation of the GTPase-activating complex. *J Neurosci* 27:1151–1160.
14. Kerov V, et al. (2005) Transducin activation state controls its light-dependent translocation in rod photoreceptors. *J Biol Chem* 280:41069–41076.
15. Zhang H, et al. (2003) Light-dependent redistribution of visual arrestins and transducin subunits in mice with defective phototransduction. *Mol Vis* 9:231–237.
16. Fung BK, Hurley JB, Stryer L (1981) Flow of information in the light-triggered cyclic nucleotide cascade of vision. *Proc Natl Acad Sci USA* 78:152–156.
17. Mendez A, Lem J, Simon M, Chen J (2003) Light-dependent translocation of arrestin in the absence of rhodopsin phosphorylation and transducin signaling. *J Neurosci* 23:3124–3129.
18. Kefalov V, Fu Y, Marsh-Armstrong N, Yau KW (2003) Role of visual pigment properties in rod and cone phototransduction. *Nature* 425:526–531.
19. Fu Y, Kefalov V, Luo DG, Xue T, Yau KW (2008) Quantal noise from human red cone pigment. *Nat Neurosci* 11:565–571.
20. Shi G, Yau KW, Chen J, Kefalov VJ (2007) Signaling properties of a short-wave cone visual pigment and its role in phototransduction. *J Neurosci* 27:10084–10093.
21. Shi GW, et al. (2005) Light causes phosphorylation of nonactivated visual pigments in intact mouse rod photoreceptor cells. *J Biol Chem* 280:41184–41191.
22. Ma J, et al. (2001) A visual pigment expressed in both rod and cone photoreceptors. *Neuron* 32:451–461.
23. Flannery JG, et al. (1997) Efficient photoreceptor-targeted gene expression in vivo by recombinant adeno-associated virus. *Proc Natl Acad Sci USA* 94:6916–6921.
24. Haire SE, et al. (2006) Light-driven cone arrestin translocation in cones of postnatal guanylate cyclase-1 knockout mouse retina treated with AAV-GC1. *Invest Ophthalmol Vis Sci* 47:3745–3753.
25. Sillman AJ, Ito H, Tomita T (1969) Studies on mass receptor potential of isolated frog retina. 1. General properties of response. *Vision Research* 9:1435–1442.
26. Pugh EN, Jr, Lamb TD (1993) Amplification and kinetics of the activation steps in phototransduction. *Biochim Biophys Acta* 1141:111–149.
27. Pepperberg DR, et al. (1992) Light-dependent delay in the falling phase of the retinal rod photoresponse. *Vis Neurosci* 8:9–18.