G-Protein βγ-Complex Is Crucial for Efficient Signal Amplification in Vision

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A fundamental question of cell signaling biology is how faint external signals produce robust physiological responses. One universal mechanism relies on signal amplification via intracellular cascades mediated by heterotrimeric G-proteins. This high amplification system allows retinal rod photoreceptors to detect single photons of light. Although much is now known about the role of the α-subunit of the rod-specific G-protein transducin in phototransduction, the physiological function of the auxiliary βγ-complex in this process remains a mystery. Here, we show that elimination of the transducin γ-subunit drastically reduces signal amplification in intact mouse rods. The consequence is a striking decline in rod visual sensitivity and severe impairment of nocturnal vision. Our findings demonstrate that transducin βγ-complex controls signal amplification of the rod phototransduction cascade and is critical for the ability of rod photoreceptors to function in low light conditions.

Introduction

Retinal rod photoreceptors rely on the prototypical GPCR-mediated pathway to detect light (Stryer, 1986). They present a unique opportunity to address the physiological roles of individual subunits of heterotrimeric G-proteins because their phototransduction cascade is mediated by a single G-protein transducin (Gt) that consists of Gtα1 (Gtα), Gtβ1 (Gtβ), and Gtγ1 (Gtγ) isoforms. Photoactivated rhodopsin (R*) binds to Gt and activates it by triggering the exchange of GDP for GTP on Gtα. On activation, the G-protein dissociates into Gtα–GTP and Gtβγ. In turn, Gtα–GTP activates the effector enzyme phosphodiesterase (PDE6), which hydrolyzes cGMP. The resulting closure of cGMP-gated channels on the plasma membrane of the photoreceptor outer segment hyperpolarizes the cell and produces the light response. The activation of Gt represents the first amplification step in the rod phototransduction cascade. In rods, a single R* molecule activates 20–100 Gt molecules during its lifetime (Leskov et al., 2000; Heck and Hofmann, 2001; Krispel et al., 2006). The resulting overall amplification allows rods to achieve the highest physically possible sensitivity and detect a single photon of light (Baylor et al., 1979).

Phototransduction in rods is mediated exclusively by Gtα, as its deletion completely abolishes rod-driven photoreceptor activity (Calvert et al., 2000). In contrast, the Gtβγ complex has no established role in phototransduction in vivo. Early biochemical studies have suggested that Gtβγ might participate in transducin activation (Fung, 1983). However, although it is now believed that Gtβγ is necessary for maintaining the inactive state of Gtα and facilitating heterotrimer interactions with R* (Oldham and Hamm, 2008; Wensel, 2008), these conclusions are based on in vitro experiments performed under unphysiological conditions, with protein concentrations 1000-fold less (micromolar range) compared with those found in intact rods (Fu and Yau, 2007; Nickell et al., 2007). Furthermore, several biochemical experiments have suggested that, although effective R*-Gt coupling depends on the βγ-complex at low concentrations of rhodopsin, at higher bleached pigment concentrations, this dependence is lost and maximal activation of Gtα could be achieved without Gtβγ (Navon and Fung, 1987; Phillips et al., 1992; Kisselev et al., 1999; Herrmann et al., 2006). Thus, it remains an open question whether Gtβγ is required for effective signal amplification in intact rods, and the physiological role of the Gtβγ complex in vision is still unclear. An earlier attempt to address this question using a commercially available (Deltagen) Gtγ knock-out mouse strain was hampered by early onset of photoreceptor degeneration, which complicated its biochemical and physiological anal-
yisis, and resulted in the conclusion that Gtβγ does not have any specific role in visual signaling (Lobanova et al., 2008). Here, we used a different approach to create Gtγ-deficient mice with no discernible retinal degeneration during the early stages of postnatal life. Our behavioral, physiological, and biochemical analysis of these mice demonstrates that Gtβγ is crucial for the high amplification of the signaling cascade in intact rods required to support the high sensitivity of rod-mediated night vision.

Materials and Methods

Generation of Gngt1 knock-out mice. All experiments were performed in accordance with the policy on the Use of Animals in Neuroscience Research and were approved by the Saint Louis University Institutional Animal Care and Use Committee and the Washington University Animal Studies Committee. Unless otherwise specified, all mice were age-matched 2- to 3-month-old littermates of either sex; they were kept under the standard 12 h dark/light cycle and dark-adapted overnight before all experiments.

The mouse rod Gtγ gene, Gngt1, was isolated and mapped by screening the mouse phage library. It contains three exons and two introns (Hurley et al., 1984; Yatsunami et al., 1985; Tao et al., 1993; Scherer et al., 1996; Downes and Gautam, 1999) (see Fig. 1A). The targeting construct was designed to replace all three exons with a Neo cassette to eliminate the coding region of Gtγ. The conventional targeting vector was constructed by using a 1.6 kb DNA fragment as the short homology arm (SA). It was amplified by PCR using primers located 1.9 and 0.2 kb upstream of exon 1. SA was subcloned at the 5'-end of the Neo cassette in the 5'-3' orientation using MluI sites. The long homology arm (LA), a 7.3 kb XbaI fragment isolated from a lambda phage clone, was inserted at the 3'-end of the Neo cassette in the 5'-3' orientation using AvrII sites. The targeting vector was confirmed by restriction analysis and sequencing. This transgenic design is notably different from the commercially available Deltagen Gngt 1/−/− mouse (Deltagen; target ID 408), in which Gngt1 was targeted by a gene trap replacement of a part of the Gtγ coding sequence (amino acids 17-44) and intron 2 by the IRES-LacZ-Neo cassette.

The Gngt1 knock-out construct was electroporated into the 129 strain of ES cells, and G418-resistant clones were identified by PCR, DNA sequencing, and Southern blotting (inGenious Targeting Laboratory). Positive clones were injected into blastocysts to generate chimeric mice. Germline transmission in F1 and in subsequent generations derived by crossings with C57BL/6 was confirmed by PCR using primers A1/N1 for the 1.8 kb knock-out (KO) allele and WT1/WT2 for the 460 bp wild-type (WT) allele (data not shown). The forward A1 primer (5'-GGAGAACACTCATGAGGAVGACT-3') was just outside of SA, and the reverse N1 primer (5'-CCAGGGCCACTTGTGAGC-3') was within the Neo gene. The forward WT1 primer (5'-GTAAGTGCAAAGCAGAGGCATGGGCTGCCTG-3') and the reverse WT2 primer (5'-CCGGATCCAAGTGTGGCTCTTTGCCTGTTTTGGTACGAC-3') were injected into blastocysts to generate chimeric mice. Germline transmission in F1 and in subsequent generations derived by crossings with C57BL/6 was confirmed by PCR using primers A1/N1 for the 1.8 kb knock-out (KO) allele and WT1/WT2 for the 460 bp wild-type (WT) allele (data not shown). The forward A1 primer (5'-GGAGAACACTCATGAGGAVGACT-3') was just outside of SA, and the reverse N1 primer (5'-CCAGGGCCACTTGTGAGC-3') was within the Neo gene. The forward WT1 primer (5'-GTAAGTGCAAAGCAGAGGCATGGGCTGCCTG-3') and the reverse WT2 primer (5'-CCGGATCCAAGTGTGGCTCTTTGCCTGTTTTGGTACGAC-3') was inside intron 1, and the reverse WT2 primer (5'-CCGGATCCAAGTGTGGCTCTTTGCCTGTTTTGGTACGAC-3') was inside intron 2.

Antibodies and Western blotting. Rabbit antibodies sc-389-Gtα, sc-390-Gtα, sc-390-Gtα, sc-380-Gtβ, sc-381-Gtβ, sc-374-Gtγ, sc-376-Gtγ, sc-377-Gtγ, sc-15382-rhodopsin, sc-28850-phosducin, as well as goat antibodies sc-26477-Gtγ, sc-8143-RGS9, and mouse antibodies sc-8004-GRK1, sc-73044-SNAP25 were from Santa Cruz Biotechnology. Rabbit antibodies against Gγ and PDEα, PDEβ, and PDEγ were from CytoSignal Research Products. Rabbit antibodies against GCα1, GCα2, and retGC1 were a gift from A. M. Dizhoor (Pennsylvania College of Optometry, Elkins Park, PA). Rabbit antibodies against M-opsin and S-opsin were a gift from C. M. Craft (Zhu et al., 2003) (Mary D. Allen Laboratory for Vision Research, Doheny Eye Institute, University of Southern California, Los Angeles, CA). Rabbit antibodies against Gβ1 and Gγ were a gift from N. Gautam (Washington University, St. Louis, MO). Rabbit Gβ2 antibody was a gift from W. F. Simonds (National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD). Mouse antibody for rod arrestin was a gift from W. C. Smith (University of Florida, Gainesville, FL). Rabbit antibody against PhLP was a gift from B. M. Willardson (Brigham Young University, Provo, UT). Rabbit antibody body AB5585-recoverin was from Millipore. Secondary HRP antibodies were from Jackson ImmunoResearch Laboratories. Blots were developed using Pierce Femto Supersignal kit. Signal intensity of the protein bands on x-ray film was quantified by densitometry using Image Gauge (FujiFilm).

Light microscopy, electron microscopy, and immunohistochemistry. For immunolabeling, eyes were fixed in freshly prepared 0.1 M phosphate buffer, pH 7.4, containing 2% paraformaldehyde and 0.1% glutaraldehyde and embedded in LR White. Semithin 0.5 μm sections were cut in the dorsal-to-ventral direction through the optic nerve and immunostained essentially as previously described (Naash et al., 2004) followed by silver intensification using an IntenSE M Silver Enhancement Kit (GE Healthcare). For electron microscopy, ultrathin 0.1 μm sections were picked up on uncoated 75/300 mesh nickel grids, stained with uranyl acetate and lead citrate, and exposed to OsO4 vapor for 30 min. For measurements of rod outer segment (ROS) length, the embedded blocks were sectioned in the dorsal-to-ventral direction through the optic nerve. Twenty independent measurements were made starting at −500 μm from the edge of the optic nerve head on both sides with 2 μm steps between individual measurements, and the mean and SEM values were calculated for each specimen.

Protein quantification and transducin membrane partitioning. Retinas and ROS disk preparations used for Western blotting were from 2-month-old mice. Dark-adapted mouse ROS disc membranes were prepared from 50 to 150 mouse retinas, as previously described (Papamaster and Dreyer, 1974). Purified ROS disk membrane pellets contained only membrane-bound transducin subunits. They were aliquoted and stored at −80°C until protein quantification or biochemical experiments. Soluble transducin fraction was lost during the ROS disk membrane purification procedure and thus was not considered in additional analysis. Contamination by the inner segment marker, cytochrome c, was undetectable. Bovine Gtγ and Gtβγ subunits were purified and total ROS disk membrane protein and rhodopsin concentration were measured as previously described (Kisselev, 2007). Using rhodopsin or total ROS disk membrane protein measurements as loading controls produced similar results.

For partitioning experiment, R*-Gt binding measurements in fully bleached ROS disk membranes were performed as described previously (Kisselev, 2007), with the following modifications: mouse ROS disk membranes were resuspended at 3 μM rhodopsin in 10 mM Tris-HCl, pH 7.4, 100 mM NaCl, 5 mM MgCl2, 1 mM DTT, and 0.1 mM PMSF, to establish a new equilibrium between the membrane and soluble Gt. After light activation, samples were incubated on ice for 10 min, and supernatant and pellet were separated by centrifugation at 100,000 × g at 4°C for 10 min in a TLA-100.3 rotor. Gtγ content in both fractions was analyzed by quantitative immunoblotting.

Histology. Retinal and 2-month-old mice because of the lack of early retinal degeneration. Animals were dark-adapted overnight and anesthetized by subcutaneous injection of ketamine (80 mg/kg) and xylazine (15 mg/kg). Mice were euthanized with 1% isoflurane. During testing, a heating pad controlled by a rectal temperature probe maintained body temperature at 37–38°C. Full-field ERGs were recorded using a UTAS-E3000 apparatus (LKC Technologies) and platinum corneal electrodes, as described (Brantley et al., 2008; Kolesnikov et al., 2010). Reference and ground electrode needles were inserted under the skin at the skull and the tail, respectively. Test flashes of 15–650 μs white light were applied either in darkness (scotopic conditions) or in the presence of steady background illumination (200 cd m−2), after 5 min adaptation to the background light (photopic conditions). Responses from several trials were averaged and the intervals between trials were adjusted so that responses did not decrease in amplitude over the series of trials for each step. The recorded responses were bandpass filtered at 0.05–1500 Hz.

Single-cell electrophysiology. In contrast to the previously characterized Deltagen Gngt 1/−/− model (Lobanova et al., 2008), suction recordings could be performed easily from the rods of our 2- to 3-month-old Gngt 1/−/− mice because of the lack of early retinal degeneration. Animals were dark-adapted overnight and the retinas were removed, chopped into small pieces, and transferred to a perfusion chamber. A single rod outer segment was drawn into a glass microelectrode filled with solution containing 140 mM NaCl, 3.6 mM KCl, 2.4 mM MgCl2, 1.2 mM CaCl2, 3
mM HEPES, pH 7.4, 0.02 mM EDTA, and 10 mM glucose. The perfusion solution contained 112.5 mM NaCl, 3.6 mM KCl, 2.4 mM MgCl2, 1.2 mM CaCl2, 10 mM HEPES, pH 7.4, 20 mM NaHCO3, 3 mM Na succinate, 0.5 mM Na glutamate, 0.02 mM EDTA, and 10 mM glucose. The perfusion solution was bubbled with 95% O2/5% CO2 mixture and heated to 37–38°C.

Light stimulation was applied by 20 ms test flashes of calibrated 500 nm light. Photoresponses were amplified, low-pass filtered (30 Hz, 8-pole Bessel), and digitized (1 kHz). Dominant recovery time constant (τ90%) was determined from supersaturating flashes (Pepperberg et al., 1992), using a 10% criterion for photocurrent recovery from saturation. The amplification of the rod phototransduction cascade was evaluated from test flash intensities that produced identical rising phases of dim flash responses. This approach was preferred to the Lamb and Pugh determination of the amplification constant (Pugh and Lamb, 1993) because of the relatively long duration of test flashes and the effect of low-pass filtering on the response front.

Spatial contrast sensitivity measured from optomotor responses. Spatial contrast visual sensitivity of age-matched 2- to 3-month-old mice was measured using a two-alternative forced-choice protocol (Umino et al., 2008). The optomotry system (Cerebral Mechanics) consisted of a square array of four computer monitors with a pedestal in the center where the mouse was placed. An infrared-sensitive television camera and a round array of six infrared LEDs mounted above the animal were used to observe the mouse but not the monitors. Using a staircase paradigm, rotating stimuli (sine wave vertical gratings) were applied on the monitors where they formed a virtual cylinder around the mouse (Prusky et al., 2004). Mice responded to the stimuli by reflexively rotating their head in the corresponding direction. Contrast sensitivity was defined as the inverse of contrast threshold for optomotor responses. Responses were measured over a range of background light intensities, from ~6.25 to 1.85 log cd m⁻². Background monitor luminance was controlled by neutral density film filters (E-Color 211 0.9 ND; Rosco Laboratories). Temporal frequency (f) was fixed at its optimal value of 0.75 Hz for all background conditions. Spatial frequency (fj) was varied in the range of 0.014–0.481 cyc/deg, and speed of the stimuli was adjusted based on the following equation: fj = s × fj (Umino et al., 2008). For determination of maximal contrast sensitivity under each condition, data were fitted with mouse contrast sensitivity model (Umino et al., 2008), using parameters adjusted for best fit (r² > 0.8). All data were analyzed using independent two-tailed Student’s t test, with accepted significance level of p < 0.05.

Mathematical modeling of phototransduction. A mathematical model of phototransduction (Kuzmin et al., 2004) was used. This model includes all firmly established biochemical mechanisms of phototransduction and its regulation by calcium feedback. Basic equations of the model are similar to those used in many previous works (Hammer, 2000a; Nikonov et al., 2000; Hammer et al., 2003, 2005). However, our treatment of Ca²⁺ regulation and Ca²⁺ turnover differs slightly from that used before. Therefore, we present here the full set of equations comprising the model.

Number of active rhodopsin molecules, R*, is determined by a balance between its generation by light, I(t), and quenching by phosphorylation with rhodopsin kinase. We omit the detailed description of Ca²⁺ regulation of rhodopsin kinase via recoverin (Hammer et al., 2003, 2005) and instead use an empirical Hill-like relationship (the term in parentheses on right side of Eq. 1) (Calvert et al., 1998) as follows:

\[ \frac{dR^*}{dt}(t) = I(t) - \left( k_{\text{bmin}} + \frac{k_{\text{bmax}} - k_{\text{bmin}}}{1 + (Cat(t)/K_{\text{cat}})^{n_c}} \right) \cdot R^*(t). \]

Here, \( k_{\text{bmin}} \) and \( k_{\text{bmax}} \) are minimum and maximum rate constants of phosphorylation (in seconds⁻¹) at very high and zero Ca²⁺ concentrations, respectively. \( K_{\text{cat}} \) is the half-saturating Ca²⁺ concentration, and \( n_c \) is the Hill’s coefficient of regulation.

Number of activated phosphodiesterase molecules \( E^* \) is given by the following:

\[ \frac{dE^*}{dt}(t) = v_{\text{cat}}R^*(t) - k_{\text{e}}E^*(t), \]

where \( v_{\text{cat}} \) is the rate of PDE activation by single \( R^* \) (in seconds⁻¹), and \( k_{\text{e}} \) is the rate constant of \( E^* \) turnover (in seconds⁻¹).

cGMP turnover is described by the following:

\[ \frac{dcG}{dt}(t) = \alpha(t) - \beta(t), \]

where \( \alpha(t) \) is the rate of cGMP production by guanylate cyclase, and \( \beta(t) \) is the rate of its hydrolysis by phosphodiesterase. Here, cGMP concentration is expressed in moles ⋅ liter⁻¹, and \( \alpha(t) \) and \( \beta(t) \), in moles ⋅ liter⁻¹ ⋅ second⁻¹.

Guanylate cyclase activity is under calcium control, in the form similar to that for \( R^* \) turnover as follows:

\[ \alpha(t) = \alpha_{\text{min}} + \frac{\alpha_{\text{max}} - \alpha_{\text{min}}}{1 + (Cat(t)/K_{\text{cyc}})^{n_c}}. \]

Notice that, in this formulation, like in the study by Nikonov et al. (2000) (Eq. A10), the extent of guanylate cyclase regulation is limited by the range between \( \alpha_{\text{max}} \) and \( \alpha_{\text{min}} \) in contrast to most recent models (Burns et al., 2002; Hammer et al., 2003, 2005) that assume infinite regulation range (\( \alpha_{\text{min}} = 0 \)).

The rate of cGMP hydrolysis is as follows:

\[ \beta(t) = \left( \beta_{\text{dark}} + \frac{k_{\text{ct}}}{V_{\text{ct}}N_A} E^* (t) \right) \frac{cG(t)}{cG(t) + K_{\text{ct}}}. \]

Here, \( \beta_{\text{dark}} \) is the steady PDE activity in darkness, and the second term in parentheses yields light-induced activity. \( k_{\text{ct}} \) is the catalytic activity of a single light-activated PDE subunit (in seconds⁻¹), whereas ROS cytoplasmic volume \( V_{\text{ct}} \) and Avogadro’s number \( N_A \) convert the number of photoactivated PDE molecules into concentration. Hydrolysis of cGMP is supposed to proceed in accord with Michaelis kinetics, with the half-saturating cGMP concentration \( K_{\text{ct}} \). Again, we do not make the simplifying assumption \( cG(t) \ll K_{\text{ct}} \) common in recent models.

The ROS membrane current is a sum of two components, the current flowing through cGMP-gated channels \( j_{\text{cG}}(t) \) and the current carried by the Ca²⁺/Na⁺ exchanger \( j_{\text{CaEx}}(t) \):

\[ j_{\text{cG}}(t) = \frac{cG(t)^{\text{max}}}{cG(t)^{\text{max}} + K_{\text{ct}}^{\text{max}}} - j_{\text{CaEx}}(t). \]

where \( j_{\text{cGmax}} \) is maximum current at saturating cGMP concentrations, \( K_{\text{ct}}^{\text{max}} \) is a half-saturating concentration, and \( n_{\text{cG}} \) is the Hill’s coefficient of the regulation of the channels.

Free Ca²⁺ turnover is described by the following:

\[ \frac{dCat(t)}{dt} = \frac{1}{FB + 1} \left( \frac{1}{3} \cdot V_{\text{cyto}} \cdot j_{\text{cG}}(t) - j_{\text{CaEx}}(t) - k_{\text{cat}} \cdot Cat(t) (B_{\text{max}} - Cat_{\text{bmax}}) + k_{\text{cat}} \cdot Cat_{\text{bmax}}(t) \right). \]
Table 1. Experimental parameters of single-cell responses and model parameters that were varied to simulate the effects of Gβγ deletion on flash responses

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wild type</th>
<th>Gnt1T179A (n = 50)</th>
<th>Gnt1T179A (n = 27)</th>
<th>Gnt1T179A (n = 41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response parameter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tflash (μA)</td>
<td>15.7 ± 0.3</td>
<td>14.9 ± 0.4 NS</td>
<td>14.9 ± 0.3 NS</td>
<td></td>
</tr>
<tr>
<td>tpeak (μA)</td>
<td>93 ± 2</td>
<td>130 ± 10**</td>
<td>8408 ± 553**</td>
<td></td>
</tr>
<tr>
<td>tfade (ms)</td>
<td>152 ± 2</td>
<td>121 ± 2**</td>
<td>92 ± 2**</td>
<td></td>
</tr>
<tr>
<td>tint (ms)</td>
<td>260 ± 9</td>
<td>246 ± 14 NS</td>
<td>132 ± 5**</td>
<td></td>
</tr>
<tr>
<td>τmem (ms)</td>
<td>190 ± 10</td>
<td>184 ± 13 NS</td>
<td>121 ± 5**</td>
<td></td>
</tr>
<tr>
<td>Model parameter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>νFG (s⁻¹)</td>
<td>307</td>
<td>305</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>Kmax (s⁻¹)</td>
<td>11.4</td>
<td>22.3</td>
<td>63.5</td>
<td></td>
</tr>
<tr>
<td>Kj (s⁻¹)</td>
<td>6.5</td>
<td>5.4</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>FB</td>
<td>59.4</td>
<td>32.1</td>
<td>30.1</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM. Experimental parameters were as follows: tflash, Dark current measured from saturated responses; tpeak, half-saturating light intensity; ttime-to-peak (tpeak), and integration time (tint), refer to responses whose amplitudes were <0.1 μA and fell within the linear range; τmem, time constant of single-exponential decay of dim flash response recovery phase; τr, dominant time constant of recovery after supersaturating flashes determined from the linear fit to time in saturation versus intensity semilog plots (Pepperberg et al., 1992). Model parameters were as follows: νFG, rate of PDE activation by single R*; Kmax, maximum rate constant of R* turnoff at zero Ca2+; Kj, rate constant of PDE turnoff/FB, buffering power of fast Ca2+ buffer. Values were determined for population-averaged dim flash responses normalized to amplitudes of corresponding saturated responses.

In the present paper, we set BSR = 0 (see Table 2). With properly chosen FB, this does not significantly affect the quality of fitting of flash responses but reduces the number of free model parameters.

The exchange current is defined in Michaelis’ manner (Cervetto et al., 1989) as follows:

\[ j_{ex}(t) = j_{max}(t) + \frac{Cat(t)}{Cat(t) + K_{cat}} \]  

The parameters of the model were chosen to be within empirical biochemical and biophysical values when such data were available (Tables 1, 2). This provided a great flexibility of fitting, allowing virtually perfect simulation of photoresponses (coefficient of correlation between experimental and model traces r > 0.999 in wild-type and Gnt1 T179A, and r > 0.997 in Gnt1T179A rods). However, our goal was not to produce the best fit of the experimental data, but rather to determine what parameters of the phototransduction cascade must be modified to account for the effects of Gtγ deletion. Thus, a group of parameters that was unlikely to be affected by the lack of Gtγ was kept constant among wild-type, Gnt1T179A, and Gnt1T179A rods. These included properties of the ROS plasma membrane, namely, surface density, ionic selectivity, and affinity to cGMP of the cGMP-gated channels, and properties of the Ca²⁺/K⁺ Na⁺ exchanger. Since the dark current was virtually unchanged in genetically manipulated rods, the above assumptions imply that the dark concentrations of cGMP and Ca²⁺ also remained constant. Although a constant dark cGMP level does not necessary mean that dark guanylate cyclase and phosphodiesterase activities remained unchanged, the two parameters were fixed as well. Furthermore, parameters of the Ca²⁺ modulation of the cascade components (fold regulation, affinities, and Hill’s coefficients in Eqs. 1 and 4) were also kept constant.

For proper comparison with experimental responses, model curves were Gauss-filtered, with smoothing window of 23 ms equivalent to experimental 30 Hz Bessel filter.

**Results**

**Lack of early retinal degeneration in Gtγ-deficient mice**

To investigate the function of Gtγ in the phototransduction pathway in vivo, we generated a mouse line lacking the retinal rod-specific Gtγ subunit (Gnt1T179A) (Fig. 1A). Morphological and ultrastructural analysis of 1- to 2-month-old Gnt1T179A retinas by light microscopy (Fig. 1B) and transmission electron microscopy (data not shown) demonstrated normal retinal development and photoreceptor maturation. This result clearly indicates that the Gtγ complex is not required for the formation of the rod outer segments. Although Gnt1T179A retinas showed slow progressive retinal degeneration with an onset at 3–4 months, this effect was negligible at early adult ages (Fig. 1C). Notably, the rate of late rod degeneration was similar to that observed in Gtγ-deficient (Gnat1T179A) mice (Calvert et al., 2000) indicating that Gtγ- and Gtα-deficient rods are only weakly susceptible to degeneration. This result argues against the notion that Gtγ is critical for rod viability (Lobanova et al., 2008), which, in addition, may be influenced by the choice of targeting construct and genetic background of the mice.

We used retina extracts, as well as highly purified Gnt1T179A ROS disk membrane preparations, to analyze the protein composition of Gnt1T179A rods. The ROS disks contained no contamination by rod inner segment (RIS), as demonstrated by the absence of the RIS marker cytochrome c (cyt c) (Fig. 1D). Consistent with the normal retinal morphology of Gnt1T179A retinas, the deletion of Gtγ had no effect on the level of rhodopsin expression (Fig. 1E). As ex-

**Table 2. Definition of model parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Meaning</th>
<th>Units</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>νFG</td>
<td>Rate of PDE activation by fully active R*</td>
<td>s⁻¹</td>
<td>1.4 × 10⁻⁴</td>
</tr>
<tr>
<td>k_j</td>
<td>Rate constant of PDE quenching</td>
<td>s⁻¹</td>
<td>5 × 10⁻²</td>
</tr>
<tr>
<td>cGMP turnover</td>
<td>Concentration of free cGMP in darkness</td>
<td>M</td>
<td>3 × 10⁻⁶</td>
</tr>
<tr>
<td>α_min</td>
<td>Minimum rate of cGMP synthesis</td>
<td>M⁻¹ s⁻¹</td>
<td>3.48 × 10⁻⁸</td>
</tr>
<tr>
<td>α_max</td>
<td>Maximum rate of cGMP synthesis</td>
<td>M⁻¹ s⁻¹</td>
<td>3.48 × 10⁻⁶</td>
</tr>
<tr>
<td>K_j</td>
<td>Half-saturating Ca for regulation of α</td>
<td>M</td>
<td>4.8 × 10⁻⁷</td>
</tr>
<tr>
<td>η_Ca</td>
<td>Hill’s coefficient for Ca regulation of α</td>
<td></td>
<td>3.5</td>
</tr>
<tr>
<td>β_Dark</td>
<td>Rate of cGMP hydrolysis in darkness</td>
<td>M⁻¹ s⁻¹</td>
<td>7 × 10⁻⁵</td>
</tr>
<tr>
<td>K_cat</td>
<td>Catalytic activity of single PDE subunit</td>
<td></td>
<td>2200</td>
</tr>
<tr>
<td>PDE’s Michaelis constant</td>
<td></td>
<td>M</td>
<td>10⁻⁵</td>
</tr>
<tr>
<td>Photocurrent control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I_Ca</td>
<td>Dark current</td>
<td>A</td>
<td>1.5 × 10⁻¹¹</td>
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Numerical values are given for the parameters that were kept constant in all three mouse strains.
Impaired rod function in Gty-deficient mice

To determine how the deletion of Gty affects the overall functionality of mouse vision, we first performed behavioral tests based on the ability of mice to reflexively respond to computer-generated visual stimuli. We observed a dramatic reduction in the ability of Gty-knockout mice to respond to visual stimuli, indicating a significant impairment in rod function. This impairment was consistent across different light conditions and was evident even in early stages of development, suggesting a critical role for Gty in the maturation and function of rod photoreceptors.

Among 19 other major phototransduction proteins examined, all but phosducin and PDEγ had expression levels similar to those in wild-type rods (Fig. 1F). Phosducin levels in retinas of Gngt1−/− mice were downregulated to 83 and 62% of those in control retinas, respectively. ROS-localized phosducin was reduced even more dramatically, to 69 and 35%, respectively (Fig. 1F). This effect appears to be reciprocal to the observed reduction of the Gty expression in the phosducin knock-out mouse line (Krügel et al., 2007). Interestingly, we also observed an unexpected twofold and threefold increase in the levels of inhibitory PDEγ subunit in Gngt1−/− and Gngt1−/− mice, respectively (Fig. 1F). Finally, we consistently detected residual amounts of Gγ3 and Gγ5 in our ROS preparations, possibly because of contamination with ROS plasma membranes or other retinal subcellular elements. None of the following Gγ subunits were detected: Gγ1, Gγ2, Gγ4, Gγ6, Gγ10, Gγ11, Gγ12, and Gγ13 (data not shown). The lack of early retinal degeneration and the normal expression levels of most transduction proteins in our mice allowed us to quantitatively characterize how the deletion of Gty affects their visual function as well as the phototransduction properties of individual rods.
Figure 2. Impairment of visual function in Gtγ-deficient mice. A, Spatial contrast sensitivity functions (CSFs) of wild-type (left; n = 3), Gngt1−/− (middle; n = 3), and Gnat1−/− (right; n = 3) mice. Temporal frequency (f) was fixed at its optimal value of 0.25 Hz for all light intensities. To determine the maximal contrast sensitivity under each condition, data were fitted with a mouse contrast sensitivity model (Umino et al., 2008). All data points below unity indicate no detectable optomotor responses. B, Averaged amplitudes of spatial CSFs as functions of background light intensity. All values are means ± SEM (n = 3 for all groups). C, Families of ERG responses from wild-type (left) and Gngt1−/− (right) animals. D, Intensity–response relationships for scotopic a-waves (left) and b-waves (right). Data were fitted with hyperbolic functions that yielded scotopic a-wave half-saturating light intensities of $0.39 \pm 0.08 \text{ cd m}^{-2}$ (wild type, n = 7) and $13.0 \pm 3.1 \text{ cd m}^{-2}$ ($Gngt1^{-/-}$, n = 7), and b-wave maximum amplitudes of $382 \pm 41 \text{ µV}$ (wild type, n = 7) and $207 \pm 15 \text{ µV}$ ($Gngt1^{-/-}$, n = 7). Fitting the b-wave data yielded half-saturating light intensities of $0.009 \pm 0.002 \text{ cd m}^{-2}$ (wild type, n = 7) and $0.34 \pm 0.04 \text{ cd m}^{-2}$ ($Gngt1^{-/-}$, n = 7), and b-wave maximum amplitudes of $912 \pm 69 \text{ µV}$ (wild type, n = 7) and $821 \pm 90 \text{ µV}$ ($Gngt1^{-/-}$, n = 7). Values are means ± SEM.

generated rotating sine wave gratings (Prusky et al., 2004) (Fig. 2A). The absolute contrast sensitivity of $Gngt1^{-/-}$ mice was unaltered in the photopic region ($-1 \text{ log cd m}^{-2}$ and brighter) where vision is maintained by cone photoreceptors (Umino et al., 2008) as rods become saturated. This result implies normal cone function and the absence of cone degeneration, consistent with the normal levels of cone M/L- and S-opsins and cone-specific transducin Gto x subunit in Gngt1−/− retinas (Fig. 1J) and the unaltered photopic ERG b-wave amplitudes (data not shown). In contrast, the scotopic (rod-mediated) spatial contrast sensitivity of Gty-deficient mice was shifted ~100-fold to brighter light conditions (Fig. 2B), indicating substantial rod desensitization in the absence of Gty. Gngt1−/− rods still contributed to mouse vision as spatial contrast sensitivity of $Gngt1^{-/-}$ mice was approximately twofold ($p < 0.05$) higher compared with that in Gtox-deficient ($Gnat1^{-/-}$) animals, where rods are not functional (Calvert et al., 2000). Thus, although Gty-deficient mice retained rod vision, their visual sensitivity under dim light conditions was severely reduced.

The effect of Gty deletion on retinal function was further assessed by full-field ERGs. In agreement with our behavioral results, the scotopic visual sensitivity of 2-month-old Gngt1−/− animals was markedly reduced compared with wild-type age-matched controls (Fig. 2C). The sensitivity of the photoreceptor-driven a-wave in Gngt1−/− mice was greatly decreased (by ~33-fold), and its maximal amplitude was also ~2-fold smaller compared with wild-type animals (Fig. 2D, left). The reduction in amplitude of the scotopic b-wave, dominated by rod bipolar cells, in Gngt1−/− animals was less dramatic (~10%), but the b-wave sensitivity was decreased by ~38-fold (Fig. 2D, right), in a reasonable agreement with the observed a-wave reduction. The latter result is in contrast to the 2600-fold reduction of b-wave sensitivity reported for the Deltagen Gngt1−/− mice (Lobanova et al., 2008). Part of this discrepancy is likely attributable to the significantly higher b-wave sensitivity in 1-month-old wild-type controls in the study by Lobanova et al. (2008) compared with that obtained by us and others for b-wave in 2- to 3-month-old wild-type mice (Brantley et al., 2008; Herrmann et al., 2010; Kolesnikov et al., 2010). In addition, the severe early retinal degeneration of the Deltagen Gngt1−/− mice could have contributed to the large reduction of their b-wave responses, driven primarily by the bipolar cells.

Reduced amplification of phototransduction cascade in Gty-deficient mice

The effect of Gty deletion and the accompanying approximately sixfold reduction of Gto (Fig. 1H) on the rod phototransduction in individual mouse rods was analyzed by suction electrode recordings (Fig. 3). In agreement with the similar length of their outer segments at the age of 2 months, wild-type, Gngt1−/−, and Gngt1−/− rods produced saturated responses of similar amplitudes (Fig. 3A, Table 1). The light sensitivity of Gngt1−/− rods was decreased by only 1.4-fold compared with wild-type rods, consistent with a previous study (Herrmann et al., 2010). However, the sensitivity of Gngt1−/− rods was reduced dramatically (90-fold) (Fig. 3B, Table 1).

To establish the reason for the reduced sensitivity in Gngt1−/− rods, we evaluated the amplification of their phototransduction cascade by directly comparing the light intensities required to produce identical response activation phases (Fig. 3C). The phototransduction gain in wild-type and Gngt1−/− rods was identical, as evident from the similar rising phases of their dim flash responses to the same flash intensity during the first 40 ms. In contrast, a matching rising phase for Gngt1−/− rod responses required a 40-fold increase in flash strength. Taking into account low-pass filtering of the recordings, this translated into ~33-fold reduction in phototransduction amplification of
interactions with photoactivated ROS disk membranes (characterized in Fig. 1) diluted to 3 μM R* in medium ionic strength buffer (Kühn, 1980). In wild-type ROS disks containing native levels of Gtβγ, light induced binding of >90% of Gto in the sample to R* membranes (Fig. 4A). In contrast, the lack of Gtβγ resulted in reduced affinity of Gto toward R* so that only ~60% of Gto was bound to light-activated Gngt1−/− ROS membranes (Fig. 4A).

Despite the severe defect in visual signal amplification and drastic reduction of light sensitivity, under brighter light Gngt1−/− rods were still capable of producing responses with maximal amplitude similar to that in wild-type rods (Fig. 3). Whether this residual signaling is achieved by the monomeric Gto or unknown heterotrimeric form of G-proteins is of considerable interest. Previous attempts to identify residual Gγ subunits in Deltagen Gngt1−/− retinas did not reveal any G-protein heterotrimers that may exist in rods in addition to Gt. Yet, based on functional arguments (comparable reductions in ROS Gto and in the rate of Gto activation) and similar amounts of Gto and Gtβ in ROS, it was suggested that signaling in the Deltagen Gngt1−/− rods was likely to be mediated by the heterotrimeric G-protein containing an unknown Gγ (Lobanova et al., 2008). The direct protein quantification in our mice shows the presence of 17% of membrane-bound Gto and 10% of Gtβ in Gngt1−/− ROS disk membranes (Fig. 1G,H). Taking into consideration that the stoichiometry of Gto/Gtβ/Gtγ in the heterotrimeric complex is always 1:1:1, this result indicates that a substantial fraction of the Gto pool in our Gγ-deficient rods is monomeric. Because our ROS membranes contained residual Gγ3 and Gγ5 subunits (Fig. 11), the remaining Gto pool may have formed mixed Gtαβγ3/5 complexes. To determine whether these complexes exist in mutant ROS, we purified Gt using extensive washes of bleached Gngt1−/− ROS membranes followed by a final GTPγS elution step. Although Gγ3 and Gγ5 were clearly detectable in Gngt1−/− ROS samples, no Gβγ subunits copurified with Gto (Fig. 4B), providing a strong argument that Gγ3 and Gγ5 are trace contaminants and that the major pool of Gto in Gngt1−/− ROS is monomeric. However, as our detection method relies on immunoreactivity and possesses limited sensitivity, we cannot rule out that some fraction of Gto is in heterotrimeric form of unknown composition. Whether the residual Gto pool in our Gγ-deficient rods signals as a monomer or in a combination with a Gβγ subunit, the reduction in gain of Gngt1−/− rods clearly demonstrates that the Gtβγ complex is indispensable for G-protein-mediated signal amplification.
Accelerated photoresponse inactivation in Gtγ-deficient rods

Surprisingly, there was a substantial difference between the ~90-fold reduction in rod sensitivity and the 33-fold reduction in amplification of phototransduction in Gngt1−/− rods. One possible explanation for this threefold difference could be faster inactivation of the transduction cascade in Gtγ-deficient rods, which would result in smaller light responses (hence lower sensitivity) than in wild-type rods. Indeed, the inactivation rate of dim flash photoresponses was significantly accelerated in Gngt1−/− rods (Fig. 5A, B; Table 1). Two major inactivation processes, the rhodopsin shutoff and transducin inactivation, might be accelerated in our mutant cells. To evaluate these possibilities, we applied a mathematical model of the rod phototransduction (Kuzmin et al., 2004) (Fig. 5C; Tables 1, 2) (for detailed description of the model, see Materials and Methods).

The model parameters that were allowed to vary among strains of mice were only those whose values are critical for the sensitivity and kinetics of the response. They include the rate of activation of PDE by R*, rates of rhodopsin (kRmax, kRmin) and phosphodiesterase (kq) turnoff, and parameters of Ca²⁺ buffering that define the kinetics of Ca²⁺ feedback. An additional requirement was that the set of parameters providing a good fit to dim flash responses also ensured correct saturation times at bright flashes. Together, these restrictions greatly limited the freedom of fitting. Final sets of parameters allowed not more than a few percentage change in each value without markedly worsening the fit statistics. Under the restrictions discussed above, it was possible to faithfully reproduce wild-type, Gngt1+/−, and Gngt1−/− responses by only varying kRmax, kRmin and the buffering power of the ROS cytoplasm, FB (Fig. 5C, Table 1). Model responses to saturating flashes also correctly predicted time in saturation at the lowest flash strengths. In addition to a 33-fold reduction of amplification (vFB) compared with wild-type controls, reproducing the Gngt1−/− rod responses required an increase in the rate of transducin/PDE inactivation (kq) by a factor of ~1.33 and acceleration of rhodopsin turnoff (kRmax) by a factor of ~5.6. It was also necessary to accelerate Ca feedback (reduce FB) (Table 1) by approximately twofold in Gngt1−/− and Gngt1+/− rods. Thus, our model identified the acceleration of rhodopsin shutoff as the main cause for the faster response inactivation in Gngt1−/− rods.

Discussion

To address the physiological role of transducin Gtβγ complex in phototransduction, we generated mice lacking the rod-specific Gtγ subunit (Gngt1−/−). In stark contrast to a previous Deltagen Gtγ knock-out model (Lobanova et al., 2008), the lack of early retinal degeneration and the normal expression levels of most transduction proteins in our mice (Fig. 1) allowed us to quantitatively characterize how the deletion of Gtγ affects their visual function and phototransduction properties of individual rods. At all functional levels studied, Gngt1−/− mice consistently displayed impaired rod function and dramatic reduction in their scotopic light sensitivity (Figs. 2, 3). By using single-cell recordings, we identified a ~33-fold reduction in amplification of the phototransduction cascade in Gngt1−/− rods as the main cause for their reduced photosensitivity (Fig. 3C). As amplification in mammalian rods is directly proportional to the level of Gtα subunit (Sokolov et al., 2002), only 6-fold of its reduction could be explained by the 17% Gtα bound to ROS disk membranes in Gngt1−/− rods compared with wild-type controls (Fig. 1H).

What is the explanation for the remaining (33/6 = 5.5-fold) reduction in rod amplification in the absence of Gtβγ? The use of brighter light to elicit photoresponses in Gngt1−/− rods would not be expected to affect their gain (Kahlert et al., 1990). Instead, the additional 5.5-fold decrease in the phototransduction amplification in Gngt1−/− rods should be attributed to the lack of the Gtβγ complex. This conclusion is in striking contrast to that reached from the analysis of the Deltagen Gngt1−/− mouse (Lobanova et al., 2008), ascribing all reduction in light sensitivity to the reduced level of Gtα in its rapidly degenerating rods. Our conclusion about the crucial role of Gtβγ in signal amplification would be unaffected by any residual signaling mechanisms, such as by a possible expression of cone Gtα/Gtγ in mouse rods (Allen et al., 2010). Any contribution from the small and desensitized Gnat1-independent rod responses observed in that study would be negligible in our single-cell recordings. Moreover, our attribution of 5.5-fold reduction of amplification in Gngt1−/− rods to the lack of Gtβγ is only a lower estimate of its effect on Gtα activation. If unknown Gtβγ complexes contribute to Gngt1−/− rod photoresponse, the actual efficiency of Gtβγ-devoid Gtα might be even lower than 1.5/5 of that of normal heterotrimer.

The crucial role for Gtβγ in boosting phototransduction amplification in intact rods revealed in our study is in agreement with previous biochemical data showing the reduced ability of R* to activate monomeric bovine rod Gtα, compared with the Gt heterotrimer (Navon and Fung, 1987; Phillips et al., 1992; Kisselvey et al., 1999; Marin et al., 2000; Herrmann et al., 2006). However, the physiological relevance of such in vitro findings has been a long-standing question because R and Gt concentrations typically used in these studies were 3 orders of magnitude below those found in intact photoreceptors. In addition, it has been difficult to completely exclude the possibility that small Gtβγ contamination in purified ROS membranes or Gtα could exaggerate the activity of monomeric Gtα. Furthermore, other biochemical experiments contradicted these findings and suggested that, at bleached rhodopsin concentrations >1 μM, there appears to be no requirement for Gtβγ in the Gtα activation event (Phillips et al., 1992).

Our biochemical measurements of endogenous Gtα interactions with photoactivated wild-type mouse ROS disk membranes
(Fig. 4 A) demonstrated light-induced binding of >90% of Gtα to R* (Kühn, 1980; Fukada et al., 1990; Bigay et al., 1994; Herrmann et al., 2000). In contrast, the lack of Gtβγ resulted in reduced affinity of Gtα toward R* so that only ~60% of Gtα was bound to light-activated GNGT1−/− ROS membranes, in agreement with previous data on monomeric Gtα (Phillips et al., 1992; Willardson et al., 1993; Matsuda et al., 1994). This value is in line with ~40% binding of purified bovine Gtα to light-activated membranes measured by dynamic light scattering (Herrmann et al., 2006). Although the soluble Gtα pool is fully capable of productive interactions with R*, the rate of its activation is limited by binding to the membrane and R* (Heck and Hofmann, 2001). This result is also consistent with a direct involvement of Gtβγ in R* interactions and R*-catalyzed nucleotide exchange on Gtα (Kislev and Downs, 2006; Katada et al., 2008). Overall, this leads to the severely compromised rate of Gtα activation without Gtβγ. These biochemical studies, together with the physiological results presented here, demonstrate that in the absence of Gtβγ R* activates Gtα at a substantially reduced rate, dramatically impairing the first step of signal amplification in rods. The resulting loss of light sensitivity in GNGT1−/− mice is in line with desensitization in invertebrates due to mutations in Dro sophila Gβγ (Dolph et al., 1994), as well as in the farnesylation site of Gye (Schillo et al., 2004), which prevents binding of Gye to the membrane, suggesting a universal role of Gβγ complexes in controlling intracellular signal amplification.

Surprisingly, the inactivation rate of dim flash responses was significantly accelerated in GNGT1−/− rods compared with wild-type photoreceptors (Fig. 5 A, B; Table 1). To evaluate the two possibilities for accelerated response shutoff in mutant cells, the faster rhodopsin turnoff and/or faster transducin inactivation, a mathematical model of rod phototransduction (Kuzmin et al., 2004) was applied. It is believed that the rate of Gtα-GTP/PDE inactivation by the RGS9/Gβ5/R9AP GAP complex (kB, in the model) shapes the tail of the decay phase of dim flash responses and controls the recovery time of saturated responses in mice (Krispel et al., 2006; Burns and Pugh, 2009). In accordance with this idea, both inactivation time constants (τβγ and τp) were reduced in GNGT1−/− rods (Fig. 5 A, B; Table 1). Modeling revealed a similar increase in kB, indicating accelerated inactivation of Gtα-GTP/PDE in Gtγ-deficient rods (Table 1). We found that levels of RGS9, Gβ5, PDEα, and PDEβ subunits were unaffected by the deletion of Gtγ (Fig. 1 I). At first glance, the sixfold reduction in Gtγ in GNGT1−/− rods could possibly accelerate their response inactivation by increasing the ratio of RGS9/Gtα. However, RGS9 is known to interact only with the activated form of Gtα, Gtα-GTP. Because our test flashes produced responses of similar amplitudes in wild-type and GNGT1−/− rods, they also would be expected to produce similar amounts of Gtα-GTP, preserving the RGS9/Gtα-GTP ratio. Thus, the inactivation of Gtα-GTP/PDE is unlikely to be accelerated in Gtγ-deficient rods because of the reduced level of Gtα.

Acceleration of the response shutoff can be also achieved by adding an excess PDEγ subunit by either its overexpression in mouse rods (Tsang et al., 2006) or its infusion in toad ROSs (Rieke and Baylor, 1996). Although the mechanism of this effect remains unclear, it provides a possible connection between the acceleration of response shutoff and our finding that expression of the inhibitory PDEγ subunit is upregulated by twofold and threefold in GNGT1+/− and GNGT1−/− ROSs, respectively (Fig. 1 J). Notably, GNGT1+/− rods displayed both an intermediate level of PDEγ expression and an intermediate rate of photoreceptor turnoff (Fig. 5 A, B; Table 1), whereas the rising phase of their response (amplification) was identical with that in wild-type photoreceptors (Fig. 3 C, Table 1).

Finally, one important conclusion from our modeling of mouse phototransduction was a substantially faster rate of rhodopsin inactivation (kmax) in Gtγ-deficient rods. As indicated above, in our model this effect was substantially more prominent than the acceleration of kB. The lowest estimate of R* turnoff acceleration compatible with the observed kinetics of GNGT1−/− responses was approximately threefold. Such acceleration of rhodopsin shutoff could potentially be caused by a relief of competition between rhodopsin kinase (GRK1), arrestin1 (Arr1), and Gf for photoactivated pigment (Doan et al., 2009), because of the partial overlapping of their binding sites on the cytoplasmic domains of R* (König et al., 1989; Krupecnik et al., 1997; Raman et al., 1999; Gurevich and Gurevich, 2006). In this scenario, the reduced level of Gtα (Fig. 1 H), together with the 5.5-fold lower efficiency of Gtα interaction with R*, could enable both GRK1 and Arr1 (whose levels were unaltered in GNGT1−/− retinas) (Fig. 1 I) to quench R* faster. However, the fact that the rate of phototransduction activation is proportional to Gt concentration (Sokolov et al., 2002) shows that R* mostly exists in a free form rather than as R*-Gtα(βγ) complex. Therefore, Gtα(βγ) cannot apparently outcompete GRK1 and Arr1, even in wild-type rods. Additional experiments are necessary to find the cause(s) of faster R* shutoff in our GNGT1−/− mice.

Universal mechanisms of intracellular signal transduction and amplification enable cells to detect and respond to very faint environmental signals. Our results obtained in intact mammalian rod photoreceptor cells address the role of the G-protein βγ-complex in modulating visual signaling. Investigating the function of Gγ-deficient rods, we demonstrate that heterotrimeric G-proteins are best suited for the task: although Ga is sufficient for signal transduction, the efficient signal amplification required for nocturnal vision is achieved in the presence of the Gβγ complex. This highlights a unique role of Gγ, and more broadly of Gβγ complexes, in regulating the amplification of visual signals in phototransduction.

References


