G-protein betagamma-complex is crucial for efficient signal amplification in vision

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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 βγ-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 Gtxα (Gtx), Gtxβ (Gtxβ), and Gtyγ (Gtyγ) isoforms. Photoactivated rhodopsin (R*) binds to Gt and activates it by triggering the exchange of GDP for GTP on Gtxα. On activation, the G-protein dissociates into Gtxα-GTP and Gtxβγ. In turn, Gtxα-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 Gtxα, as its deletion completely abolishes rod-driven photoreception (Calvert et al., 2000). In contrast, the Gtxβγ complex has no established role in phototransduction in vivo. Early biochemical studies have suggested that Gtxβγ might participate in transducin activation (Fung, 1983). However, although it is now believed that Gtxβγ is necessary for maintaining the inactive state of Gtxα 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 Gtxα could be achieved without Gtxβγ (Navon and Fung, 1987; Phillips et al., 1992; Kisselev et al., 1999; Herrmann et al., 2006). Thus, it remains an open question whether Gtxβγ is required for effective signal amplification in intact rods, and the physiological role of the Gtxβγ complex in vision is still unclear. An earlier attempt to address this question using a commercially available (Deltagen) Gtxγ knock-out mouse strain was hampered by early onset of photoreceptor degeneration, which complicated its biochemical and physiological anal-

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ysis, 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 Gt- 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 Xbal 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 Gngt1-/- 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 G418-resistant clones were identified by PCR, DNA sequencing.

Laboratory for Vision Research, Doheny Eye Institute, University of Delaware, and UC Irvine (Doheny Eye Institute, UC Irvine). Rabbit antibodies against M-opsin, GCAP2, and retGC1 were a gift from A. M. Dizhoor (Pennsylvania College of Medicine). Rabbit antibodies against GCAP1, 8004-GRK1, sc-73044-SNAP25 were from Santa Cruz Biotechnology. Rabbit antibodies against Gtβ1, Gtβ2, and Gtγ were from N. Gautam (Washington University, St. Louis, MO). Rabbit Gβ1 antibody was a gift from W. F. Simonds (National Eye Institute, Bethesda, MD). Mouse antibody for rod arrestin was a gift from W. C. Smith (University of Florida, Gainesville, FL). Rabbit antibody against phospho-PKG1 was a gift from B. M. Willardson (Brigham Young University, Provo, UT). Rabbit antibody to β ARS mouse 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% glutaraldehyde 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 sections 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 (Papermaster 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 Gtα 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, Rb-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. Gtα content in both fractions was analyzed by quantitative immunoblotting.

Immunohistochemistry. Animals were dark-adapted overnight and anesthetized by subcutaneous injection of ketamine (80 mg/kg) and xylazine (15 mg/kg). Pupils were dilated with 1% atropine sulfate. 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 (KLC 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 Gngt1-/- model (Lobanova et al., 2008), suction recordings could be performed easily from the rods of our 2- to 3-month-old Gngt1-/- 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. Photoreponses were amplified, low-pass filtered (30 Hz, 8-pole Bessel), and digitized (1 kHz). Dominant recovery time constant \( \tau_d \) was determined from supersaturating flashes (Pepperberg et al., 1992), using a 10% criterion for photorecovery 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 (sinewave 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 \( f_j \) was fixed at its optimal value of 0.75 Hz for all background conditions. Spatial frequency \( f_j \) varied in the range of 0.014 – 0.481 cyc/deg, and speed of the stimuli was adjusted based on the following equation: \( f_j = s_j \cdot f_1 \) (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 \( r^2 > 0.8 \). 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,b; Nikonov et al., 2000; Hamer et al., 2003, 2005). However, our treatment of Ca²⁺ regulation 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 (Hamer 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:

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

(1)

Here, \( k_{R_{\text{min}}} \) and \( k_{R_{\text{max}}} \) 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 \( n_{\text{cat}} \) is the Hill’s coefficient of regulation.

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

\[
\frac{dE^*(t)}{dt} = v_E R^*(t) - k_E E^*(t),
\]

(2)

where \( v_E \) is the rate of PDE activation by single \( R^* \) (in seconds⁻¹), and \( k_E \) is the rate constant of \( E^* \) turnover (in seconds⁻¹).

**cGMP turnover is described by the following:**

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

(3)

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:

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

(4)

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; Hamer et al., 2003, 2005) that assume infinite regulation range \( \alpha_{\text{min}} = 0 \).

The rate of cGMP hydrolysis is as follows:

\[
\beta(t) = \left( \beta_{\text{max}} + \frac{k_{\text{el}}}{V_{\text{cyt}}N_{\text{Av}}E^*(t)+t} \right) \cdot cG(t) + K_{\text{cat}}.
\]

(5)

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

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

\[
j_{cG}(t) = j_{cG_{\text{max}}} \cdot \frac{cG(t)}{cG(t)+K_{\text{cat}} } + j_{cG_{\text{max}}} t,
\]

(6)

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

Free Ca²⁺ turnover is described by the following:

\[
\frac{dCa(t)}{dt} = \frac{1}{FB + 1} \left( \frac{1}{2} \cdot f_{\text{el}}(t) \cdot \text{Cat}(t) - j_{k_{1}} \cdot \text{V}_{\text{cyt}} \cdot k_{1} \cdot \text{Cat}(t) \right) + j_{k_{1}} \cdot \text{Cat}(t) \left( B_{\text{max}} - \text{Cat}(t) \right) + j_{k_{2}} \cdot \text{Cat}(t) \left( B_{\text{max}} - \text{Cat}(t) \right) + j_{k_{1}} \cdot \text{Cat}(t) \left( B_{\text{max}} - \text{Cat}(t) \right).
\]

(7)

Here, \( f_{\text{el}}(t) \) is the fraction of the ROS current carried by Ca²⁺, \( j_{k_{1}} \) is Ca²⁺ extrusion current carried by Ca,K/Na exchanger, and \( FB \) is Faraday’s number [cf. Hamer et al. (2005), their Eq. A9]. In accordance with experimental data on amphibian rods (McCarthy et al., 1996; Younger et al., 1996; Govardovskii and Kuzmin, 1999), ROSs are supposed to contain a two-component Ca²⁺ buffer. One of the components exchanges with free Ca²⁺ quickly, so its effect on free Ca²⁺ turnover can simply be characterized by its buffering power, FB (Lagnado et al., 1992). The second, slowly exchangeable component is characterized by its binding capacity \( B_{\text{max}} \) and two rate constants, \( k_{1} \) for binding and \( k_{1} \) for releasing Ca²⁺, \( \text{Cat}(t) \) is the concentration of calcium bound to slow buffer and is described by the following:

\[
\frac{d\text{Cat}(t)}{dt} = k_{-1} \cdot \text{Cat}(t) \left( B_{\text{max}} - \text{Cat}(t) \right) - k_{-1} \cdot \text{Cat}(t) \left( B_{\text{max}} - \text{Cat}(t) \right).
\]

(8)
Table 1. Experimental parameters of single-cell responses and model parameters that were varied to simulate the effects of Grβ deletion on flash responses

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wild type</th>
<th>Gntg1&lt;sup&gt;1→−&lt;/sup&gt;</th>
<th>Gntg1&lt;sup&gt;1→−&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 50)</td>
<td>(n = 27)</td>
<td>(n = 41)</td>
<td></td>
</tr>
</tbody>
</table>

**Response parameter**
- \( I_{\text{dark}} (pA) \)
  - Values: 15.7 ± 0.3, 14.9 ± 0.4<sup>NS</sup>, 14.9 ± 0.3<sup>NS</sup>

- \( I_{1/2} (pH \mu m^{-2}) \)
  - Values: 93 ± 2, 130 ± 10<sup>**</sup>, 8408 ± 533<sup>**</sup>

- \( I_{\text{peak}} (ms) \)
  - Values: 152 ± 2, 121 ± 2<sup>**</sup>, 99 ± 2<sup>**</sup>

- \( I_{\text{imp}} (ms) \)
  - Values: 260 ± 9, 246 ± 14<sup>NS</sup>, 132 ± 5<sup>**</sup>

- \( \tau_{\text{rise}} (ms) \)
  - Values: 190 ± 10, 184 ± 13<sup>NS</sup>, 121 ± 5<sup>**</sup>

- \( \tau_{\text{model}} (ms) \)
  - Values: 200 ± 13 (16), 161 ± 10 (19)<sup>**</sup>, 141 ± 9 (23)<sup>**</sup>

**Model parameter**
- \( \nu_{\text{ex}} (s^{-1}) \)
  - Values: 307, 305, 9.4

- \( k_{\text{in}} (s^{-1}) \)
  - Values: 11.4, 22.3, 63.5

- \( k_{j} (s^{-1}) \)
  - Values: 6.5, 5.4, 8.3

- FB
  - Values: 59.4, 32.1, 30.1

**Values are means ± SEM. Experimental parameters were as follows: \( I_{\text{dark}} \). Dark current measured from saturated responses; \( I_{1/2} \). Half-saturating light intensity; \( I_{\text{peak}} \) and \( I_{\text{imp}} \) represent to responses where amplitudes were <0.2I<sub>max</sub> and fell within the linear range; \( \tau_{\text{rise}} \), time constant of single-exponential decay of dim flash response recovery phase; \( \tau_{\text{model}} \), 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: \( \nu_{\text{ex}} \), rate of PDE activation by single R*; \( k_{\text{in}} \), maximum rate constant of R* turnoff at zero Ca<sup>2+</sup>; \( \nu_{\text{exsat}} \), rate constant of Ca<sup>2+</sup> buffer; FB, buffering power of fast Ca<sup>2+</sup> buffer. Values were determined for population-averaged dim flash responses normalized to amplitudes of corresponding saturated responses.**

**Results**

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

To investigate the function of Grβ in the phototransduction pathway in vivo, we generated a mouse line lacking the retinal rod-specific Gtβ subunit (Gntg1<sup>1→−</sup>) (Fig. 1A). Morphological and ultrastructural analysis of 1- to 2-month-old Gntg1<sup>1→−</sup> 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 Grβγ complex is not required for the formation of the rod outer segments. Although Gntg1<sup>1→−</sup> 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 the Gtα-deficient (Gnat1<sup>−/−</sup>) 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 Gntg1<sup>1→−</sup> ROS disk membrane preparations, to analyze the protein composition of Gntg1<sup>1→−</sup> 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 Gntg1<sup>1→−</sup> retinas, the deletion of Gtγ had no effect on the level of rhodopsin expression (Fig. 1E). As ex-
Figure 1. Genetic, morphological, and biochemical characterization of Gngt1−/− mice. A, Schematic representation of WT and KO alleles. WT gene exons 1–3 are shown by tall white boxes. The Gγ-γ protein coding region in exons 2 and 3 is shown in black. SA is the 1.6 kb short homology arm. LA is the 7.3 kb long homology arm. The 3.4 kb region of the Gngt1 gene encompassing exons 1, 2, and 3 was replaced by a 1.8 kb Neo cassette. For PCR genotyping, a 460 bp DNA fragment in the WT allele was amplified by WT1 and WT2 primers, and a 1.8 kb fragment in the KO allele was amplified by A1 and N1 primers (data not shown). The arrows show the position of the primers. B, Immunostaining of wild-type and Gngt1−/− retinas with anti-Gγ epitope antibody: immunogold staining with silver enhancement, LR white embedment, toluidine blue counterstaining. Scale bar, 20 μm. C, Average number of rows of outer nuclear layer (ONL) nuclei (means ± SD; n = 15) as a function of age. The cyan circles (Deltagen) represent comparative data reconstituted from Figure 2 in the study by Lobanova et al. (2008). D, Immunoblotting of whole retina extracts and purified ROS disk membranes from Gngt1−/− retinas using anti-opsin and anti-cytochrome c antibodies. The lack of cyt c in ROS membranes demonstrates that they were not contaminated by the RIS material. E–H, Expression of phototransduction proteins determined by quantitative immunoblotting: rhodopsin in the retina (E), Gγ (F), Gβ (G), and Gα (H) in ROS disk membranes isolated from Gngt1−/− retinas. Levels of rhodopsin and transducin α and β subunits in whole mouse retinas and ROS disk preparations were determined based on quantitative calibrations with highly purified bovine rhodopsin and transducin standards. The data represent means ± SD from three independent experiments. I, Effects of Gγ deletion on the expression of phototransduction proteins in rods. Whole retina and ROS samples were prepared from 2-month-old wild-type, Gngt1+/−, and Gngt1−/− mice. The sample rhodopsin level was used as a loading control for quantification of phototransduction proteins. Similar results were obtained when total protein was used as the loading control (data not shown).

predicted, purified ROS were lacking Gγ (Fig. 1 F). Because all Gβγ complexes function as nondissociable dimers, the deletion of Gγ resulted in a dramatic reduction in Gβγ as well, with only 10% remaining in Gngt1−/− rods (Fig. 1 G). The level of Gββγ expression showed a clear gene titration effect, as the levels of Gγβ and Gγγ subunits in Gngt1−/− ROS disks were reduced to 61 and 50%, respectively (Fig. 1 F,G). Finally, we also observed a decrease in the levels of Gαβ bound to dark-adapted Gngt1−/− and Gngt1+/− ROS disk membranes to 67 and 17%, respectively (Fig. 1 H). A decrease of similar magnitude was also observed by immunohistochemical analysis of the Gngt1−/− retinas (data not shown). Notably, the ROS disk membrane-bound fraction of Gγ in our animals (17% of wild-type levels) was substantially higher than the expression of Gγ in the ROS of the commercial Deltagen Gngt1−/− mice (2%) (Lobanova et al., 2008). A possible reason for this prominent difference could be the significantly faster rate of retinal degeneration in the Deltagen Gngt1−/− mutant, even at very early ages.

Among 19 other major phototransduction proteins examined, all but phosducin and PDEγ had expression levels similar to those in wild-type rods (Fig. 1 F). Phosducin levels in retinas of Gngt1+/− and 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. 1 F). This effect appears to be reciprocal to the observed reduction of the Gβγ expression in the phosducin knock-out mouse line (Krispel et al., 2007). Interestingly, we also observed an unexpected twofold and threefold increase in the levels of inhibitory PDEγ subunit in Gngt1−/− and Gngt1+/− ROS, respectively (Fig. 1 F). 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: Gγ2, Gγ4, Gγ6, Gγ8, Gγ9, 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 Gγ affects their visual function as well as the phototransduction properties of individual rods.

Impaired rod function in Gγ-deficient mice

To determine how the deletion of Gγ affects the overall functionality of mouse vision, we first performed behavioral tests based on the ability of mice to reflexively respond to computer-
Reduced amplification of phototransduction cascade in Gtγ-deficient mice

The effect of Gtγ deletion and the accompanying approximately sixfold reduction of Gto 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, Gntγ+−/−, and Gntγ−/− rods produced saturated responses of similar amplitudes (Fig. 3A, Table 1). The light sensitivity of Gntγ+−/− 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 Gntγ−/− rods was reduced dramatically (90-fold) (Fig. 3B, Table 1).

To establish the reason for the reduced sensitivity in Gntγ−/− 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 Gntγ+−/− 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 Gntγ−/− 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

generated rotating sine wave gratings (Prusky et al., 2004) (Fig. 2A). The absolute contrast sensitivity of Gntγ−/− mice was unaltered in the photopic region (~1 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 Gtα subunit in Gntγ−/− retinas (Fig. 1J) and the unaltered photopic ERG b-wave amplitudes (data not shown). In contrast, the scotopic (rod-mediated) spatial contrast sensitivity of Gtγ-deficient mice was shifted ~100-fold to brighter light conditions (Fig. 2B), indicating substantial rod desensitization in the absence of Gtγ. Gntγ+−/− rods still contributed to mouse vision as spatial contrast sensitivity of Gntγ−/− mice was approximately twofold (p < 0.05) higher compared with that in Gtα-deficient (Gntγ−/−) animals, where rods are not functional (Calvert et al., 2000). Thus, although Gtγ-deficient mice retained rod vision, their visual sensitivity under dim light conditions was severely reduced.

The effect of Gtγ 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 Gntγ−/− animals was markedly reduced compared with wild-type age-matched controls (Fig. 2C). The sensitivity of the photoreceptor-driven a-wave in Gntγ−/− 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 Gntγ−/− 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 Gntγ−/− 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 Gntγ−/− mice could have contributed to the large reduction of their b-wave responses, driven primarily by the bipolar cells.
and Gngt1

We performed biochemical measurements of endogenous Gt

with anti-G

corresponding maximum dark currents, 39, 125, 444, and 1406 photons of all three responses to coincide. Correspondingly scaled light intensities were 1:1:0.025 (wild type: old wild-type (left), photoactivated ROS disk membranes. Top, Representative Western blot of the membrane (M) and soluble (S) fractions using antibody; the bottom portions were stained with anti-Gt

subunits. Bottom, Corresponding densitometry results for the Gt

2). [35] demonstrates that the Gt

5 are trace contaminants and that the major pool of Gt

The red traces show responses to identical light intensity (1406 photons for wild-type (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 Gtx/Gtβ/Gtγ in the heterotrimeric complex is always 1:1:1, this result indicates that a substantial fraction of the Gtx 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 Gtxβγ3γ5 complexes. To determine whether these complexes exist in mutant ROS, we purified Gt using extensive washes of bleached Gngt1−/− ROS disk membranes followed by a final GTPγS elution step. Although Gγ3 and Gγ5 were clearly detectable in Gngt1−/− ROS samples, no Gβγ subunits copurified with Gtx (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 was necessary, mostly caused by the low-pass filtering of the recordings. Gngt1−/− rods. Thus, although Gγ-deficient rods were still able to respond to light, their sensitivity was severely reduced mostly because of a decrease in the amplification of their phototransduction cascade.

Reduced affinity of Gto toward R* in Gγ-deficient mice

To determine the mechanism leading to the reduced amplification in Gngt1−/− rods, we investigated whether the absence of Gtβγ affects the binding efficiency of mouse Gto to R* in ROS disk membranes purified from wild-type and Gngt1−/− retinas. We performed biochemical measurements of endogenous Gto

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 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 Gtx/Gtβ/Gtγ in the heterotrimeric complex is always 1:1:1, this result indicates that a substantial fraction of the Gtx 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 Gtxβγ3γ5 complexes. To determine whether these complexes exist in mutant ROS, we purified Gt using extensive washes of bleached Gngt1−/− ROS disk membranes followed by a final GTPγS elution step. Although Gγ3 and Gγ5 were clearly detectable in Gngt1−/− ROS samples, no Gβγ subunits copurified with Gtx (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* (vPDE), rates of rhodopsin (kRmax, kRmin) and phosphodiesterase (kE) turnover, 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 vPDE, kE, kRmax, 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 (vPDE) compared with wild-type controls, reproducing the Gngt1−/− rod responses required an increase in the rate of transducin/PDE inactivation (kE) by a factor of ~1.33 and acceleration of rhodopsin turnover (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 (Kahler 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-deficient 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; Kisselev 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 Gtβ to R* (Kühn, 1980; Fukada et al., 1990; Bigay et al., 1994; Herrmann et al., 2006). In contrast, the lack of Gtβ-Y results in reduced affinity of Gtβ 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; Matsuuda 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 Gtβ in R* interactions and R*-catalyzed nucleotide exchange on Gtα (Kislev and Downs, 2006; Katadae et al., 2008). Overall, this leads to the severely compromised rate of Gtα activation without Gtβ. These biochemical studies, together with the physiological results presented here, demonstrate that in the absence of Gtβ the 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 Drosofília Gβe (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 Gβe complexes in controlling intracellular signal amplification.

Surprisingly, the inactivation rate of dim flash photoresponses 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 (kR 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 (ταs and ταu) were reduced in Gngt1−/− rods (Fig. 5 A, B; Table 1). Modeling revealed a similar increase in kR, indicating accelerated inactivation of Gtα-GTP/PDE in Gtβ-deficient rods (Table 1). We found that levels of RGS9, Gβ5, PDEα, and PDEβ subunits were unaffected by the deletion of Gtβ (Fig. 1 I). At first glance, the sixfold reduction in Gtα 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 Gtβ-deficient rods because of the reduced level of Gtα.

Acceleration of the response shutoff can be also achieved by adding an excess PDEβ 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 PDEβ subunit is upregulated by twofold and threefold in Gngt1+/− and Gngt1−/− ROS, respectively (Fig. 1 J). Notably, Gngt1−/− rods displayed both an intermediate level of PDEβ 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 Gtβ-deficient rods. As indicated above, in our model this effect was substantially more prominent than the acceleration of kR. 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 Gt 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; Krupnick 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*-Gtβ(β) complex. Therefore, Gtα(β) 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


