Coordinated control of sensitivity by two splice variants of Go in retinal ON bipolar cells

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The high sensitivity of scotopic vision depends on the efficient retinal processing of single photon responses generated by individual rod photoreceptors. At the first synapse in the mammalian retina, rod outputs are pooled by a rod “ON” bipolar cell, which uses a G-protein signaling cascade to enhance the fidelity of the single photon response under conditions where few rods absorb light. Here we show in mouse rod bipolar cells that both splice variants of the Gα subunit, Gαo1 and Gαo2, mediate light responses under the control of mGluR6 receptors, and their coordinated action is critical for maximizing sensitivity. We found that the light response of rod bipolar cells was primarily mediated by Gαo1, but the loss of Gαo2 caused a reduction in the light sensitivity. This reduced sensitivity was not attributable to the reduction in the total number of Gα subunits, or the altered balance of expression levels between the two splice variants. These results indicate that Gαo1 and Gαo2 both mediate a depolarizing light response in rod bipolar cells without occluding each other’s actions, suggesting they might act independently on a common effector. Thus, Gαo2 plays a role in improving the sensitivity of rod bipolar cells through its action with Gαo1. The coordinated action of two splice variants of a single Gα may represent a novel mechanism for the fine control of G-protein activity.

INTRODUCTION

At the first synapse of the visual system, the output of the photoreceptor cells is segregated into ON and OFF pathways, which respond to increments and decrements of light intensity, respectively. ON bipolar cells use a G protein-coupled receptor-signalizing pathway to signal light-evoked reductions in glutamate release from the rod photoreceptor spherule. However, unlike the phototransduction cascade, many of the components of the bipolar signaling cascade have yet to be identified. What is known is that a metabotropic glutamate receptor, mGluR6 (Nakajima et al., 1993; Nomura et al., 1994; Masu et al., 1995), senses glutamate release from photoreceptors and conveys this activity through a heterotrimeric G protein, Go (Nawy, 1999; Dhingra et al., 2000), to close nonselective cation channels, recently identified to be TRPM1 (Bellone et al., 2008; Koike et al., 2009; Morgans et al., 2009; Shen et al., 2009). However the target of the G protein and the gating particle controlling the TRPM1 current remain unidentified.

Despite the lack of identity of key signaling components in the mGluR6 pathway, work on mammalian rod ON bipolar cells has led to several insights about the pathway’s functional properties. For instance, rod bipolar cells generate responses to light that are briefer than the response of rods (Field and Rieke, 2002; see also Sampath et al., 2005). In addition, a nonlinear threshold for signal transmission between rods and rod bipolar cells (van Rossum and Smith, 1998; Field and Rieke, 2002; Bernston et al., 2004a) produced by saturation of the mGluR6 signaling cascade (Sampath and Rieke, 2004) improves the signal-to-noise ratio of the single photon response by preserving responses in rods absorbing photons while eliminating noise from the majority of rods that do not. These properties are ultimately dependent on the speed and sensitivity of G-protein signaling in the rod bipolar dendrites.

Here we investigated the role played by the Go splice variants in setting the properties of the light response in mouse rod bipolar cells. The expression of Go in the mouse retina is mainly restricted to ON bipolar cells, with little or no expression in the photoreceptors (Vardi et al., 1993; Vardi, 1998; Dhingra et al., 2000;
Dhingra et al., 2002). Two splice variants of the Goα subunit (Goαo1 and Goαo2) are found in mouse ON bipolar cells (Dhingra et al., 2002). However, the expression of Goαo2 is much lower than Goαo1, and electroretinography from knockout mice for each splice variant suggests that rod bipolar responses appeared to require Goαo1, but not Goαo2 (Dhingra et al., 2002). We find surprisingly that both Goαo2 and Goαo1 contribute to dark-adapted responses of rod bipolar cells. Rod bipolar cells in mice lacking Goαo2 exhibited reduced light sensitivity. The reduction in sensitivity was not attributable to the reduction in the retinal expression level of Goα protein, as ~50% reduction in total Goα expression for Goαo2−/− mice did not alter light sensitivity. Furthermore light sensitivity was not affected by the altered balance of retinal expression levels between two splice variants in Goαo2−/− mice. These data indicate that the saturation within the mGlur6 signaling cascade that separates the rod single photon response from rod noise is not set by Goα concentration, and that Goαo2 works in a coordinated manner with Goαo1 to improve the light sensitivity of rod bipolar cells.

**MATERIALS AND METHODS**

**Animals and preparation**

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Southern California (Protocol 10890) and followed guidelines set by the National Institutes of Health on the care and use of animals. Several lines of mice were crossed and used in these experiments, including mice lacking Goα (Jiang et al., 1998), lacking either Goα splice variants Goαo3 or Goαo4 (Dhingra et al., 2002), or lacking the gap junction subunit connexin 36 (Deans et al., 2002). Wild-type (WT), Cx36−/−, Goαo3−/−, Goαo4−/−, and Goαo2−/− mice were used between 6 wk and 3 mo of age. Goαo3−/−, Goαo4−/−, and Goαo2−/− Cx36−/− mice rarely survived more than 4 wk and were used at the age of 3–4 wk when their retina reached maturity as assessed by morphological maturation criteria, such as for cells in Gαo3−/− and Gαo4−/− mice, they were confirmed by visualizing the axonal stratification within the inner plexiform layer with 100–200 µm Alexa 750 (Invitrogen) added to the internal solution. Full-field 10-ms flashes were delivered from a blue LED (λmax ~ 470 nm, FWHM ~ 30 nm) and focused onto the retinal slice with 20X 0.75NA objective (Nikon). Light-evoked currents were low-pass filtered at 300 Hz with an 8-pole Bessel filter and digitized at 1 kHz. The series resistance in these recordings was 10–25 MΩ and was uncompensated. Light intensity was calibrated daily and converted to an effective photon flux at the peak of spectral sensitivity for mouse rhodopsin (λmax ~ 501 nm) by convolving the power-scaled LED output spectrum with the normalized spectral sensitivity curve for mouse rhodopsin. The number of activated rhodopsins per rod for a given flash was calculated by multiplying this effective photon flux with the estimated collecting area of mouse rods in retinal slices, which we calculated in the experimental setup to be 0.18 µm2 (Cao et al., 2008; Okawa et al., 2010).

**Western blotting**

Isolated retinas were homogenized in lysis buffer containing protease inhibitor (Roche), 50 mM Tris-HCl (pH 8), 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, and 0.1% SDS. The homogenate was treated with 100 U/ml DNase for 30 min at room temperature. The protein concentration was checked using a BCA Protein Quantification Assay (Thermo Fisher Scientific). The extracted protein was run on a 10% NuPAGE Bis-Tris gel (Invitrogen) and transferred to a nitrocellulose membrane using a Transphor Electrophoresis Unit (Hoefer). The membrane was blocked in 10% milk in Tris-buffered saline with Tween-20 (TBST) for 1 h at room temperature and incubated in a Goα rabbit polyclonal antibody (Santa Cruz Biotechnology, Inc.) in TBST (1:200), or in a Goαo2 mouse monoclonal antibody (clone#101.4, provided by R. Jahn [Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany] and G. Ahnert-Hilger [Medical University of Berlin, Berlin, Germany]; see Winter et al., 2005) in TBST (1:200), or in a Goαo2 mouse monoclonal antibody (clone#101.4, provided by R. Jahn [Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany] and G. Ahnert-Hilger [Medical University of Berlin, Berlin, Germany]; see Winter et al., 2005) in TBST (1:5,000) at 4°C overnight. The membrane was washed with TBST and incubated with IRDye 800 CW anti-rabbit antibody or anti-mouse antibody (LI-COR) in TBST (1:20,000) for 1 h at room temperature and then washed with TBST. The positive bands were detected and expression quantified using an Odyssey Infrared Image System (LI-COR), with the expression of b-actin used as a loading control for total protein.

**Online supplemental material**

The supplemental material (Fig. S1) is available online at http://www.jgp.org/cgi/content/full/jgp.201010477/DC1. Fig. S1 A displays the average response to the dimmest flash tested in WT and Goαo2−/− rod bipolar cells. Fig. S1 B documents the relationship between the maximal response to light and the flash strength that evokes a half-maximal response across all WT rod bipolar cells in this study.

**RESULTS**

**Residual responses in Goαo2−/− rod bipolar cells are mediated by Goαo2**

Experimental evidence suggests strongly that Goαo2 is responsible for transduction channel closure (Nawy, 1999; Dhingra et al., 2000, 2002; Koike et al., 2009), with a splice variant of Goαo, Goαo3, mediating the ON bipolar
response (Dhingra et al., 2002). We recorded from rod bipolar cells (Fig. 1 A) in G_α_2_1/- mice in an attempt to characterize the influence of G_α_2 on transduction channel gating. Fig. 1 B shows the average response to the first flash for nine rod bipolar cells from the G_α_2_1/- retina after achieving the whole-cell voltage-clamp recording (one such cell is visualized). Surprisingly, we found that ON responses persisted in the absence of G_α_2. In G_α_2_1/- retinas that showed light responses, rod bipolar cell responses were typically small in amplitude (5.3 ± 0.8 pA; n = 9) and decayed quickly after establishing the whole-cell configuration (Fig. 1 B). For comparison, the maximal amplitude of WT rod bipolar responses routinely exceeds several hundred picamperes (see Table I). Thus, the electroretinography appears to have failed to detect this small remaining ON response (see Dhingra et al., 2002).

Previous work indicated that ON bipolar cells also express at a lower level the splice variant G_α_2 in addition to G_α_1 (Dhingra et al., 2002). To determine if G_α_2 generated the small residual response in G_α_1/- mice, we recorded from rod bipolar cells in the full G_α knockout

Figure 1. Rod bipolar responses are partially mediated by G_α_2. (A) Schematic of the mammalian rod bipolar pathway. Rod photoreceptors (R) synapse onto rod bipolar cells (RB), which in turn synapse onto AIIACs (All). Signals from AIIACs, which are coupled to one another by Cx36 gap junctions (Deans et al., 2002), send light-driven signals to ON cone bipolar cells (ON BC) through gap junctions composed of Cx36 on the All side, and make glycinergic (-) synapses with OFF cone bipolar cells (OFF BC). Each bipolar cells synapses with its respective ganglion cell (GC). Cone photoreceptors (C) are also depicted. (B) A representative G_α_1/- rod bipolar cell visualized with Alexa 750 and the average flash response of 9 G_α_1/- rod bipolar cells immediately after whole-cell break in (0 s), and 15 s and 2 min later. The flash strength was 15 Rh*/rod, a strength that saturates WT rod bipolar cells. (C) A representative G_α_2/- rod bipolar cell visualized with Alexa 750 did not generate light responses to flashes producing 32 Rh*/rod. In every rod bipolar cell tested from G_α_2/- mice, rod bipolar light responses were never observed. (D) To confirm viability within the retinal slice, a G_α_2/- Off-bipolar cell located near rod bipolar cell was visualized with Alexa 750, and displayed normal response families, indicating that the lack of rod bipolar responses was not due to the conditions of the retinal slice. Flash strengths were 0.5, 1.0, 2.0, 4.0, 8.0, 16, and 32 Rh*/rod.

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despite the loss of Gαo indicating that transduction channels remained closed and are not statistically different from that in WT cells (Table I).

Characterization of Gαo-mediated rod bipolar responses

Rod bipolar responses of Gαo−/− and AIIACs in different mouse lines

<table>
<thead>
<tr>
<th>Rod bipolar</th>
<th>Gαo+/+</th>
<th>Gαo−/−</th>
<th>Gαo−/− Cx36+/−</th>
<th>Gαo+/+ Cx36+/−</th>
</tr>
</thead>
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<tr>
<td>I_max (pA)</td>
<td>420 ± 38 (14)</td>
<td>250 ± 22 (15)</td>
<td>490 ± 36 (16)</td>
<td>350 ± 28 (17)</td>
</tr>
<tr>
<td>I_max (pA)</td>
<td>−33 ± 4.2 (14)</td>
<td>−28 ± 3.3 (15)</td>
<td>−25 ± 5.0 (16)</td>
<td>−27 ± 2.4 (17)</td>
</tr>
<tr>
<td>σ2 (pA2)</td>
<td>14 ± 3.1 (13)</td>
<td>6.4 ± 1.6 (15)</td>
<td>2.2 ± 0.3 (10)</td>
<td>9.6 ± 1.3 (14)</td>
</tr>
<tr>
<td>T_peak (ms)</td>
<td>2.5 ± 0.13 (14)</td>
<td>2.5 ± 0.17 (15)</td>
<td>2.8 ± 0.12 (16)</td>
<td>2.5 ± 0.15 (17)</td>
</tr>
<tr>
<td>n</td>
<td>1.5 ± 0.04 (14)</td>
<td>1.6 ± 0.06 (15)</td>
<td>1.4 ± 0.05 (16)</td>
<td>1.5 ± 0.07 (17)</td>
</tr>
<tr>
<td>τ_int (ms)</td>
<td>120 ± 10 (9.1)</td>
<td>100 ± 8 (10.0)</td>
<td>110 ± 6 (11.7)</td>
<td>120 ± 6 (10.3)</td>
</tr>
<tr>
<td>T_peak (ms)</td>
<td>120 ± 4 (9.1)</td>
<td>130 ± 5 (10.0)</td>
<td>120 ± 5 (11.7)</td>
<td>120 ± 4 (10.3)</td>
</tr>
<tr>
<td>% I_sat</td>
<td>16 ± 1.2 (9.1)</td>
<td>15 ± 2.0 (10.0)</td>
<td>16 ± 1.4 (11.7)</td>
<td>16 ± 1.7 (10.3)</td>
</tr>
</tbody>
</table>

The effective number of cells was used to calculate the SEM of I_max and T_peak. Rod bipolar AIIAC responses were plotted versus the flash strength and revealed that the rod bipolar cell inputs are active (Pang et al., 2004; Dunn et al., 2006), their light responses will reflect subtle changes in the rod bipolar response. In addition, AIIACs are not subject to washout because their response is mediated by ionotropic glutamate receptors (Boos et al., 1993; Hartveit and Veruki, 1997). To isolate the direct output of rod bipolar cells, we eliminated input to the recorded AIIACs from neighboring AIIACs and ON cone bipolar cells by crossing Gαo−/− mice with Cx36+/− mice (Deans et al., 2002; see Fig. 1 A).

Fig. 2 (A and B) shows voltage-clamped (V_m = −60 mV) response families to flashes of increasing strength from Gαo+/+ Cx36+/− and Gαo−/− Cx36+/− AIIACs. The maximum response amplitude among all the Gαo+/+ Cx36+/− AIIACs tested was ~200 pA (n = 9), indicating that even small rod bipolar responses mediated by Gαo can produce more substantial changes in downstream signals. In Fig. 2 C, the normalized response amplitude is plotted versus the flash strength and reveals that response families in Gαo−/− Cx36+/− AIIACs are shifted to ~10-fold brighter flash strengths compared with Gαo+/+ Cx36+/− AIIACs. Furthermore, the maximal response amplitude of Gαo−/− Cx36+/− AIIACs was, on the other hand, ~200 pA (n = 9), indicating that even small rod bipolar responses mediated by Gαo can produce more substantial changes in downstream signals.

Characterization of Gαo2-mediated rod bipolar responses in All amacrine cells

The Gαo2-mediated ON response in Gαo2−/− rod bipolar cells was small and decayed too quickly to be characterized. To assess the sensitivity of the Gαo2-mediated response in rod bipolar cells we instead recorded their output in the postsynaptic All amacrine cells (AIIAC; see Fig. 1 A). Because AIIACs are more sensitive than rod bipolar cells and operate at light levels where few of the rod bipolar cell inputs are active (Pang et al., 2004; Dunn et al., 2006), their light responses will reflect subtle changes in the rod bipolar response. In addition, AIIACs are not subject to washout because their response is mediated by ionotropic glutamate receptors (Boos et al., 1993; Hartveit and Veruki, 1997). To isolate the direct output of rod bipolar cells, we eliminated input to the recorded AIIACs from neighboring AIIACs and ON cone bipolar cells by crossing Gαo−/− mice with Cx36+/− mice (Deans et al., 2002; see Fig. 1 A).

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and the $G_{o2}$ mediate a depolarizing light response in rod bipolar cells through the activity of mGluR6.

Reduced amplitude and sensitivity of light responses in $G_{o2}$–/– rod bipolar cells

We assessed the functional role played by $G_{o2}$ on the dark-adapted response of rod bipolar cells in $G_{o2}$–/– mice (Fig. 3 A). Response families in $G_{o2}$–/– rod bipolar cells appeared similar to WT, with statistically indistinguishable maximal amplitudes (Table I). The time-to-peak and integration time (defined as the integral of the dim flash response divided by its peak amplitude) of the dim flash response was also statistically indistinguishable from WT rod bipolar cells (Fig. 3 B; see Table I). However, the loss of $G_{o2}$ caused a reduction in the amplitude of the $G_{o2}$–/– dim flash responses (Fig. 3 B; see also Fig. S1 A), which led to an overall reduction of light sensitivity of rod bipolar cells, as seen by the shift to higher flash strengths in the plot of normalized response amplitude average, approximately twofold smaller than $G_{o1}$+/+ $G_{o2}$–/– AIIACs (Table I). Provided that AIIACs provide an accurate measure of rod bipolar sensitivity, this suggests that the rod bipolar response mediated by $G_{o2}$ alone is ~20-fold less sensitive than the response mediated by both $G_{o1}$ and $G_{o2}$. Interestingly we find that dark-adapted light responses to the strongest flashes in the $G_{o1}$–/– $G_{o2}$–/– AIIACs lacked the initial nose seen under normal circumstances (Nelson, 1982), suggesting that rod bipolar responses mediated by $G_{o2}$ alone are not able to fully drive glutamate release from the rod bipolar synaptic terminal.

$G_{o2}$–/–mediated responses were also controlled by mGluR6. Fig. 2 D plots the maximal inward response amplitude during the application of the mGluR6 agonist, 1-2-aminophosphonobutyric acid (APB), for $G_{o1}$–/– $G_{o2}$–/– AIIACs. APB (8 μM) completely suppressed the response in $G_{o1}$–/– $G_{o2}$–/– AIIACs, an effect that was reversible after washout (Fig. 2 E). Thus, both the $G_{o1}$ and the $G_{o2}$ mediate a depolarizing light response in rod bipolar cells through the activity of mGluR6.

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versus flash strength (Fig. 3 C). The half-maximal flash strength provides a robust measure of the sensitivity of rod bipolar cells that is independent of the maximal response amplitude (Fig. S1 B). Thus the presence of G\(_{\alpha_{02}}\) increases the sensitivity of the average response to a dim flash in rod bipolar cells of WT mice.

To determine how the decreased amplitude of the dim flash response influenced its detection, we characterized how the absence of G\(_{\alpha_{02}}\) impacted the dark noise. We calculated the total variance (0–300 Hz) of the noise in darkness for G\(_{\alpha_{02}}^-/-\) and WT rod bipolar cells in the 5 s immediately after establishing the whole-cell recording for the cells shown in Fig. 3. The total variance of the dark noise in WT rod bipolar cells was 11.5 ± 2.0 pA\(^2\) (\(n = 14\)) and in G\(_{\alpha_{02}}^-/-\) rod bipolar cells was 12.7 ± 1.8 pA\(^2\) (\(n = 16\)) (mean ± SEM; \(P = 0.67\)), values that are indistinguishable statistically. The loss of G\(_{\alpha_{02}}\) appears then to cause a reduction in the amplitude of the light response with the magnitude of the dark noise remaining unchanged, resulting in an overall reduced signal-to-noise ratio in G\(_{\alpha_{02}}^-/-\) rod bipolar cells.

Reducing the total expression of G\(_{\alpha}\) does not alter rod bipolar responses

Reduced sensitivity in G\(_{\alpha_{02}}^-/-\) rod bipolar cells may be simply due to the decrease in the total amount of G\(_{\alpha}\) protein rather than any specific role played by G\(_{\alpha_{02}}\). To test whether the concentration of G\(_{\alpha}\) influenced response sensitivity, we recorded rod bipolar responses from heterozygous mice for G\(_{\alpha}\) (G\(_{\alpha}\)^+/− littermate). As shown in

Figure 3. G\(_{\alpha_{02}}^-/-\) rod bipolar cells exhibited reduced light sensitivity. (A) Responses to a family of flashes producing 0.29, 0.59, 1.2, 2.3, 4.7, 9.4, and 19 Rh*/rod were recorded in a WT (i.e., G\(_{\alpha_{02}}^+/-\) littermate) and a G\(_{\alpha_{02}}^-/-\) rod bipolar cell. (B) Normalized rod bipolar response to dim flashes producing 1 Rh*/rod was estimated by averaging normalized responses to dim flashes casing 5–25% of maximal responses and dividing those by the average flash strength, which was 0.60 Rh*/rod for WT and 0.72 Rh*/rod for G\(_{\alpha_{02}}^-/-\) rod bipolar cells. The WT response is the average of 332 dim flash responses across 15 cells from 8 mice, and G\(_{\alpha_{02}}^-/-\) response is the average of 321 dim flash responses across 16 cells from 6 mice. (C) Normalized response amplitudes from individual families were averaged across cells for WT (\(n = 15\)) and G\(_{\alpha_{02}}^-/-\) rod bipolar cells (\(n = 16\)), and plotted as a function of flash strength. Half-maximal flash strengths estimated from the Hill curve fits were 2.2 ± 0.15 vs. 2.6 ± 0.19 Rh*/rod, and the Hill exponents were 1.51 ± 0.02 vs. 1.55 ± 0.05 for WT vs. G\(_{\alpha_{02}}^-/-\) rod bipolar cells, respectively (mean ± SEM). While differences in the Hill exponent were not statistically significant (\(P = 0.13\)), the shift in half-maximal flash strengths was significant (\(P = 0.047\)).
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(Fig. 4, A and D; Table I). While the average time-to-peak was delayed slightly in Ga_o^+/− rod bipolar cells (from 118 to 133 ms; see Table I), the integration time of the dim flash response was statistically indistinguishable from WT rod bipolar cells (Fig. 4 B; Table I). Thus, the reduced sensitivity in Ga_o^+/− rod bipolar cells appears instead to result from a specific effect of Ga_o, and not from a reduction in the overall Ga_o level. Furthermore, Fig. 4 D shows the Hill exponent between WT and Ga_o^+/− rod bipolar cells are statistically identical, indicating that saturation within the mGluR6 cascade is not set by the Ga_o concentration (see Discussion).

Figure 4. Reduced Ga_o expression in Ga_o^+/− mice does not alter rod bipolar responses (A) Responses to a family of flashes producing 0.59, 1.2, 2.4, 4.7, 9.4, and 19 Rh*/rod were recorded in a WT (Ga_o^+/+ littermate), and a Ga_o^+/− rod bipolar cell. (B) Normalized rod bipolar response to dim flashes producing 1 Rh*/rod was estimated by averaging normalized responses to dim flashes casing 5–25% of maximal responses and dividing it by the average dim flash strength, which was 0.73 Rh*/rod for WT and 0.79 Rh*/rod for Ga_o^+/−. The WT response is the average of 437 dim flash responses across 14 cells from 3 mice, and the Ga_o^+/− response is the average of 271 dim flash responses across 15 cells from 3 mice. (C) The total amount of Ga_o and Ga_o2 proteins in WT and Ga_o^+/− retinas were compared using Western blot analysis. The amount of Ga_o2 proteins in Ga_o2^−/− retinas was also examined to check the specificity of the antibody. The protein level of Ga_o^+/− retina was normalized to that of WT retina for a pair of WT and Ga_o^+/− mice used in one experiment, and the collected results are shown in the bar graph. The error bars are the SEM. The Ga_o protein levels were 1 vs. 0.52 ± 0.02 (n = 4) and the Ga_o2 protein levels were 1 vs. 0.46 ± 0.04 (n = 3) (mean ± SEM, WT vs. Ga_o^+/−). (D) Normalized response amplitudes from individual families were averaged across cells for WT rod bipolar cells (n = 14) and Ga_o^+/− rod bipolar cells (n = 15) and plotted as a function of flash strength. Half-maximal flash strengths estimated from the Hill curve fits were 2.5 ± 0.13 vs. 2.5 ± 0.17 Rh*/rod, and the Hill exponents were 1.54 ± 0.04 vs. 1.62 ± 0.06 (mean ± SEM, WT vs. Ga_o^+/−).
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We tested how the ratio of Go$_{o1}$ to Go$_{o2}$ influences the properties of rod bipolar light responses in Go$_{o1}^{-/-}$ mice. Fig. 5 C shows that the total Go$_{o}$ expression was decreased by $\sim$60% in these mice, whereas Go$_{o2}$ expression was increased by $\sim$25% compared with WT retinas. Overall, the ratio of Go$_{o2}$ expression over Go$_{o1}$ increased approximately threefold in Go$_{o1}^{-/-}$ retinas compared with WT. Since the presence of Go$_{o2}$ increased the sensitivity of the light response (Fig. 3), increasing the relative ratio of Go$_{o2}$ to Go$_{o1}$ might further increase the sensitivity of rod bipolar cells. However, neither the

Altering the balance of expression between Go$_{o1}$ and Go$_{o2}$ does not alter rod bipolar responses

Splice variants of G proteins typically display alterations in cellular functions, and frequently act on different effectors in the same cell. Go$_{o1}$ and Go$_{o2}$ both mediate depolarizing light responses in rod bipolar cells (Figs. 1 and 2), suggesting in the simplest scheme that they act on a common effector in the mGluR6-signaling cascade, although actions on different effectors cannot be ruled out. Regulation of the effector might then be dependent on the relative ratios of each of these splice variants.

Figure 5. Altered Go$_{o1}$ vs. Go$_{o2}$ expression in Go$_{o1}^{-/-}$ mice does not alter rod bipolar responses (A) Responses to a family of flashes producing 0.4, 0.8, 1.6, 3.2, 6.4, and 13 Rh*/rod were recorded in a WT (Go$_{o1}^{+/+}$ littermate) and a Go$_{o1}^{-/-}$ rod bipolar cell. (B) Normalized rod bipolar response to dim flashes producing 1 Rh*/rod was estimated by averaging normalized responses to dim flashes casing 5–25% of maximal responses and dividing it by the average dim flash strength, which was 0.70 Rh*/rod for WT and 0.72 Rh*/rod for Go$_{o1}^{-/-}$. The WT response is the average of 351 dim flash responses across 16 cells from 4 mice and the Go$_{o1}^{-/-}$ response is the average of 331 dim flash responses across 17 cells from 4 mice. (C) The total amount of Go$_{o}$ and Go$_{o2}$ proteins in WT, Go$_{o1}^{-/-}$, Go$_{o2}^{-/-}$, and Go$_{o2}^{+/+}$ retinas were compared with Western blot analysis. The protein levels were normalized to those of WT retinas for a group of mice used in one experiment, and the results of repeated experiments are shown in the bar graph. The error bars show SEM. The Go$_{o}$ protein levels were 1 vs. 0.42 ± 0.02 vs. 0.05 ± 0.01 vs. 0.05 ± 0.01 (n = 3), and the Go$_{o2}$ protein levels were 1 vs. 1.27 ± 0.11 vs. 1.84 ± 0.16 vs. 0.01 ± 0.01 (n = 3) (mean ± SEM, WT vs. Go$_{o1}^{-/-}$ vs. Go$_{o2}^{-/-}$ vs. Go$_{o2}^{+/+}$). (D) Normalized response amplitudes from individual families were averaged across cells for WT rod bipolar cells (n = 16) and Go$_{o1}^{-/-}$ rod bipolar cells (n = 17) and plotted as a function of flash strengths. Half-maximal flash strengths estimated from the Hill curve fits were 2.81 ± 0.12 vs. 2.47 ± 0.14 Rh*/rod, and the Hill exponents were 1.43 ± 0.05 vs. 1.54 ± 0.07 (mean ± SEM, WT vs. Go$_{o1}^{-/-}$), and are statistically indistinguishable (P = 0.12 for half-maximal flash intensity values, and P = 0.19 for Hill exponents between WT and Go$_{o1}^{-/-}$ rod bipolar cells).
components of the signaling cascade that allow mGluR6 receptors through the action of Gαo to close TRPM1 transduction channels (for reviews see Okawa and Sampath, 2007; Snellman et al., 2008). Here we have studied how Gαo activity influences the dark-adapted light response in mouse rod bipolar cells and found the following: (a) the coordinated action of two splice variants of Gαo (Gαo1 and Gαo2) maximizes light sensitivity, (b) reductions in the concentration of Gαo do not influence the open probability of transduction channels, and (c) the nonlinear threshold due to the saturation of the transduction cascade does not depend on the Gαo concentration.

**Figure 6.** Proposed mGluR6-signaling cascade in rod bipolar cells. mGluR6 receptors activated upon binding glutamate released from rods exchange GTP for GDP on both Gαo1 and Gαo2, which leads to the closure of nonselective cation channels (TRPM1) through an unknown downstream cascade. The efficiency of the Gαo2 pathway is lower than that of the Gαo1 pathway, as represented by the thin arrow leading to the putative effector (E?). While a single effector is shown, this work does not exclude the possibility that Gαo1 and Gαo2 act on separate effectors that lead to the coordinated closure of TRPM1 gating. Arrows show that nonlinearity in the signaling cascade might reside at several locations.
a region known to link αo subunits to their receptors and effectors (for review see Clapham and Neer, 1997). Goα splice variants have typically been assigned with different or redundant functions. For instance, in the rat pituitary GH3 cells, Goα1 and Goα2 mediate Ca2+ channel inhibition through muscarinic and somatostatin receptors, respectively (Kleuss et al., 1991, 1993; Degtiar et al., 1997). In rod bipolar cells, both Goα1 and Goα2 are controlled by the mGluR6 receptor and mediate the depolarizing light response (Figs. 1 and 2) without occluding each other’s actions (Fig. 5). The most parsimonious explanation for these two facts are that both splice variants act independently on a common effector, as diagrammed in Fig. 6, however, we cannot rule out actions on different effectors.

Although Goα2-mediated signals are much less efficient than Goα1-mediated signals, a feature that may result from differing affinities of each splice variant for mGluR6 or the effector, the reduced efficiency likely reflects the relatively low expression of Goα2 compared with Goα1 (Dhingra et al., 2002). However, given that AIIACs are highly sensitive to rod bipolar cell input (Dunn and Rieke, 2008; Tian et al., 2010), any subtle variation in the rod bipolar response should result in detectable changes in AIIAC activity. We find that the amplitude of dim flash responses per photon in rod bipolar cells of Goα2−/− mice is ~25% smaller on average than in WT rod bipolar cells (Fig. 3 B; Fig. S1 A; Table I). This reduced sensitivity is attributable to a Goα2-specific effect because it cannot be explained either by the total Goα concentration or the balance of expression between Goα1 and Goα2. Thus, the light response in rod bipolar cells is primarily mediated by Goα1, but Goα2 is necessary to increase the magnitude of the response without increasing the dark noise, thereby increasing the signal-to-noise ratio. Such coordination between two splice variants of a single Go may represent a novel mechanism that fine tunes the functional properties of signaling cascades.

TRPM1 channels remain closed in the absence of Goαo activity

A surprising finding of this work is that reductions of Goα concentration (Figs. 4 and 5), or even the elimination of Goαo entirely (Fig. 1), does not appear to influence the amplifier holding current for voltage-clamped (V_m = −60 mV) rod bipolar cells (see Table I). The interpretation of this result is that reductions in Goα concentration do not correspond to increases in the nonselective cation current of TRPM1 channels. Previous studies for TRPM1 channels expressed in CHO cells (Koike et al., 2009) suggest that these channels are constitutively open, with the presumed role of Goαo to close them (Nawy, 1999; Dhingra et al., 2000, 2002; Koike et al., 2009). The lack of influence of Goαo on the open probability of TRPM1 channels argues that this scheme is more complicated in situ, and may require additional factors for TRPM1 opening (Fig. 6). Alternatively, strong Ca2+-dependent reductions in TRPM1 open probability (Nawy 2000, 2004; Berntson et al., 2004b) may relegate these channels closed even in the absence of Goαo.

Goαo concentration does not set the nonlinear thresholding of rod signals

Our most sensitive vision is encoded in a specialized retinal circuit that pools rod signals, known as the rod bipolar pathway (see Fig. 1 A; Dacheux and Raviola, 1986; Smith et al., 1986). Under conditions where few rod photoreceptors receive photons, downstream cells must discriminate between rods that absorb light from those that do not. The optimization of signal transfer in this pathway requires a nonlinear threshold in rod bipolar cells that separates the single photon response from noise (van Rossum and Smith, 1998; Field and Rieke, 2002), which is generated by saturation of the postsynaptic signaling cascade in the rod bipolar cell dendrites and not by mGluR6 receptor saturation (Sampath and Rieke, 2004). The molecular mechanism that underlies the nonlinear threshold is not well defined, largely due to the uncertain identity of components of this signaling cascade downstream of Goαo.

Here we show that the nonlinear threshold is not influenced by an ~50% reduction in concentration of retinal Goαo (Fig. 4), providing insight into where saturation may occur in the mGluR6 signaling cascade. If the rate of G-protein activation is saturated, such that the reduced Goαo expression does not cause an equivalent reduction in G-protein activity, these results indicate that the binding of Goαo to mGluR6 does not cause this saturation. However, we cannot eliminate the possibility that the exchange of GTP for GDP on Goαo, or the dissociation of Goαo from mGluR6, places a bottleneck on the dark steady-state G-protein activity. Experimental evidence from rod outer segment preparations indicates that transducin (Goα) activation can occur very quickly (>1000 s−1 at physiological temperatures; Bruckert et al., 1992; Heck and Hofmann, 2001), perhaps not totally surprising given the high concentration of transduction elements. However, relatively little is known about G-protein activation rates in other intact systems. It remains to be seen whether GTP exchange and Goαo dissociation limit Goαo activation on the ~120 ms integration time of dark-adapted rod bipolar light responses.

If the rate of G-protein activation by mGluR6 is not saturated in darkness, then these results would indicate that the position of the nonlinear threshold must reside downstream of Goαo activation (see Fig. 6), or alternatively that G-protein activity is sufficient to saturate a downstream component of the signaling cascade even under conditions where this activity is reduced (i.e., Goαo−/−). Saturation could potentially be in the activity of the effector molecule that controls the gating particle of...
TRPM1, or in the open probability of TRPM1 itself (compare Sampath and Rieke, 2004). For the level of saturation to be optimized with respect to the rod signal and noise, it must be set high enough to eliminate most of the continuous noise produced by spontaneous PDE activation, but not to eliminate too many single photon responses (Field and Rieke, 2002). Thus a delicate trade-off between noise and sensitivity must exist, giving great importance to identifying the component of the signaling cascade mediating this nonlinear step.

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Figure S1. Reduced light sensitivity of $\alpha_{3^{-/-}}$ rod bipolar cells. (A) Reduction in response amplitude for $\alpha_{3^{-/-}}$ versus WT rod bipolar cells for a flash strength of 0.29 Rh*/rod. The WT response is the average of 157 dim flash responses across 15 cells from 8 mice, and the $\alpha_{3^{-/-}}$ response is the average of 162 dim flash responses across 16 cells from 6 mice. (B) Half-maximal flash strength and maximal response amplitude are plotted for all WT cells in this study. The correlation coefficient was calculated as $r = 0.04$, indicating that the relationship between the half-maximal flash strength and the maximal response amplitude is uncorrelated.