Transducin Activation State Controls Its Light-dependent Translocation in Rod Photoreceptors*

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Vasily Kerov†, Desheng Chen‡, Mustapha Moussaifi‡, Yu-Jiun Chen§, Ching-Kang Chen§, and Nikolai O. Artemyev†‡

From the †Department of Physiology and Biophysics, University of Iowa College of Medicine, Iowa City, Iowa 52242 and the §Department of Biochemistry, Virginia Commonwealth University, Richmond, Virginia 23298

Light-dependent redistribution of transducin between the rod outer segments (OS) and other photoreceptor compartments including the inner segments (IS) and synaptic terminals (ST) is recognized as a critical contributing factor to light and dark adaptation. The mechanisms of light-induced transducin translocation to the IS/ST and its return to the OS during dark adaptation are not well understood. We have probed these mechanisms by examining light-dependent localizations of the transducin-α subunit (Gtα) in mice lacking the photoreceptor GAP-protein RGS9, or expressing the GTPase-deficient mutant GtαQ200L. An illumination threshold for the Gtα movement out of the OS is lower in the RGS9 knock-out mice, indicating that the fast inactivation of transducin in the wild-type mice limits its translocation to the IS/ST. Transgenic GtαQ200L mice have significantly diminished levels of proteins involved in cGMP metabolism in rods, most notably the PDE6 catalytic subunits, and severely reduced sensitivity to light. Similarly to the native Gtα, the GtαQ200L mutant is localized to the IS/ST compartment in light-adapted transgenic mice. However, the return of GtαQ200L to the OS during dark adaptation is markedly slower than normal. Thus, the light-dependent translocations of transducin are controlled by the GTP-hydrolysis on Gtα, and apparently, do not require Gtα interaction with RGS9 and PDE6.

Heterotrimeric GTP-binding proteins (G proteins) propagate a variety of hormonal and sensory signals from specific cell surface receptors to intracellular effectors (1–3). The visual transduction cascade in vertebrate photoreceptors has served for many years as a paradigm for G protein signaling. In rod photoreceptor cells, illuminated rhodopsin stimulates GTP-GDP exchange on the retinal G protein, transducin (Gt),2 resulting in dissociation of GtαGTP from Gtβγ and rhodopsin. Gtα in the GTP-bound conformation stimulates the effector enzyme, cGMP phosphodiesterase (PDE6), by displacing the inhibitory γ-subunits (Pγ) from the PDE6 catalytic core (PDE6αβ). cGMP hydrolysis by active PDE6 results in closure of cGMP gated channels in the plasma membrane (4, 5). The turn-off phase of the visual signal is determined by reactions controlling the lifetimes of photoexcited rhodopsin (R*) and activated transducin. The catalytic function of R* is blocked by the rhodopsin-kinase mediated phosphorylation and the binding of arrestin to phosphorylated R* (6–8). The lifetime of GtαGTP is controlled by intrinsic GTPase activity. Hydrolysis of GTP switches the Gtα molecule to the inactive GDP-bound conformation and allows rehydration of PDE6βγ by Py. RGS9-1, a photoreceptor-specific member of the RGS (regulators of G protein signaling) family complex, in the complex with Gtβγ. Gtβγ acts as a GTPase-activating protein for transducin and thus is a major regulator of the turn-off kinetics of the visual signal (9–11). The RGS9-1/Gtβγ complex is anchored to disc membranes through the interaction with R9AP (RGS-2–1-anchor protein) that enhances the complex GAP activity (12–14). The recovery in the visual transduction cascade and light adaptation of photoreceptors are coupled to the changes in the intracellular Ca2+ concentration. A decrease in intracellular Ca2+ concentration resulting from the closure of cGMP-gated channels leads to activation of guanylate cyclase by specific Ca2+-binding guanylate cyclase-activating proteins (GCAPs) and restoration of cGMP levels (15, 16). Enhanced guanylate cyclase activity in light-adapted photoreceptors adjusts the sensitivity of photoreponses and expands the receptive range of light intensities.

The light-induced movement of transducin from the rod outer segments (OS) into the inner segments (IS) and synaptic terminals (ST) of photoreceptor cells, and the movement of arrestin in the opposite direction were known for a long time (17–19). This phenomenon has attracted renewed attention with the application of quantitative approaches involving tangential microsectioning of flat mounted retinas (20). A reduction in the transducin content in the rod outer segment because of its translocation was correlated with a decrease in the amplification of the photoreponse, suggesting that the protein redistribution is a novel mechanism contributing to light adaptation (20). A recent study concluded that the distribution of arrestin is controlled by its interactions with rhodopsin in the OS and microtubules in the IS, and movement of arrestin occurs by simple diffusion (21). The mechanisms of transducin translocation to the IS/ST in the light and back to the OS during dark adaptation remain largely unknown. Diffusion and active transport represent two potential general modes for the transducin translocation (20). In a diffusion model, the light-induced dissociation of GtαGTP and Gtβγ from the disc membranes simply allows them to diffuse into the IS/ST. In the IS/ST, Gtα is converted into the GDP-bound state, forms a heterotrimer with Gtβγ, and returns by diffusion to the OS during dark adaptation. Gt accumulates in the OS in the dark due to its affinity for the disc membranes and, perhaps, binding to the ground-state rhodopsin (22). Based on the model, the kinetics and/or the illumination threshold for Gtα movement to the IS should depend on the rate of transducin inactivation. GTPase-deficient mutants of Gtα can be predicted to undergo a rapid translocation to the IS and have slower return kinetics during dark adaptation. We have examined the mechanism of transducin movement using the RGS9 knock-out (11) and GTPase-deficient GtαQ200L mouse models. In agreement with the diffusion hypothesis, the illumination threshold for the Gtα movement to the IS/ST is lower in the RGS9−/− mice, and the return of GtαQ200L to the OS during dark
adaptation of the transgenic mice is markedly slower than normal. The GtoQ200L mice show dramatic down-regulation of the rod PDE6 catalytic subunits. Therefore, the observation of light-dependent movement of transducin in these mice suggests that the translocation mechanism is independent of the transducin/PDE6 interaction.

**EXPERIMENTAL PROCEDURES**

**Generation of EE-tagged Gtα and GtαQ200L Transgenic Mice—**All experimental procedures involving the use of mice were carried out in accordance with the NIH guidelines and the protocols approved by the University of Iowa, University of Utah, and the Utah Institutional Animal Care and Use Committees. A pBRH Gtα transgenic construct containing the mouse Gtα genomic sequence of ~5.5 kb (23) flanked by the 4.4-kb mouse opsin promoter fragment (24) and polyadenylation signal was kindly provided by Dr. J. Lem (New England Medical Center). The Glu-Glu (EE) monoclonal antibody epitope was introduced into Gtα to quantitatively assess the level of expression of the transgene (see Fig. 1). Two substitutions required to convert Gtα sequence 162GYVPTE167 into the epitope sequence EYMPTE were created using the QuikChange mutagenesis kit (Stratagene) according to the manufacturer’s protocol. Subsequently, a similar procedure was adapted using the QuikChange mutagenesis kit (Stratagene) according to the manufacturer’s protocol. Subsequently, a similar procedure was used to generate the transgene for expression of the EE-tagged GtαQ200L (see Fig. 1). DNA fragments of 9-kb were released from the pBRH-GtαEE and pBRH-GtαQ200L plasmids by restriction with NotI and gel purification. The GtαEE and GtαQ200L DNA fragments were microinjected into mouse embryos and implanted into pseudopregnant females in the transgenic core facilities at University of Utah. Transgenic mice were identified by PCR of mouse tail DNA with a pair of primers chosen to amplify a 300-bp fragment surrounding the junction between the Gtα and polyadenylation signal sequences. Fifteen potential GtαEE transgenic founders were generated; nine of them transmitted the transgenes after mating with C57BL/6 mice, and five transmitted the transgenes after mating with C57BL/6 mice, and five transmitted the transgenes to a F1 background. A pBRH Gtα transgenic line and one GtαQ200L line with high levels of transgene expression across the retina were selected for characterization and breeding with the rod Gtα knock-out mice (25) to move the transgenes into the hemizygous (Gtα−/−) background.

**Immunoblot Analysis and Quantification of Transgene Expression—**Total mouse retinal homogenates were obtained by solubilization of 1–2 retinas in 200 μl of 10% SDS-Na using brief sonication and heating. Protein concentrations were determined using the Bio-Rad DC protein assay and bovine serum albumin dissolved in 10% SDS-Na as a standard. Typically, a total protein content of a homogenate obtained from a single mouse retina was ~400 μg. Samples of retinal homogenates were subjected to SDS-PAGE in 10% gels, electrotransferred onto nitrocellulose membranes, and probed with antibodies against rod Tα (K-20), anti-Gβ1 (M-14), anti-Gγ1 (P-19) (Santa Cruz Biotechnology, Santa Cruz, CA), EE-epitope (CRP, Berky, CA), rod opsin (1D4) (National Cell Culture Center, Minneapolis, MN), PD66α (PA1-722), PD66β (PA1-722), GRK1 (MA1-720) (ABR, Golden, CO), RGS9-469-484 (Elmira Biologicals, Iowa City), AIP1 (gift of Dr. J. Hurley, University of Washington), Py-63-87 (gift of Dr. R. Cote, University of New Hampshire), GC-E, anti-GC-F (gift of Dr. D. Garbers, University of Texas Southwestern Medical Center), GCAP1, or GCAP2 (gift of Dr. A. Dizhoo, Pennsylvania College of Optometry) antibodies. The antibody-antigen complexes were detected using anti-rabbit, anti-mouse, or anti-sheep antibodies conjugated to horseradish peroxidase (Sigma) and ECL reagent (Amersham Biosciences). The EE-tagged transducin-like chimeric Gtα (GtαEE) and the Q200L mutant (GtαQ200L) were constructed by PCR-directed mutagenesis similarly as described previously (26). *Escherichia coli* expressed and purified GtαEE was used as a standard. In control experiments, the immunoblot signals of GtαEE were not notably affected by the presence of up to 40 μg of retinal extracts from Gtα−/− mice (not shown). Nitrocellulose membranes were exposed to film, and integrated densities of scanned individual bands were measured with Scion Image software (version 4.0.3.2).

**Immunofluorescence—**For dark adaptation, mice were kept in the dark for an indicated period of time (up to 30 days). All dark procedures were performed under infrared illumination using night vision goggles. For light adaptation, the pupils were dilated by applying a drop of 1% tropicamide, followed by a drop of 2.5% phenylephrine hydrochloride 30 min prior to light exposure. During light exposure, mice that were free to move were kept in a Styrofoam box (20 × 15 × 15 cm) covered with semi-transparent polyethylene film to diffuse light. Light from white fluorescent bulbs in the ceiling was used for strong light exposure (~500 lux, 45 min). Low level light exposures (2–8 lux, 25 min) were achieved with light from a light table reflected by a white ceiling. These conditions provided a uniform illumination from all directions as measured with a LX-1010B digital lux meter. The mice were euthanized with CO2. Mouse eyeballs were enucleated, poked through the cornea with a 21-gauge needle, and fixed in 4% formaldehyde in phosphate-buffered saline for 2 h at 22 °C. After fixation, the eyeballs were cut in half, the cornea and lens were removed, and the eyecups were submersed in a 21-gauge needle, and fixed in 4% formaldehyde in phosphate-buffered saline for 2 h at 22 °C. After fixation, the eyeballs were cut in half, the cornea and lens were removed, and the eyecups were submersed in a 30% sucrose solution in phosphate-buffered saline for 5 h at 4 °C. The eyecups were then embedded in tissue freezing medium (TBS) and frozen on dry ice. Radial sectioning (10 μm) of the retina was performed using a cryomicrotome Microm HM 505E. Retinal cryosections were air-dried and kept at ~80 °C until use. Before staining, sections were warmed up to 25 °C and incubated in 0.1% Triton/phosphate-buffered saline for 30 min followed by incubation with 2% normal goat serum/5% bovine serum albumin in phosphate-buffered saline for 30 min. Then sections were incubated with rabbit anti-rod Gtα antibody K-20 (1:2000), anti-Gβ1, M-14 (1:1000) (Santa Cruz Biotechnology, Santa Cruz, CA), a monoclonal anti-EE antibody (1:2000) (CRP, Berky, CA), or rabbit anti-γ1-63–87 antibodies (1:10,000) for 3 h. Following a 1-h incubation with goat anti-rabbit AlexaFluor 546 or goat anti-mouse AlexaFluor 488 secondary antibodies (Molecular Probes) (1:1000), the sections were visualized using a Zeiss LSM 510 confocal microscope. Fluorescence signal quantification was performed using Scion Image for Windows software (version 4.0.3.2).
Electroretinography—The mice were dark-adapted overnight and prepared for recording the next morning under infrared illumination after anesthesia with a mixture of ketamine (150 mg/kg intraperitoneal) and xylazine (10 mg/kg intraperitoneal). The body temperature was maintained at 35–37 °C using a homeothermic blanket. Pupils were dilated using equal parts of topical phenylephrine (2.5%) and tropicamide (1%). Drops of 0.9% saline were applied onto the cornea to prevent its dehydration and allow electrical contact with the recording electrode (a gold wire loop). A platinum subdermal needle was inserted under the scalp and between the two eyes to serve as the reference electrode.

**FIGURE 2.** Light-dependent translocation of Gtα in rod photoreceptors of RGS9−/− mice. A, retinal distribution of Gtα in RGS9−/− and control (WT) mice exposed to light (500 lux, 45 min) (L) and after allowing the light-adapted animals to dark adapt for 4 and 10 h (D). The retinal sections were stained with rabbit anti-rod Gtα antibody and visualized with goat anti-rabbit AlexaFluor 546 secondary antibodies. OS, outer segments; IS, inner segments; ONL, outer nuclear layer; OPL, outer plexiform layer. Bar, 10 μm. B, the immunofluorescence signals of Gtα were quantified using Scion Image software. The signal intensity in the OS layer is expressed as a percentage of the total signal intensity in the photoreceptor layer for the light-adapted mice (L) and after a 10-h dark adaptation (D). Means ± S.D. from three experiments are shown.

**FIGURE 3.** Light exposure threshold for Gtα translocation. A, retinal distribution of Gtα in C57BL/6 mice dark-adapted for 24 h and exposed for 25 min to dim light of increasing intensity. The retinal sections were stained with rabbit anti-rod Gtα antibody. Bar, 10 μm. B, the Gtα immunofluorescence signal intensity in the OS layer is expressed as a percentage of the total signal intensity in the photoreceptor layer. Means ± S.D. from three experiments are shown.
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Amplification (at 1–1000-Hz bandpass, without notch filtering), stimulus presentation, and data acquisition were performed on the UTA-E 3000 visual diagnostic system from LKC Technologies Inc. Scotopic ERG responses to single flash presentations (10-μsec duration) at increasing intensities covering a range from −3.6 to 1.4 log cd s/m² were recorded. To ensure the dark-adapted state of the animals, interstimulus intervals have been programmed to increase from 10 s at lowest stimulus intensity to 2 min at highest stimulus intensity. Two GtαEEQ200L mice were analyzed by ERG with similar results.

Trypsin Protection Test—One GtαEE retina and one GtαEEQ200L retina from the dark-adapted mice (30-day dark adaptation) were homogenized in the dark by sonication in 400 μl of 20 mM Hepes buffer (pH = 8.0), containing 100 mM NaCl and 4 mM MgSO₄. Aliquots of retinal homogenates (15 μl) were incubated with trypsin (10 μg/ aliquot) for 30 min at 22 °C, followed by the addition of 50 μg of soy bean trypsin inhibitor. Additional aliquots of retinal homogenates were photo-bleached, followed by the addition of 100 μM GTPγS or GTP. After 5 min incubation at 22 °C, the aliquots were treated with trypsin as above and analyzed by Western blotting using anti-EE antibodies.

Single Turnover GTPase Assay—Single turnover GTPase activity measurements were carried out in suspensions of uROS membranes (5 μM rhodopsin) reconstituted with purified recombinant Gtα or GtαQ200L (0.5 μM) and Gtβγ (0.5 μM) as previously described (27, 28). The GTPase reaction was initiated by the addition of 50 nM [γ-32P]GTP (~4 × 10⁶ dpm/pmol). The GTPase rate constants were calculated by fitting the experimental data to an exponential function: %GTP hydrolyzed = 100(1 – e⁻ᵏᵗ), where k is a rate constant for GTP hydrolysis.

RESULTS

Transducin Translocation in RGS9 Knock-out Mice—A general translocation of Gtα in the rods of the RGS9 knock-out mice was examined following exposure of the dark-adapted mice to moderate illumination conditions (500 lux, 45 min). This light exposure caused redistribution of Gtα from the OS to the IS/ST, similarly to that observed in control C57BL/6 mice (Fig. 2). To assess the return of Gtα to the OS, the RGS9−/− and control mice were exposed to light (500 lux, 45 min) and then dark-adapted for 4, 10, or 24 h. The immunostaining of retinal cross-sections from the dark-adapted mice demonstrated that redistribution of Gtα during dark adaptation is similar in RGS9−/− and control mice, and is largely complete within 10-h in the dark (Fig. 2). Quantification of protein distribution by immunofluorescence intensity has its limitations because of the potential antibody epitope masking and other related artifacts (20). Nonetheless, the estimates of Gtα content in the OS of the light- and dark-adapted wild type and RGS9−/− mice by immunofluorescence are similar to those obtained previously by a more quantitative procedure of tangential retina microsectioning combined with immunoblotting (Fig. 2B) (29).

The conditions of minimal illumination that cause movement of Gtα in control dark-adapted mice with dilated pupils were determined by varying the level of illumination and the time of light exposure. The distribution of Gtα in mouse retinas from C57BL/6 mice after the light exposure equivalent to 2 photopic lux over 25 min was found to be identical to the Gtα distribution in the dark-adapted animals (Fig. 3). Small, but detectable movement of Gtα to the IS was observed in the control retinas using illumination of 4 and 8 lux for 25 min (Fig. 3). In contrast to control mice, the lowest level of light exposure (2 lux, 25 min) induced a considerable translocation of Gtα to the rod IS/ST in the RGS9−/− mice (Fig. 4).

Transgenic Mouse Lines Expressing EE-tagged Gtα and the Q200L Mutant—To monitor the localization and translocation of the GTPase-deficient GtαQ200L mutant in the presence of native transducin the Glu-Glu (EE) antibody epitope was introduced into the Gtα helical domain. The EE-tag at the selected position had no effect on the signaling activity of different G protein α subunits including Gaα1, Gaα12, and Gaα13 (30, 31). We first confirmed that the EE-epitope does not affect the key biochemical properties of Gtα, whereas the Q200L mutation
The Q200L mutation reduced the rate of GTP hydrolysis by Gt. Impaired transducin-like light-induced translocation of Gt in rods—The immunofluorescence staining of retinal sections from the transgenic mice expressing GtQ200L or GtEEQ200L mice was used to assess the localization and translocation of Gt in the retinal layers. The immunoblot analysis of total and Gt EE levels revealed that Gt EE is primarily localized to the IS/ST compartments in the transgenic mice. The reduction in the PDE6 content caused by the GTPase-deficient mutant is in agreement with a similar observation made in transgenic mice expressing the cone GtQ200L heterozygous GtQ200L to the OS during dark adaptation of the transgenic mice.

After breeding the transgenic mice with the Gt knock-out mice, the levels of total Gt, native Gt plus mutant, GtEE, and GtEEQ200L were assessed in mice with the hemizygous GtEEQ200L animals at 1 month of age was found to be largely normal (Fig. 6A). The levels of GC-1, GC-2, GCAP-1, and GCAP2 were also significantly decreased (Fig. 6A). The reduction in the PDE6 content caused by the GTPase-deficient mutant is in agreement with a similar observation made in transgenic mice expressing the cone GtQ200L heterozygous GtQ200L to the OS during dark adaptation of the transgenic mice.

Examination of the levels of major phototransduction proteins revealed no significant differences between the GtEE mice and control non-transgenic animals (not shown). In contrast, the rod PDE6 catalytic subunits were dramatically down-regulated in the GtEEQ200L mice (Fig. 6A). The levels of GC-1, GC-2, GCAP-1, and GCAP2 were also significantly decreased (Fig. 6A). The reduction in the PDE6 content caused by the GTPase-deficient mutant is in agreement with a similar observation made in transgenic mice expressing the cone GtQ200L heterozygous GtQ200L to the OS during dark adaptation of the transgenic mice.

Two transgenic mouse lines expressing the highest levels GtEEQ200L or GtEE were selected for the initial characterization. The expression of transducin-like light-induced translocation of Gt in rods—The immunofluorescence staining of retinal sections from the transgenic mice expressing the cone GtQ200L heterozygous GtQ200L to the OS during dark adaptation of the transgenic mice.

**FIGURE 6.** Characterization of transgenic GtEEQ200L mice. A, Western blot analyses of proteins involved in cGMP metabolism in rod photoreceptors. Each sample contained 10 μg of retinal extract of 1-month-old GtEEQ200L or control non-transgenic littermate mice. B, retinal morphology of 1-month-old GtEEQ200L and control non-transgenic littermate animal. C, electrophotoreceptors of control (WT) and GtEEQ200L mice. Scotopic ERG responses of dark-adapted 6-week-old animals to flashes of increasing intensities ranging from −3.6 log cd/m² (dimest) to 1.4 log cd/m² (brightest).

**FIGURE 7.** Expression of GtEE and GtEEQ200L in transgenic mice. Immunoblot analysis with anti-rod Gtα (K-20) (top) and anti-EE epitope (bottom) antibodies. GtαEE, purified recombinant His6- and EE-tagged Gtα, was used as a standard. Retinas from hemizygous GtEE, GtEEQ200L, and control (WT) mice were homogenized and subjected to SDS-PAGE and Western blotting.
was markedly slower. Only a partial relocalization of GtαEEQ200L to the OS was observed after a 5-day period of dark adaptation. The mutant Gtα apparently continued to accumulate in the OS during extended dark adaptation for 30 days, but its return was still incomplete with a fraction of GtαEEQ200L present in the IS/ST (Fig. 8). The distribution of Gtβγ in the light- (500 lux, 45 min) and dark-adapted GtαEE and GtαEEQ200L mice was probed using anti-Gtβ antibodies (Fig. 9). This analysis confirmed light-dependent translocation of transducin in rods expressing the GTPase-deficient mutant. A fraction of Gtβγ also remained in the IS/ST after a 30-day dark adaptation of the transgenic animals (Fig. 9).

Mouse retinal homogenates were obtained from the GtαEEQ200L and GtαEE mice dark-adapted for 30 days to determine the nucleotide occupancy of the Q200L mutant. The dark-kept or light-exposed retinal samples in the absence or presence of GTP or GTPγS were tested for Gtα sensitivity to trypsin using immunoblotting with anti-EE antibodies. The tryptic cleavage site at Arg-204 is protected in the activated GTP/GTPγS-bound Gtα leading to the formation of ~30-kDa proteolytic product. The trypsin protection test demonstrated that GtαEE is...
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DISCUSSION

Light-dependent redistributions of transducin and arrestin in photoreceptor cells represent a novel mechanism of light/dark adaptation. The movements of transducin and arrestin are unaffected in mice lacking arrestin and transducin, respectively, demonstrating that the translocations of the two proteins are independent processes (36, 37). Two general mechanisms of light-dependent redistribution of Gtα are plausible: diffusion and active transport with the help of motor proteins. Supporting the diffusion mechanism, the movement of arrestin, and perhaps transducin, is reported to be energy-independent (21). The diffusion mechanism is also favored for the recently discovered light-dependent translocation of recoverin (38). The rate of diffusion of soluble proteins in photoreceptors is fast (21), whereas the time course of Gtα translocation to the IS/ST upon exposure to light is relatively slow (20). If the activated GtαGTP moves by simple diffusion, then the rate of its translocation to the IS/ST must be limited by the fast inactivation of Gtα by the RGS9 GAP complex. Consequently, the movement of an individual Gtα molecule during continuous illumination would be composed of multiple steps of the Gt activation, release, and diffusion of GtαGTP followed by the GTP hydrolysis and rebinding of Gtα to a disc membrane more proximate to the IS. The RGS9 knock-out mouse is a well-characterized model with a slower GTP hydrolysis on Gtα and slowed recovery of photoresponses (11). Equivalent levels of illumination are expected to produce a larger steady-state concentration of GtαGTP in the RGS9−/− mice than in control mice and lead to a greater translocation of Gtα according to the diffusion mechanism. The analysis of Gtα redistribution in the RGS9−/− mice demonstrated that the Gtα translocation does not require RGS9, and the illumination threshold for this translocation is indeed lower. The redistribution of Gtα from the IS/ST to the IS during dark adaptation was unaffected in the RGS9−/− mice. This observation is in accord with the cellular localization of RGS9-1 exclusively in the OS. Absent of the RGS9 GAP complex, GtαGTP inactivates in the IS/ST and forms a heterotrimer with Gtβγ with the same rate in wild type and RGS9−/− mice. Supporting the notion that Gtα and Gtβγ translocate to the OS in the dark as a heterotrimer, the time courses of Gtα and Gtβγ return to the outer segment are similar (20), whereas in the dark- or light-adapted Gtα knockout mice, Gtβγ is distributed throughout the photoreceptor cells (37).

Gtα moves against the concentration gradient when the translocation to the IS is nearly complete (20). This would indicate a presence of Gtα binding sites in the inner segments or an involvement of active transport of some sorts. A potential binding partner for GtαGDP, a G protein modulator LGN, has been identified in the IS of photoreceptor cells (39, 40). Alternatively, Gtβγ itself might be a binding “sink” for Gtα. The time course of Gtβγ return to the OS during dark adaptation is much slower than the light-induced translocation of Gtα and Gtβγ (20). A slower transport of Gtαβγ to the OS may be because of the greater size and lipophilic nature of the heterotrimer and may lead to its accumulation in the IS/ST. Interesting insights into the mechanism of Gt translocation to the IS/ST were revealed from the GTPase-deficient GtαEEQ200L mutant mouse model. In the light-adapted transgenic mice, GtαEEQ200L accumulates in the IS/ST similarly to Gtα in control mice. Yet in the light-exposed retinal samples, this mutant is GTP-bound. This observation suggests that Gtα may concentrate in the IS independently of the complex formation with Gtβγ and indicates a possible binding partner for GtαGTP. Although the GTP hydrolysis may not be essential for the accumulation of Gtα in the IS/ST, the GtαEEQ200L model demonstrates that it is necessary for the relocalization of Gtα to the OS during dark adaptation. The return of GtαEEQ200L to the OS during dark adaptation of the transgenic mice

GDP-bound (not protected) in the dark and GTPγS-bound (protected) in the light in the presence of GTPγS (Fig. 10). However, a small fraction of GtαEEQ200L was GTP-bound in the dark. Unlike GtαEE, GtαEEQ200L was protected in the light not only in the presence of GTPγS, but GTP as well (Fig. 10), which is a clear indication of the reduced GTPase activity (32). A partial protection of GtαEEQ200L because of endogenous GTP was detected in the light-exposed sample in the absence of added GTP (Fig. 10). The presence of the GTP-bound GtαEEQ200L after extended dark adaptation indicates that the impairment of GTPase activity because of the QL substitution in native Gtα is much more severe than that in the chimeric Gtα in vitro.

Localization of Py in GtαEEQ200L, RGS9−/−, and Wild Type Mice—Intriguingly, the down-regulation of the rod PDE6 catalytic subunits in the GtαEEQ200L mutant mice did not alter the inhibitory Py subunit (Fig. 6A). Perhaps, this Py was stabilized by the interaction with the GTP-bound Gtα mutant. Free Py is a small, soluble, and, presumably, readily diffusible protein. With the markedly reduced levels of PDE6αβ in the OS, free Py would be expected to distribute evenly between the OS and the IS/ST. However, the immunofluorescence staining of retinal sections from the light-adapted GtαEEQ200L mice showed localization of Py mainly to the IS/ST, which is consistent with the complex between Py and the QL mutant (Fig. 11). Py partially moved to the OS after 30 days of dark adaptation (Fig. 11). Evidently, the pool of GTP-bound QL mutant in the IS/ST becomes sufficiently small during prolonged dark adaptation to cause the release of Py. The subsequent partial movement of Py to the OS might be because of simple equilibration of free Py between the IS and OS and/or the interaction with the small amount of PDE6αβ in the OS of Q200L mice. This observation and earlier studies showing the release of a soluble complex Py-GtαGTPγS from the membrane-bound PDE6 in vitro (35) pointed toward a possibility that Py might co-translocate with the light-activated Gtα. Yet the immunofluorescence analysis of the retinal sections of the wild type, RGS9−/− (Fig. 11), and GtαEE mice (not shown) showed that Py is localized in the dark in the OS and does not appreciably translocate with Gtα in response to light.

FIGURE 11. Localization of Py in retinas of light- and dark-adapted GtαEEQ200L, wild type, and RGS9−/− mice. Retinal distribution of Py in light-adapted (L) GtαEEQ200L mice, control C57BL/6, and RGS9−/− mice (500 lux, 45 min) and after allowing the light-adapted animals to dark adapt (D) for 30 days (GtαEEQ200L) or 24 h (C57BL/6, and RGS9−/−). The retinal sections were stained with rabbit anti-Pγ63–87 antibody. Bar, 10 μm. Similar distributions of Gtβγ were observed in three separate experiments. ONL, outer nuclear layer; OPL, outer plexiform layer.
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was markedly slower. This deficient relocalization of GtαEEQ200L was because of slow GTP hydrolysis by the mutant rather than because of potentially compromised interaction with Gtβγ. The complex of Gtα with Gtβγ is required for Gt activation by R*. Because GtαEEQ200L readily becomes activated in the light-exposed retinal preparations from the dark-adapted transgenic mice, the mutant Gtα is competent of interaction with Gtβγ. A fraction of GtαEEQ200L remains in the GTP-bound state after prolonged dark adaptation, providing an explanation for the residual presence of the mutant Gtα and Gtβγ in the IS/ST of transgenic rods.

The analysis of the transgenic GtαEEQ200L mice revealed a dramatic down-regulation of the PDE6 catalytic subunits. This finding is in agreement with the previously reported reduction in PDE6αβ levels by expression of the GTPase-deficient cone Gtα in mouse rods (34). Moreover, GtαEEQ200L mice are highly insensitive to light, essentially lacking the rod ERG responses. Therefore, the light-dependent translocation of the native and mutant Gtα in GtαEEQ200L mice suggests that the movement of transducin does not require its interaction with PDE6 and the downstream signaling events. Interestingly, the Py levels are unchanged in GtαEEQ200L mice. In the absence of an equivalent concentration of PDE6αβ, Py is concentrated in the IS/ST of light-adapted Q200L rods, apparently in the complex with the mutant Gtα. Py partially diffuses to the OS during dark adaptation of Q200L mice. However, in the WT and RGS9−/− mice with normal levels of PDE6αβ, Py constantly resides in the OS and does not translocate with activated Gtα to the IS/ST.

The analysis of the RGS9−/− and GtαQ200L mice suggests that transducin translocation is controlled by the nucleotide-bound state of Gtα. Reduced Gtα GTPase activity in the OS facilitates the light-induced transduction of GtαGTP to the IS/ST. In a Gtα mutant with severely impaired GTPase activity, such as GtαQ200L, the GTP hydrolysis becomes a rate-limiting step in the relocalization of transducin from the IS/ST to the OS during dark adaptation.

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