



Light-dependent redistribution of visual arrestins and transducin subunits in mice with defective phototransduction

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Purpose: The light-dependent redistribution of phototransduction components in photoreceptor cells plays a role in light adaptation. Upon illumination, rod and cone arrestins (Arr and cArr) translocate from the inner to the outer segments while transducin subunits (T α , T $\beta\gamma$) translocate in the opposite direction. The underlying translocation mechanisms are unclear. This study examines these previously demonstrated translocation in mice with defective phototransduction.

Methods: The distribution of Arr, cArr, T α , and T $\beta\gamma$ was examined using immunoblotting and immunocytochemistry in dark- and light-adapted single knockout mice lacking G-protein coupled receptor kinase 1 (*Grk1*^{-/-}) and double knockout mice lacking GRK1 and transducin α subunit (*Grk1*^{-/-}/*Gnat1*^{-/-}), or lacking GRK1 and arrestin (*Grk1*^{-/-}/*Arr*^{-/-}).

Results: Arr redistributed in the light to the outer segments in *Grk1*^{-/-} mice as well as in *Grk1*^{-/-}/*Gnat1*^{-/-} double knockout retinas. Immunoblotting revealed that approximately 25-50% of Arr associated with the membrane in light-adapted wild-type, *Grk1*^{-/-} and *Gnat1*^{-/-}/*Grk1*^{-/-} mouse retinas. In contrast, cArr did not stably associate with light-adapted membranes in either wild-type or *Grk1*^{-/-} retinas under our experimental conditions, but redistributed to the cone outer segments in a light-dependent manner. The redistribution of transducin subunits to the inner segments in light occurred in both wild-type and *Grk1*^{-/-}/*Arr*^{-/-} double knockout photoreceptors. However, T $\beta\gamma$ subunits did not redistribute in the absence of T α , suggesting that transducin only translocates as an intact heterotrimer.

Conclusions: We conclude that in rods, Arr redistribution requires neither rhodopsin phosphorylation nor phototransduction, suggesting the presence of another light-dependent pathway to trigger translocation. In cones, the light-dependent movement of cArr appears to be independent of stable association with the cone pigments. The light-dependent translocations of Arr and transducin subunits in opposite directions appear to be based on independent mechanisms.

Retinal photoreceptors are highly differentiated sensory neurons where conversion of light energy into neuronal signals takes place through a mechanism called phototransduction [1-3]. In rod photoreceptors, the absorption of photons elicits a response culminating in rapid hydrolysis of cGMP, the second messenger of phototransduction, and hyperpolarization of the cell. Rod photoreceptors are able to retain light responsiveness at different levels of illumination, a process known as light adaptation that requires feedback mechanisms to control the sensitivity and gain of the phototransduction cascade. Most of these mechanisms operate on a fast timescale using Ca²⁺ as an internal messenger [4,5]. Another slower mechanism of light adaptation (within minutes) involves the light dependent redistribution of distinct phototransduction proteins such as arrestin and transducin in and out of the outer segment where phototransduction occurs. This phenomenon was initially reported by several groups in the late 80's [6-11], but only recently unambiguously confirmed in both vertebrate and invertebrate photoreceptors [12,13]. It was shown that such

movements enable the photoreceptor to modulate its signaling capacities by changing the effective concentrations of these proteins in the outer segment. The mechanism for this light-dependent redistribution is unclear, although the involvement of cytoskeleton [14] and lipid rafts [15] has been postulated.

In this report we use mice lacking proteins involved in phototransduction or inactivation of the visual pigments [16-19] to study the light-dependent redistribution of rod transducin subunits and visual arrestins. The mutant mice used are either single knockouts lacking expression of GRK1 [19], or double knockouts lacking GRK1/Arr [18] or GRK1/ T α expression [17]. We show that the redistribution of rod arrestin and transducin does not depend on one another and that phosphorylation of the receptor is not necessary for either rod or cone arrestin translocation. Furthermore, rod arrestin translocates in the absence of a functional phototransduction cascade. We also show that the T $\beta\gamma$ subunits of rod transducin do not translocate in the absence of T α , suggesting that T α is necessary for the transport of T $\beta\gamma$ to photoreceptor outer segments. Our data predict the existence of unidentified light-dependent pathways in retinal photoreceptors that couple the absorption of photons to the redistribution of visual arrestins and rod transducin.

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METHODS

Animals: All experimental procedures involving the use of laboratory mice complied with NIH guidelines as approved by the Institutional Animal Care and Use Committee of the University of Utah. All knockout mice used were in a mixed background of C57BL/6 and 129SvEv, except for *Grk1*^{-/-}/*Gnat1*^{-/-} mice which were in a mixed C57BL/6, 129SvEv, and BALB/c background. These mice were raised in the dark to preserve their retinal photoreceptors. The wild type mice, in a mixed background of C57BL/6 and 129SvEv, were raised in 12/12 light-dark cycles. Mice in the age of 1-3 months were used. To dark adapt, the control mice were placed in the dark for more than 12 h. Approximately 20 min prior to light exposure, the pupils were dilated by topical treatment of 1% tropicamide (Bausch & Lomb, Tampa, FL) and 2.5% phenylephrine (Alcon, Ft. Worth, TX). The mice were then exposed to about 2500 lux fluorescent white light for 30 min at room temperature with unrestricted access to food and water. The mice were euthanized by CO₂ inhalation. All dark procedures were performed under infrared illumination.

Protein quantification and immunoblot analysis: Retinas derived from dark- and light-adapted mice were homogenized in eppendorf tubes using disposable pestles (Kontes, Vineland, NJ) followed by sonication in RIPA buffer (150 mM NaCl, 1% Nonidet P-40 (NP40), 0.5% deoxycholate, 0.1% SDS, 50 mM Tris-HCl pH 7.2). The protein levels in the resulting retinal extracts were determined by BioRad Protein Assay dye using bovine serum albumin (BSA) as standards. Precisely 70 µg of protein was subject to 10% SDS-PAGE followed by immunoblot analysis. To measure the relative amount of Arr and cArr bound to the membrane, retinas derived from dark- and light-adapted mice were homogenized in 100 µl/retina Buffer A (phosphate buffered saline (PBS) containing 5 mM ATP, 1 mM DTT and protease inhibitors (Roche Applied Science, Mannheim, Germany)) either as described above or by 30 s homogenization in eppendorf tubes with disposable pestles on ice. The resulting protein extracts were subject to ultracentrifugation either at 250,000x g at 25 °C for 10 min or at 20,000x g at 4 °C for 10 min. The pellets were resuspended in 100 µl/retina Buffer A. Approximately one twentieth of the supernatant and resuspended pellet derived from two retinas were applied onto 10% SDS-PAGE gels [20], and the relative amounts of Arr and cArr in soluble and membrane fractions were determined by immunoblotting using C10C10 (anti-Arr [21], used at 1:5,000 dilution) and LUMIJ (anti-cArr [22], used at 1:5,000 dilution) antibodies, followed by horseradish peroxidase (HRP) conjugated donkey anti-rabbit (for LUMIJ, used at 1:2000; Amersham, Piscataway, NJ) and HRP conjugated goat anti-mouse (for C10C10, used at 1:5000, Santa Cruz Biotechnology, Santa Cruz, CA) secondary antibodies. The Western Lightning Chemiluminescence Reagent Plus Kit (PerkinElmer Life Sciences, Boston, MA) was used to detect the signals which were then digitized and quantified using Kodak 1D image analysis program on a Kodak IS440 imaging station (PerkinElmer Life Sciences).

Immunocytochemistry: Mouse eyeballs were enucleated and fixed for 2 h in 4% paraformaldehyde in PBS at room temperature. After fixation, the cornea and the lens were removed and the eyecups were immersed in 10% sucrose prepared in PBS for 1 h, 20% sucrose in PBS for 1 h, and 30% sucrose in PBS for overnight at 4 °C. The eyecups were embedded in TBS (Triangle Biomedical Sciences, Durham, NC) and 14 µm frozen sections were cut and mounted onto Superfrost/Plus slides (Fisher Scientific, Pittsburgh, PA). The sections were blocked with PBS containing 1% BSA, 0.1% Triton X-100 and 10% goat serum, and probed with various primary antibodies, including A9C6 (anti-Arr [21], 1:400 dilution), LUMIJ (anti-cArr [22], 1:250), Tα1A (anti-Tα [16], 1:500), BN1 (anti-Tβ [23], 1:800) and GN2 (anti-Tγ [24], 1:1,200), respectively. Rhodamine-conjugated goat-anti-rabbit (for LUMIJ, Tα1A, BN1 and GN2) and goat-anti-mouse (for A9C6) secondary antibodies (Southern Biotechnology Associates, Birmingham, AL) were used (1:2,000 dilution) to visualize the signals under a Nikon Eclipse E600 fluorescent microscope.

RESULTS

Light-dependent membrane association of rod arrestin: In mouse retinas, G protein coupled receptor kinase 1 (GRK1) is required for the deactivation of light activated visual pigments in rods and cones [19,25,26]. In rods, the phosphorylation of activated rhodopsin (R*) by GRK1 facilitates its interaction with rod Arr, which prevents further interaction of R* with transducin, a key event in turning off the cascade [27,28]. Stable and light-dependent binding of Arr to phosphorylated pigment and hence the reduction of soluble Arr in the outer segment may create a gradient that triggers redistribution of Arr from the inner segment to the outer segment. We tested the light-dependent binding of Arr in *Grk1*^{-/-} photoreceptors in which rhodopsin cannot be phosphorylated, and in *Grk1*^{-/-}/*Gnat1*^{-/-} photoreceptors lacking both phosphorylation and a functional phototransduction cascade. The expression level of Arr in wild-type and mutant retinas was not affected by either the inactivation of the *Grk1* gene alone, or by the inactivation of both the *Grk1* and the *Gnat1* genes (Figure 1A). In the dark-adapted state, regardless of the genetic background, Arr was nearly completely soluble (>95%, Figure 1B, upper panel). In light-adapted retinas, in contrast, the amount of soluble Arr appeared to depend on the genetic background (Figure 1B, lower panel). Approximately 53% of Arr in wild-type, 74% in *Grk1*^{-/-}, and 57% in *Grk1*^{-/-}/*Gnat1*^{-/-} mouse photoreceptors remained soluble (Figure 1B, bargraph). These results indicate that the deletion of the *Grk1* gene reduces the binding of Arr to rhodopsin in light-adapted retina, but does not completely eliminate the binding. The more efficient binding of Arr to the *Grk1*^{-/-}/*Gnat1*^{-/-} membrane, as compared with *Grk1*^{-/-} retina, may result from the lack of competition from Tα to bind to unphosphorylated R* in the *Grk1*^{-/-}/*Gnat1*^{-/-} double knockout retina.

Light-dependent redistribution of Arr in mouse *Grk1*^{-/-} and *Grk1*^{-/-}/*Gnat1*^{-/-} photoreceptors: It was shown that Arr and transducin translocated within photoreceptors upon illumina-

tion in opposite directions [7,14,29]. To examine whether the reciprocal redistribution of Arr and transducin is coupled and the relationship between rhodopsin phosphorylation and Arr redistribution, we compared the redistribution of Arr in wild-type mice with that in *Grk1*^{-/-} and *Grk1*^{-/-}/*Gnat1*^{-/-} mice (Figure

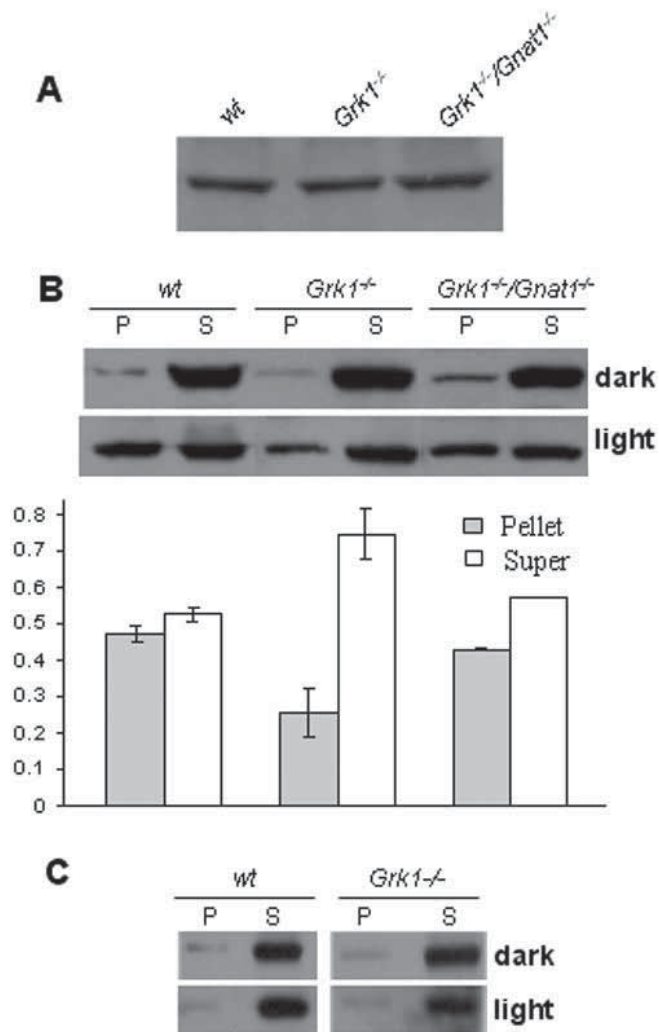


Figure 1. Light-dependent membrane association of rod/cone arrestins in dark- and light-adapted retinas from various genetic backgrounds. **A:** Comparable levels of rod arrestin are present in wild type (wt), GRK1 knockout (*Grk1*^{-/-}), and GRK1/Gnat1 (*Grk1*^{-/-}/*Gnat1*^{-/-}) double knockout retinas. **B:** Relative levels of rod arrestin in pellet (P) and in soluble (S) fractions in dark-adapted (upper panel) and light-adapted (lower panel) retinas derived from the indicated genetic backgrounds. One representative set of immunoblots are shown. The amount of arrestin in light-adapted retinas was quantified as described in Methods and shown (bargraph, n=3, error bars represent the standard error of the mean). **C:** Immunoblots showing the relative amount of cone arrestin in pellet and in soluble fractions from indicated genetic backgrounds. Shown here is one representative experiment from five separate trials. Only small amounts of cone arrestin are present in membrane fractions (P), nearly all cone arrestin is present in the supernatant (S), independent of dark- and light-adaptation or genetic backgrounds.

2). We found that in dark-adapted wild type, *Grk1*^{-/-} and *Grk1*^{-/-}/*Gnat1*^{-/-} retinas, the majority of Arr was localized to rod inner segments (RIS) where biosynthesis takes place (a small fraction of Arr was localized to synaptic terminals). In the light-adapted retinas, the majority of Arr redistributed to rod outer segments (ROS). These results show that the light-dependent redistribution of Arr does not require the phosphorylation of light-activated rhodopsin. The weaker association of Arr with membranes in *Grk1*^{-/-} retina appeared to have little effect on the light-dependent redistribution of arrestin. Since T α is an essential component of the phototransduction cascade, the light-dependent redistribution of arrestin in *Grk1*^{-/-}/*Gnat1*^{-/-} retina indicates that downstream signaling events in phototransduction are not required for Arr redistribution.

Light-dependent movement of cone arrestin in Grk1^{-/-} mice: It was previously reported that bovine [30] or salamander cArr [31] have low binding affinities compared to rod Arr for light-activated phosphorylated and unphosphorylated rhodopsin. It was also shown that cArr did not bind to heparin [30] as rod Arr does, although rod and cone arrestin amino acid sequences are highly conserved suggesting a closely related structure. The light-dependent redistribution of cArr in mouse and

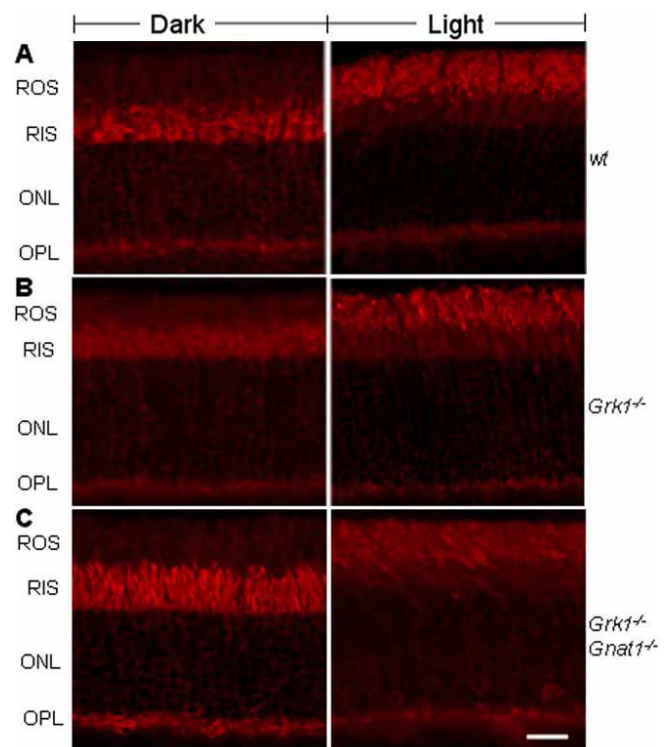


Figure 2. The light-dependent distribution of rod arrestin in dark- and light-adapted retinas. Independent of the genetic background, rod arrestin is predominantly localized in the rod inner segments (RIS) and outer plexiform layer (OPL) in the dark. In the light, however, the majority of rod arrestin is localized to rod outer segments (ROS). **A:** Wild type (wt) retinas. **B:** GRK1 knockout (*Grk1*^{-/-}) retinas. **C:** GRK1/Gnat1 double knockout (*Grk1*^{-/-}/*Gnat1*^{-/-}) mice. ONL, outer nuclear layer. Bar represents 40 μ m.

bovine cones has been observed [22,32] suggesting that cArr may quench the cone phototransduction cascade similarly as Arr does in rods. Because GRK1 is expressed in both rod and cone photoreceptors in mouse [25], and it has recently been shown that GRK1 is required for light-dependent phosphorylation of mouse cone visual pigments [33], we examined the light-dependent membrane association and photoreceptor redistribution of cArr in *Grk1*^{-/-} mice. We found that the majority of cArr remained in the soluble fraction in both wild-type and *Grk1*^{-/-} retinas independent of light history under our experimental conditions (Figure 1C), but the light-dependent redistribution of cArr was evident and indistinguishable between wild type and *Grk1*^{-/-} retinas (Figure 3). These results suggest that, similar to what we observed for Arr, neither stable membrane association of cArr nor phosphorylation of light-activated cone opsin by GRK1 is required for light-dependent cArr redistribution.

Light-dependent movement of transducin in the absence of arrestin and GRK1: In addition to arrestin, light also triggers the translocation of transducin subunits from the outer segment in the dark, to the inner segment in the light (Figure 4, top panels). Transducin, the heterotrimeric G protein of the rod phototransduction cascade, is activated by interacting with R*. The phosphorylation of R* weakens the interaction between transducin and rhodopsin, and the binding of Arr to P-R* prevents further interaction between rhodopsin and transducin. To investigate how phosphorylation of R* and arrestin binding to P-R* affect the movement of T α , we examined light-dependent redistribution of T α in *Grk1*^{-/-}/*Arr*^{-/-} retinas. As shown in Figure 4, the majority of T α resides in

ROS in dark-adapted retina, whereas in the light-adapted state T α is found throughout the photoreceptor layer, including the RIS and the synapse, similar to the distribution of T α in wild type mouse retinas. These results suggest that T α redistribution is independent of R* phosphorylation and Arr binding to phosphorylated rhodopsin.

The distribution of T β subunits in *Gnat1*^{-/-} mice: It was previously shown that all three subunits of transducin (T α , T β , T γ), undergo light-dependent redistribution in wild type retina [29]. To test the hypothesis that these three subunits translocate as a heterotrimeric complex T α β γ , we examined the light-dependent distribution of T β subunits in *Grk1*^{-/-}/*Gnat1*^{-/-} and *Grk1*^{-/-}/*Arr*^{-/-} double knockout mice (Figure 5 and Figure 6). We stained the dark- and light-adapted double knockout retinas with anti-T β and T γ antibodies, respectively (Figure 5 and Figure 6). The results demonstrate that T β subunits reside throughout the rod photoreceptors of *Grk1*^{-/-}/*Gnat1*^{-/-} mice, independent of light history, while the distribution T β subunits in *Grk1*^{-/-}/*Arr*^{-/-} resembles closely that of wild-type retina. These results show that the translocation of T β from inner segments to outer segments requires T α , and suggest that transducin translocates as an intact heterotrimer.

DISCUSSION

The purpose of this study was to provide further insight into the mechanisms of translocation of components of the phototransduction cascade using an arsenal of single and double knockout mice. The light-dependent movement of Arr from inner segment to outer segment was observed nearly two decades ago [8], and that of T α and T β from outer segments

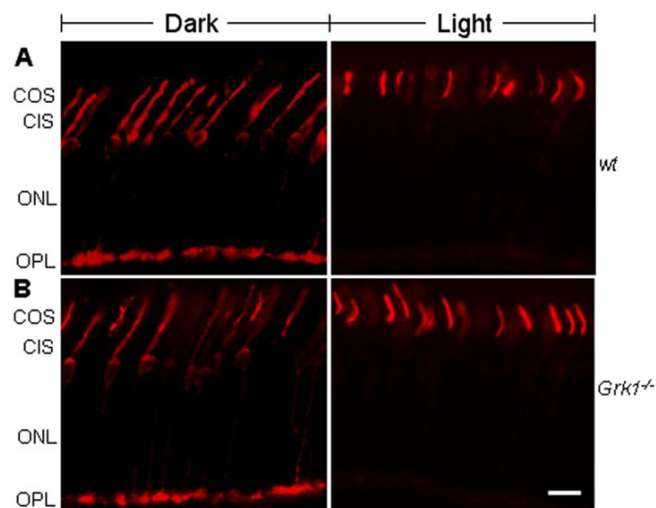


Figure 3. The distribution of cone arrestin in retinas. In dark-adapted (left panels) states, cone arrestin is present in cone outer segments (COS), cone inner segments (CIS), and the outer plexiform layer (OPL) of wild type and *Grk1*^{-/-} retinas. In the light-adapted (right panels) states, cone arrestin is predominantly in COS. Note that cone arrestin is undetectable in the OPL region (contrast to rod arrestin distribution in Figure 1). A: Wild type (wt) mice. B: *Grk1*^{-/-} mice. ONL, outer nuclear layer. Bar represents 30 μ m.

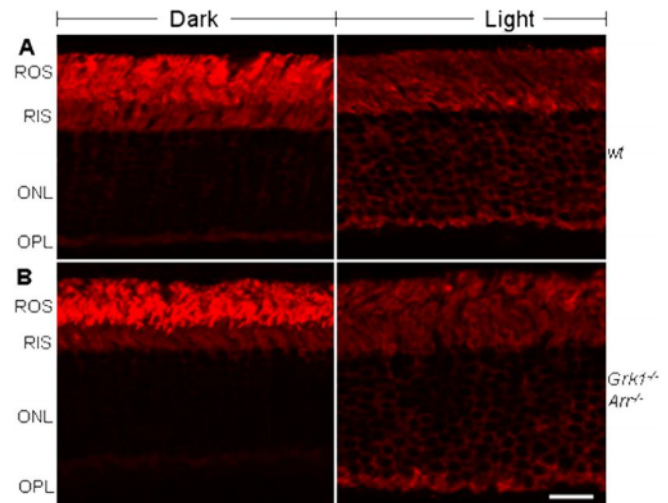


Figure 4. Light-dependent distribution of transducin α subunit (T α). In dark-adapted state (left panels), the majority of T α is distributed within rod outer segments (ROS), while in the light (right panels), a significant amount of T α is localized to rod inner segments (RIS) and outer plexiform layer (OPL). The distribution of T α in *Grk1*^{-/-}/*Arr*^{-/-} mice is indistinguishable from that of the wild type mice. A: Wild type (wt) mice. B: *Grk1*^{-/-}/*Arr*^{-/-} mice. ONL, outer nuclear layer. Bar represents 40 μ m.

to inner segments was reported shortly after [6,7]. However, the movement particularly of transducin was challenged as an artifact of epitope masking [9], a problem that hampered the progress toward understanding the movement mechanism for more than a decade. Immunoblotting of tangentially sectioned photoreceptor layers, however, proved that the light-dependent translocation of transducin indeed occurs on a time scale of minutes [12].

In the dark-adapted wild-type mouse, the majority of Arr is distributed in RIS and synaptic terminals, only small amounts of Arr are present in the ROS. Under this condition we found that >95% of Arr stays in the soluble fraction (Figure 1). Upon illumination, the majority of Arr redistributed to ROS and about 47% of Arr associated with membranes. Arr binds tightly with activated and phosphorylated rhodopsin, a process necessary for the shutoff of rod phototransduction and timely recovery of the dark-adapted state [28]. Mechanisms involved in triggering the translocation and the transport itself are unclear. It is conceivable that Arr binding to phosphorylated rhodopsin

draws the soluble Arr from the inner segment to the outer segments. Therefore, the observed translocation may simply be the result of mass action and diffusion of Arr, an event secondary to the high affinity binding of Arr to rhodopsin after photobleaching. However, in *Grk1*^{-/-} mice where light-dependent rhodopsin phosphorylation is abolished [19], we found that the membrane affinity of Arr was significantly reduced (from about 47% to 25%), but the light-dependent translocation of Arr still occurred. Because the translocation of Arr can be uncoupled from its membrane association, another light dependent mechanism that triggers massive redistribution must be present in photoreceptors. This uncoupling of translocation and membrane association is even more pronounced for cArr. GRK1 was shown to be the only GRK in mouse cones that participates in the deactivation of cone phototransduction [25,26]. Under our experimental conditions in which light-dependent membrane association of Arr can be readily observed (Figure 1B), the light-dependent membrane associa-

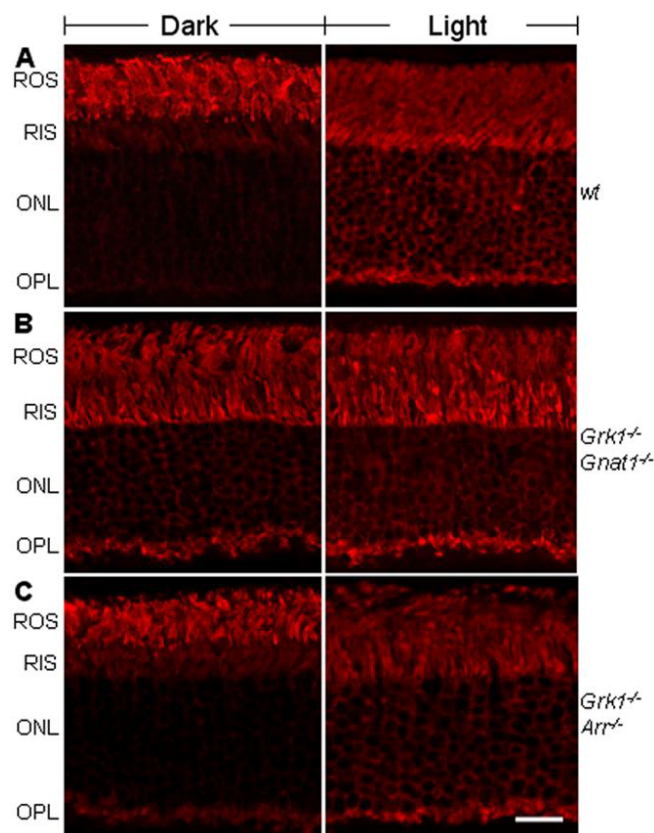


Figure 5. Light-dependent distribution of transducin β subunit ($T\beta$). In the dark (left panels), $T\beta$ is mainly distributed within the rod outer segments (ROS) in wild type and *Grk1*^{-/-}/*Arr*^{-/-} retinas. In the light (right panels), it is present throughout the entire photoreceptor layer. In *Grk1*^{-/-}/*Gnat1*^{-/-} double knockout retinas (middle panels), $T\beta$ appears equally distributed throughout the photoreceptors independent of light and dark. **A:** Wild type (wt) retinas. **B:** *Grk1*^{-/-}/*Gnat1*^{-/-} retinas. **C:** *Grk1*^{-/-}/*Arr*^{-/-} retinas. RIS, rod inner segments; ONL, outer nuclear layer; OPL, outer plexiform layer. Bar represents 40 μ m.

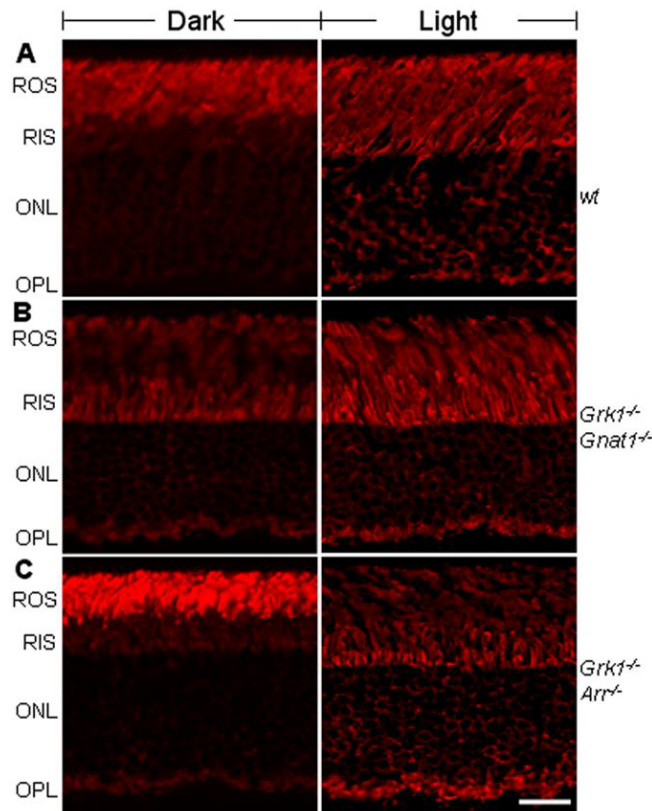


Figure 6. Light-dependent distribution of transducin γ subunit ($T\gamma$). In the dark (left panels), $T\gamma$ is mainly distributed within the rod outer segments (ROS) in wild type and *Grk1*^{-/-}/*Arr*^{-/-} retinas. In the light (right panels), it is present throughout the entire photoreceptor layer. In *Grk1*^{-/-}/*Gnat1*^{-/-} double knockout retinas (middle panels), $T\gamma$ appears equally distributed throughout the photoreceptors independent of light and dark. The distribution of $T\gamma$ is hence similar to that of $T\beta$ shown in Figure 5, consistent with the observation that $T\beta\gamma$ forms a tight heterodimeric complex. **A:** Wild type (wt) retinas. **B:** *Grk1*^{-/-}/*Gnat1*^{-/-} retinas. **C:** *Grk1*^{-/-}/*Arr*^{-/-} retinas. RIS, rod inner segments; ONL, outer nuclear layer; OPL, outer plexiform layer. Bar represents 40 μ m.

tion of cArr was not evident (Figure 1C). It is worth noting, however, under different experimental conditions using hypotonic buffer (50 mM sodium phosphate buffer, pH 6.8) and milder homogenization techniques [33] the light-dependent membrane association of cArr can be detected in wild-type mice. Under those conditions, however, the light-dependent association of cArr was not detectable in *Grk1*^{-/-} mice. Because the light-induced redistribution of cArr to cone outer segments occurs (Figure 3) in the absence of detectable light-dependent membrane association of cArr, these two light-dependent events in cone photoreceptors clearly occur independently of each other.

The light-dependent redistribution of transducin in a direction opposite to arrestins appeared normal in *Grk1*^{-/-}/*Arr*^{-/-} double knockout mice, suggesting that neither receptor phosphorylation nor arrestin binding is necessary for transducin translocation, and that the redistribution of arrestin and transducin is not coupled. This hypothesis is further supported by the observation that the light-dependent redistribution of Arr is normal in *Gnat1*^{-/-}/*Grk1*^{-/-} mice. Because transducin is required for phototransduction (there is no detectable rod-derived electroretinographic response in *Gnat1*^{-/-} mice [34]), the redistribution of Arr in *Gnat1*^{-/-}/*Grk1*^{-/-} mice hence indicates that neither receptor phosphorylation nor phototransduction is required for Arr translocation.

The lack of T β subunits accumulation in the dark-adapted *GRK1*^{-/-}/*Gnat1*^{-/-} ROS contrasts sharply to the normal light-dependent redistribution of arrestins. This not only supports the model that the movements of arrestin and transducin are not coupled, it also suggests that the redistribution may involve different mechanisms. This conclusion is consistent with an accumulation of arrestin and opsin, but not transducin, in the inner segments of photoreceptor specific *KIF3A*^{-/-} mice [35] and a recent finding that T β , but not arrestin, selectively interacts with a Ca²⁺-binding protein called centrin which is localized in the lumen of the connecting cilium [36]. The interaction between centrin and T β requires Ca²⁺-binding to centrin, an event that has been postulated to play a gate-keeping role in retaining T $\alpha\beta$ in the ROS in dark adapted state when intracellular Ca²⁺ concentration in the ROS is high. It is conceivable that in the absence of T α , the lack of phototransduction and absence of regulation of cGMP levels by light leaves a larger portion of cGMP-gated channels open and the intracellular Ca²⁺ remains high to sustain the interaction between centrin and T β and prevents the trafficking of T β through the lumen of the connecting cilium.

It is worth noting that we can not rule out the existence of extracellular mechanisms that regulate the movement of photoreceptor proteins. Such mechanisms play an important role in retinomotor movements in some vertebrates such as fish and amphibians [37]. Experiments using dimmer illumination are currently in progress to evaluate (1) the contribution of cone photoreceptors in the movement of Arr and transducin in rod and (2) the contribution of rod photoreceptors in the movement of cArr in cones (as the data reported here were obtained under a relatively intense illumination condition where both rod and cone photoreceptors were activated). Nonetheless, our

data demonstrate qualitatively that in rod photoreceptors the light-dependent redistribution of arrestin is independent of rhodopsin phosphorylation and phototransduction, and that the redistributions of transducin and Arr are not intracellularly coupled. We also show that the translocation of T β requires the presence of T α . In addition, we show that in cone photoreceptors cArr translocates in the absence of stable membrane association in both wild type and *GRK1*^{-/-} mice. Although the exact mechanisms are unclear, these results predict the existence of novel and unidentified light-dependent signaling pathways in the retina or within the photoreceptors that govern the observed light-dependent movement of these photoreceptor proteins.

Note: While this paper was under review, Mendez et al. [38] published data similar to some of the data presented here indicating that the movement of rod arrestin is independent of rhodopsin phosphorylation and phototransduction. The Mendez data set was collected by independent investigators concurrently working on the same problem.

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REFERENCES

1. Burns ME, Baylor DA. Activation, deactivation, and adaptation in vertebrate photoreceptor cells. *Annu Rev Neurosci* 2001; 24:779-805.
2. Arshavsky VY, Lamb TD, Pugh EN Jr. G proteins and phototransduction. *Annu Rev Physiol* 2002; 64:153-87.
3. Hardie RC, Raghu P. Visual transduction in *Drosophila*. *Nature* 2001; 413:186-93.
4. Krizaj D, Copenhagen DR. Calcium regulation in photoreceptors. *Front Biosci* 2002; 7:d2023-44.
5. Baehr W, Palczewski K, editors. Photoreceptors and calcium. Advances in experimental medicine and biology. Vol 514. New York: Kluwer Academic/Plenum Publishers; 2002.
6. Philp NJ, Chang W, Long K. Light-stimulated protein movement in rod photoreceptor cells of the rat retina. *FEBS Lett* 1987; 225:127-32.
7. Whelan JP, McGinnis JF. Light-dependent subcellular movement of photoreceptor proteins. *J Neurosci Res* 1988; 20:263-70.

8. Broekhuysen RM, Tolhuizen EF, Janssen AP, Winkens HJ. Light induced shift and binding of S-antigen in retinal rods. *Curr Eye Res* 1985; 4:613-8.
9. Roof DJ, Heth CA. Expression of transducin in retinal rod photoreceptor outer segments. *Science* 1988; 241:845-7.
10. Mangini NJ, Pepperberg DR. Immunolocalization of 48K in rod photoreceptors. Light and ATP increase OS labeling. *Invest Ophthalmol Vis Sci* 1988; 29:1221-34.
11. Brann MR, Cohen LV. Diurnal expression of transducin mRNA and translocation of transducin in rods of rat retina. *Science* 1987; 235:585-7.
12. Sokolov M, Lyubarsky AL, Strissel KJ, Savchenko AB, Govardovskii VI, Pugh EN Jr, Arshavsky VY. Massive light-driven translocation of transducin between the two major compartments of rod cells: a novel mechanism of light adaptation. *Neuron* 2002; 34:95-106.
13. Bahner M, Frechter S, Da Silva N, Minke B, Paulsen R, Huber A. Light-regulated subcellular translocation of Drosophila TRPL channels induces long-term adaptation and modifies the light-induced current. *Neuron* 2002; 34:83-93.
14. McGinnis JF, Matsumoto B, Whelan JP, Cao W. Cytoskeleton participation in subcellular trafficking of signal transduction proteins in rod photoreceptor cells. *J Neurosci Res* 2002; 67:290-7.
15. Nair KS, Balasubramanian N, Slepak VZ. Signal-dependent translocation of transducin, RGS9-1-Gbeta5L complex, and arrestin to detergent-resistant membrane rafts in photoreceptors. *Curr Biol* 2002; 12:421-5.
16. Calvert PD, Krasnoperova NV, Lyubarsky AL, Isayama T, Nicolo M, Kosaras B, Wong G, Gannon KS, Margolskee RF, Sidman RL, Pugh EN Jr, Makino CL, Lem J. Phototransduction in transgenic mice after targeted deletion of the rod transducin alpha-subunit. *Proc Natl Acad Sci U S A* 2000; 97:13913-8.
17. Hao W, Wenzel A, Obin MS, Chen CK, Brill E, Krasnoperova NV, Eversole-Cire P, Kleyner Y, Taylor A, Simon MI, Grimm C, Reme CE, Lem J. Evidence for two apoptotic pathways in light-induced retinal degeneration. *Nat Genet* 2002; 32:254-60.
18. Choi S, Hao W, Chen CK, Simon MI. Gene expression profiles of light-induced apoptosis in arrestin/rhodopsin kinase-deficient mouse retinas. *Proc Natl Acad Sci U S A* 2001; 98:13096-101.
19. Chen CK, Burns ME, Spencer M, Niemi GA, Chen J, Hurley JB, Baylor DA, Simon MI. Abnormal photoresponses and light-induced apoptosis in rods lacking rhodopsin kinase. *Proc Natl Acad Sci U S A* 1999; 96:3718-22.
20. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; 227:680-5.
21. Knospe V, Donoso LA, Banga JP, Yue S, Kasp E, Gregerson DS. Epitope mapping of bovine retinal S-antigen with monoclonal antibodies. *Curr Eye Res* 1988; 7:1137-47.
22. Zhu X, Li A, Brown B, Weiss ER, Osawa S, Craft CM. Mouse cone arrestin expression pattern: light induced translocation in cone photoreceptors. *Mol Vis* 2002; 8:462-71.
23. Amatruda TT 3rd, Gautam N, Fong HK, Northup JK, Simon MI. The 35- and 36-kDa beta subunits of GTP-binding regulatory proteins are products of separate genes. *J Biol Chem* 1988; 263:5008-11.
24. Gautam N, Northup J, Tamir H, Simon MI. G protein diversity is increased by associations with a variety of gamma subunits. *Proc Natl Acad Sci U S A* 1990; 87:7973-7.
25. Lyubarsky AL, Chen C, Simon MI, Pugh EN Jr. Mice lacking G-protein receptor kinase 1 have profoundly slowed recovery of cone-driven retinal responses. *J Neurosci* 2000; 20:2209-17.
26. Chen CK, Zhang K, Church-Kopish J, Huang W, Zhang H, Chen YJ, Frederick JM, Baehr W. Characterization of human GRK7 as a potential cone opsin kinase. *Mol Vis* 2001; 7:305-13.
27. Wilden U, Hall SW, Kuhn H. Phosphodiesterase activation by photoexcited rhodopsin is quenched when rhodopsin is phosphorylated and binds the intrinsic 48-kDa protein of rod outer segments. *Proc Natl Acad Sci U S A* 1986; 83:1174-8.
28. Xu J, Dodd RL, Makino CL, Simon MI, Baylor DA, Chen J. Prolonged photoresponses in transgenic mouse rods lacking arrestin. *Nature* 1997; 389:505-9.
29. McGinnis JF, Whelan JP, Donoso LA. Transient, cyclic changes in mouse visual cell gene products during the light-dark cycle. *J Neurosci Res* 1992; 31:584-90.
30. Maeda T, Ohguro H, Sohma H, Kuroki Y, Wada H, Okisaka S, Murakami A. Purification and characterization of bovine cone arrestin (cArr). *FEBS Lett* 2000; 470:336-40.
31. Smith WC, Gurevich EV, Dugger DR, Vishnivetskiy SA, Shelamer CL, McDowell JH, Gurevich VV. Cloning and functional characterization of salamander rod and cone arrestins. *Invest Ophthalmol Vis Sci* 2000; 41:2445-55.
32. Zhang H, Cuenca N, Ivanova T, Church-Kopish J, Frederick JM, MacLeish PR, Baehr W. Identification and light-dependent translocation of a cone-specific antigen (cone arrestin) recognized by monoclonal antibody 7G6. *Invest Ophthalmol Vis Sci* 2003; 44:2858-67.
33. Zhu X, Brown B, Li A, Mears AJ, Swaroop A, Craft CM. GRK1-dependent phosphorylation of S and M opsins and their binding to cone arrestin during cone phototransduction in the mouse retina. *J Neurosci*. In press 2003.
34. Lyubarsky AL, Lem J, Chen J, Falsini B, Iannaccone A, Pugh EN Jr. Functionally rodless mice: transgenic models for the investigation of cone function in retinal disease and therapy. *Vision Res* 2002; 42:401-15.
35. Marszalek JR, Liu X, Roberts EA, Chui D, Marth JD, Williams DS, Goldstein LS. Genetic evidence for selective transport of opsin and arrestin by kinesin-II in mammalian photoreceptors. *Cell* 2000; 102:175-87.
36. Pulvermuller A, Giessl A, Heck M, Wottrich R, Schmitt A, Ernst OP, Choe HW, Hofmann KP, Wolfgram U. Calcium-dependent assembly of centrin-G-protein complex in photoreceptor cells. *Mol Cell Biol* 2002; 22:2194-203.
37. Burnside B. Light and circadian regulation of retinomotor movement. *Prog Brain Res* 2001; 131:477-85.
38. Mendez A, Lem J, Simon M, Chen J. Light-dependent translocation of arrestin in the absence of rhodopsin phosphorylation and transducin signaling. *J Neurosci* 2003; 23:3124-9.

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