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Light-Dependent Compartmentalization of Transducin in Rod Photoreceptors

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Abstract Three major visual signaling proteins, transducin, arrestin, and recoverin undergo bidirectional translocations between the outer segment and inner compartments of rod photoreceptors in a light-dependent manner. The light-dependent translocation of proteins is believed to contribute to adaptation and neuroprotection of photoreceptor cells. The potential physiological significance and mechanisms of light-controlled protein translocations are at the center of current discussion. In this paper, I outline the latest advances in understanding the mechanisms of bidirectional translocation of transducin and determinants of its steady-state distribution in dark- and light-adapted photoreceptor cells.

Keywords Retina · Photoreceptor · Transducin · Arrestin · Light adaptation · Phototransduction

Introduction

Vertebrate rod and cone photoreceptors are highly polarized neurons with specialized ciliary compartments called outer segments (OS) that serve to capture photons and initiate visual signal transduction [1–3]. A majority of key photoexcitation and recovery components, such as the photon receptor rhodopsin (R), the effector enzyme cGMP-phoshodiesterase 6 (PDE6), the cGMP-gated channel/exchanger complex, the GTPase activating protein (GAP) complex RGS9-1/Gβ_5/R9AP, and retinal guanylyl cyclases (GC1 and GC2) are integral or peripheral membrane proteins that reside in the OS regardless of illumination conditions. These phototransduction proteins are constantly turned over through phagocytosis of the OS tips by RPE cells and the addition of the newly synthesized components at the base of the OS [4]. Protein synthesis in photoreceptors takes place in the inner segment (IS), a distinct compartment that contains typical cellular metabolism organelles. A slender connecting cilium joins the IS with the OS. All newly synthesized OS resident proteins must be transported within the IS and across the cilium to reach their destination. In contrast to this unidirectional IS→OS transport, three phototransduction proteins, transducin (Gt), arrestin, and recoverin, have been shown to undergo bidirectional translocations between the two compartments in a light-dependent manner [5–10]. Thus, the polarized distribution of transducin, arrestin, and recoverin is controlled by changes in illumination. In dark-adapted photoreceptors, Gt and arrestin are concentrated in the OS and IS, respectively. Sufficiently, bright light induces extensive and relatively rapid movement of the rod G protein from the OS into the IS and the synaptic terminal, whereas arrestin moves in the reverse direction from the IS into the OS [5–10]. Once in the OS, arrestin quenches activation of transducin by binding to photoexcited phosphorylated rhodopsin [1]. The light-dependent translocation of proteins is thought to contribute to light/dark adaptation and may also protect the photoreceptor cell from light- and phototransduction-dependent retinal degeneration [1, 8, 11]. Potential roles and mechanisms of light-controlled protein translocations are being actively debated and constitute the subject of comprehensive review [12]. Although a quantitative analysis of the translocation time course and light intensity dependence was first described for transducin [8], subsequent reports revealed more

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mechanistic details about the light-dependent movement of arrestin [9, 13]. More recent studies, however, have contributed important insights into the determinants of transducin compartmentalization and the mechanism of its translocation in rods [14–16]. This mini-review focuses on the latest progress in understanding bidirectional translocation of Gt.

Transducin Transport from the IS to the OS

Rod Gt is transported from the IS into the OS following two independent processes: (a) biosynthesis and subunit assembly in the IS and (b) during dark adaptation after its light-dependent translocation from the OS. Although a specific transport mechanism for newly synthesized Gt is largely unknown, it is reasonable to assume that subsequent to synthesis, processing, and assembly, transducin takes the same path as when it returns to the OS in the dark after light exposure. Translocated in light, nearly all transducin returns to the OS on a timescale of several hours [8]. Thus, the transport mechanism for transducin return has more than enough capacity to take along all newly synthesized Gt.

Lipid Modifications and Formation of Heterotrimeric Gt are Required for Its Correct Transport to the OS The membrane targeting and transport of Gt are controlled by its lipid modifications [17–19]. Rod $G\alpha_{t1}$ is heterogeneously fatty acylated at the extreme N-terminal Gly residue with C12:0, C14:0, C14:1, or C14:2 moieties [20]. This heterogeneity contrasts with the modification of other Gi family Gα subunits that are N-terminally acylated exclusively with myristate (C14:0) [17, 18]. Cone $G\alpha_{t2}$ expressed in rods was shown to be N-acylated only with C14:0 [15]. Therefore, the heterogeneous acylation of $G\alpha_{t1}$ is determined not just by availability of alternative substrates for *N*-myristoyl transferase [21] but by the N-terminal sequence of $G\alpha_{t1}$ as well [15]. N-acylation is an irreversible co-translational modification that promotes weak interaction of $G\alpha_{t1}$ with membranes in vitro [21]. Often, a single lipid modification alone is not sufficient to keep a protein attached to a membrane [17, 18, 22–24]. A second lipid anchor for Gt is provided through farnesylation of the $G\gamma_1$-subunit [25, 26]. A thioether-linked isoprenoid farnesyl (C15) is attached to the $G\gamma_1$ Cys residue within the C-terminal "CAAX" box [27]. Most studies are consistent with a model in which Gγ-subunits are isoprenylated by cytosolic prenyl transferases subsequent to the assembly of the Gβγ dimer in the cytoplasm [28]. Gβγ complexes are then targeted to the endoplasmic reticulum (ER) for further processing, which includes removal of the C-terminal tripeptide –AAX and carboxymethylation of the terminal Cys residue [29, 30]. Plentiful data suggest that formation of a heterotrimeric G protein is a prerequisite for proper G-protein targeting to the plasma membrane [27, 31, 32]. Likewise, growing evidence supports the requirement for association of $G\alpha_{t1}$ with $G\beta_1\gamma_1$ for correct transport of transducin to the OS. $G\beta_1\gamma_1$ relies on $G\alpha_{t1}$ for its localization, as it is mislocalized and distributed throughout the photoreceptor cells in dark- or light-adapted $G\alpha_{t1}$ knockout mice (Fig. 1a) [33]. Conversely, transport of $G\alpha_{t1}$ depends on its association with $G\beta_1\gamma_1$. The time course of the transfer to the OS for the GTPase-deficient $G\alpha_{t1}$ Q200L mutant is extremely slow (Fig. 1b) [34]. Apparently, the long time constant for GTP hydrolysis by $G\alpha_{t1}$Q200L exceeds the mutant lifetime in the IS, allowing only a small fraction of $G\alpha_{t1}$Q200L to hydrolyze GTP, bind $G\beta_1\gamma_1$, and escape to the OS before protein degradation. As a result, any significant accumulation of $G\alpha_{t1}$Q200L in the OS requires many days of dark adaptation of transgenic mice [34]. Also, in α-toxin-permeabilized retinas, both GTP and GTPγS allowed transducin to disperse from the OS in the light, but Gt return to the OS in the dark occurred only when GTP was used [15]. Correct targeting of Gt to the OS requires bound GDP, as the $G\alpha_{t1}$ S43C mutant with severely reduced affinity for GDP is mislocalized in transgenic mouse rods (Fig. 1b; B. Barren and N. Artemyev, unpublished). $G\alpha_{t1}$S43C retains the ability to bind $G\beta_1\gamma_1$ in vitro [35], suggesting potential mistargeting of the mutant heterotrimer lacking bound GDP. Lastly, the kinetics of return of $G\alpha_{t1}$ and $G\beta_1\gamma_1$ return to the OS in the dark are identical, indicating that Gt moves in the IS→OS direction as the heterotrimer [8]. Combining the two lipid anchors in a single holo-Gt molecule is critical for its transport. Recent analysis of the N-acylation-deficient mutant, $G\alpha_{t1}$G2A, in transgenic mice demonstrated that $G\alpha_{t1}$G2A is fully capable of interaction with $G\beta_1\gamma_1$ [16]. Yet, dark-adapted rods expressing $G\alpha_{t1}$G2A showed a severe defect in its cellular localization. $G\alpha_{t1}$G2A was found predominantly in the inner compartments of the photoreceptor cells with only 15–20% of the mutant protein present in the OS [16] (Fig. 1b). Thus, the current data on trafficking of Gt and G-proteins in general indicate that the heterotrimer formation is the triggering event for transducin transport to the OS. The two lipid anchors provide a stable interaction of transducin with an intracellular membrane [22]. Photoreceptor ER membrane represents the earliest potential site for the formation of membrane-bound holo-Gt after protein synthesis, as the processing of the prenylated $G\beta_1\gamma_1$ is completed by the ER-associated machinery. An extensive membrane network of the ER may also serve as a docking site for the translocated transducin before its return to the OS.

The IS→OS Direction: Molecular Motors or Diffusion? Two common modes of protein transfer, an energy-

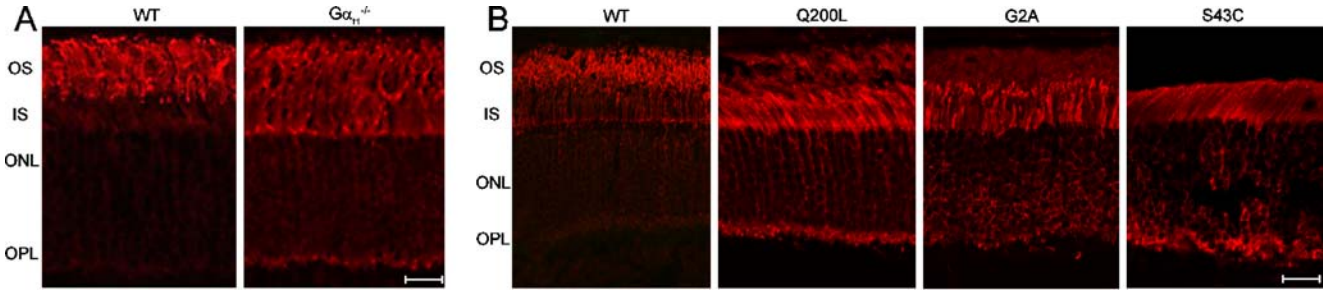

Fig. 1 Mouse models displaying mislocalization of rod transducin subunits in dark-adapted photoreceptors. **A** Immunofluorescence localization of $G\beta_1\gamma_1$ in wild-type (*WT*) and $G\alpha_{t1}$ knockout ($G\alpha_{t1}^{-/-}$) mouse retinas. **B** Immunofluorescence localization of wild-type and mutant $G\alpha_{t1}$ Q200L [34], G2A [16], and S43C subunits in mouse retinas. The mutant $G\alpha_{t1}$ subunits are expressed in the $G\alpha_{t1}^{-/-}$ background. *OS* outer segment layer, *IS* inner segment layer, *ONL* outer nuclear layer, *OPL* outer plexiform layer. Bar, 10 μm

dependent motor-driven mechanism and diffusion, are available for the IS→OS transport of Gt [8]. Conflicting reports have suggested that Gt movement to the OS is an energy-independent diffusion [9], or that it is dependent on microfilaments, and hence, is an active transport mechanism [36, 37]. As a membrane-bound protein, Gt, to diffuse, must become soluble through a protein–protein interaction, sequestering one or both of the lipid anchors. Phosducin is a well-characterized and abundant IS protein that can reduce the affinity of $G\beta_1\gamma_1$ for the membrane by inducing a farnesyl-binding cavity in the β propeller of $G\beta_1\gamma_1$ [38–40]. Modest mislocalization of $G\alpha_{t1}$ and $G\beta_1\gamma_1$ to the IS was observed in dark-adapted phosducin knockout mice [41]. However, this observation does not necessarily reflect an essential role for phosducin in the IS→OS transport because (a) phosducin prevents re-association of $G\alpha_{t1}$ and $G\beta_1\gamma_1$, and (b) expression levels of transducin subunits are reduced in the phosducin knockout model [41, 42]. Phosducin may protect $G\beta_1\gamma_1$ from proteolysis [43] and/or assist in targeting of prenylated $G\beta_1\gamma_1$ to intracellular membranes that serve as a starting point for Gt transport. Phosphorylation of phosducin in the dark allows the release of $G\beta_1\gamma_1$ and formation of heterotrimeric Gt, thereby potentially serving as an early triggering event for transducin transport to the OS [38, 44]. Another known photoreceptor prenyl-binding protein is PrBP/δ. This 17-kDa protein was originally considered the δ-subunit of PDE6 [45]. It interacts with the methylated prenylated C termini of PDE6 catalytic subunits and regulates the enzyme attachment to the membrane [46]. Subsequent studies indicated that PrBP/δ is also expressed outside photoreceptor cells and can interact with a number of prenylated and non-prenylated proteins [47]. In rods, PrBP/δ localizes mainly near the junction of the IS and OS [47]. Recent analysis of the PrBP/δ knockout mice revealed partial mislocalization of PDE6 and prenylated rhodopsin kinase (GRK1) in rods as well as severe reduction of GRK1 and cone PDE6 in the OS of cones [48]. Thus, PrBP/δ facilitates transport of a subset of prenylated proteins in photoreceptors. Interestingly, deletion of PrBP/δ did not affect the distribution of transducin [48]. Consequently, either diffusion of Gt in the IS→OS direction is mediated by an as yet unidentified binding partner or transducin utilizes a vesicle-mediated transport mechanism. A directional vesicle-mediated transport is generally thought to utilize molecular motors, which would seem to conflict with unimpaired transducin IS→OS traffic in ATP-depleted rods [9]. It is conceivable, however, that transducin-carrying vesicles move towards the cilium by diffusion in a cargo-dependent manner. A preexisting activated protein complement required for vesicle formation [49] or preformed pools of vesicles may have allowed the transport of Gt toward the connecting cilium and the OS even under conditions of severe ATP depletion. Yet, several studies indicate an active molecular-motor-mediated mode of Gt transport in the dark. These studies suggest, on the basis of the effects of cytoskeleton-disrupting drugs, that the IS→OS transport of transducin is dependent on microtubules [36] and/or actin filaments [37]. Moreover, an siRNA-induced knock down of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in photoreceptors slowed transducin translocation from the IS to OS during dark adaptation [50]. GAPDH is present in large quantities in rod OS, and some of the energy required for the synthesis of ATP and GTP is likely to be derived from glycolysis in the OS [51]. Therefore, the effect of GAPDH knockdown on translocation of Gt may reflect an energy-dependent mechanism.

Gt Transport Across the Cilium The mechanism of transducin transport through the connecting cilium is unknown. Transducin-carrying vesicles or protein complexes may diffuse across the cilium or fuse at the ciliary base with the plasma membrane. The surface of the ciliary membrane can then be utilized by mechanisms such as intraflagellar transport (IFT) complexes to move Gt to the OS. Diffusion of Gt through the inner lumen of the cilium may be controlled by centrins. A recent review provides a detailed description of protein complexes in photoreceptor cilia, including centrin–transducin complexes and their potential

functions [52]. Three isoforms of the Ca^{2+}-binding protein centrin have been localized in the cilium where they bind Gt via the $G\beta_1\gamma_1$-mediated interaction in a Ca^{2+}-dependent manner [53]. By slowing or blocking transducin movement, centrins potentially "gate" its exchange through the cilium [53]. However, in this rather passive role, centrins cannot directly contribute to the mechanism driving transducin movement across the cilium. IFT is a conserved transport mechanism required for the assembly and maintenance of eukaryotic cilia, including the photoreceptor cilium [54, 55]. The IFT system moves a large protein complex along the outer doublet microtubules in the cilium by utilizing kinesin 2 proteins as anterograde motors and dyneins as retrograde motors [54, 55]. The latest study on IFT components in photoreceptors revealed the special distribution of several IFT proteins within the photoreceptor cells [56]. Endogenous IFT proteins are concentrated in the apical part of the IS around the basal body and along the axoneme in the OS [56]. The IFT complex may mediate transport of rhodopsin through the cilium. Deletion of Kif3A, the kinesin II subunit, and mutation in the IFT protein IFT88/polaris lead to mislocalization of rhodopsin and photoreceptor cell death [55, 57]. Furthermore, rhodopsin and retinal guanylate cyclase 1 (GC1) can be immunoprecipitated together with IFT proteins [54]. Although transport of rod Gt in the Kif3A knockout [55] and GC1/GC2 double knockout mouse models [56] was largely unaffected, deletion of GC1 or both GC1/GC2 markedly impaired transport of cone Gt [58]. This observation suggests that the trafficking pathways for rod and cone transducins might be different, and cone Gt may utilize GC1- and the ITF-complex-dependent transport mechanism.

Once Gt reaches membrane discs at the base of the OS, it continues to move in the dark to populate membrane discs in the apical OS. Understanding the mode of Gt transport within the OS towards the tip of a photoreceptor cell represents a significant unresolved question. Here, an active mechanism would seem to be an attractive alternative, as it is unclear how such Gt movement can be achieved by diffusion.

Determinants of Transducin Distribution in Dark-Adapted Rods In the dark-adapted state, 80–90% of the total photoreceptor Gt concentrates in the OS (Fig. 1b) [8]. What determines this polar distribution of Gt? The two-lipid requirement for Gt localization suggests that membrane affinity is a likely determining factor for the protein accumulation in the OS. Moreover, the extent of light-induced translocation of transducin subunits is influenced by the hydrophobicity of the lipid modifications [14]. In agreement with the role of membrane affinity in Gt localization in the dark, the density of disc membranes in the OS is very high. This steady-state distribution of transducin in the dark may be consistent with either diffusion or active IS→OS transport of Gt. In the case of diffusion, the distribution would probably reflect the relative membrane abundance, and perhaps, the presence of rhodopsin in the OS. Studies using plasmon-waveguide resonance spectroscopy [59] as well as computational analyses [60] suggest that rhodopsin in its ground "dark" state maintains residual affinity for transducin and may enhance Gt binding to disc membranes. Recently, GAPDH was found to interact with transducin [50], but the enzyme plasma membrane localization indicates that it is unlikely to serve as a major binding site for Gt on the OS [51]. Active transport would bring Gt to the OS where it is trapped by binding to disc membranes. A residual fraction of transducin might be retained in the IS by membranes and/or transducin-binding proteins, such as the Leu-Gly-Asn repeat protein LGN [61, 62].

Light-Induced Movement of Transducin: Diffusion Makes Sense

Similar to the IS→OS movement of Gt in the dark, two general mechanisms, diffusion and active transport, may underlie the phenomenon of its light-dependent OS→IS translocation [8]. Both mechanisms have been actively debated, and at present, the diffusion model is supported by prevailing evidence. In rod cells, photoexcited rhodopsin (R*) stimulates GTP–GDP exchange on Gt, leading to dissociation of $G\alpha_{t1}GTP$ from $G\beta_1\gamma_1$ and from the membrane [1–3]. In a diffusion model, the light-induced dissociation of Gt subunits simply allows them to diffuse into the IS and other rod compartments [8]. A number of studies are in agreement with the general diffusion mechanism. $G\alpha_{t1}$ and $G\beta_1\gamma_1$ translocate in response to light with different time courses [8]. In addition, geranylgeranylation of $G\gamma_1$ attenuates translocation of $G\beta_1\gamma_1$, but not $G\alpha_{t1}$, in transgenic mice [63]. Phosducin binds $G\beta_1\gamma_1$ when it is dissociated from $G\alpha_{t1}$ and facilitates translocation of Gt subunits in the light [41]. All this evidence strongly suggests that Gt moves to the IS as dissociated subunits. Translocation of transducin does require photoexcitation of R and does not take place in RPE65 knockout mice in which regeneration of R from opsin is blocked [64]. Importantly, light-induced translocation of Gt occurs in rods that are depleted of ATP, indicating a passive transport mechanism such as diffusion [9, 15].

However, after activation by R*, $G\alpha_{t1}GTP$ rapidly binds and stimulates the effector enzyme cGMP phosphodiesterase (PDE6). The N-acylation of $G\alpha_{t1}$ along with lipid modifications of PDE6 provides for tight tethering of the activated $G\alpha_{t1}$/PDE6 complex to the membrane [65, 66].

The $G\alpha_{t1}$GTP/PDE6 complex is deactivated when bound GTP is hydrolyzed due to intrinsic GTPase activity of $G\alpha_{t1}$ controlled by the membrane-bound GAP complex, RGS9-1/$G\beta_5$/R9AP [1–3]. The GDP-bound $G\alpha_{t1}$ then recombines with $G\beta_1\gamma_1$, and Gt subunits rebind to the membrane as the heterotrimer. Thus, $G\alpha_{t1}$ in the activated complex with PDE6 or inactivated by the GAP complex is not free to diffuse. Two further predictions of the diffusion hypothesis must then be validated. First, there should be a light intensity threshold for Gt translocation such that the steady-state concentration of activated $G\alpha_{t1}$ at minimum exceeds PDE6 concentration. Secondly, this threshold should depend on the presence and concentration of the RGS9 GAP complex. Indeed, quantitative analysis indicated that a notable translocation of $G\alpha_{t1}$ starts only when light intensity reaches a threshold producing rhodopsin photoisomerisations at the initial rate of ~5,000 R* per rod per second [8, 12, 14]. Subsequently, it was demonstrated that compared to wild-type mice, Gt translocation in RGS9 knockout mice occurs at lower levels of illumination [34]. This observation has been recently confirmed in R9AP knockout mice, another model lacking the RGS9 GAP complex proteins [14]. In the absence of RGS9, the lifetime of diffusion-capable $G\alpha_{t1}$GTP increases, but so does the extent of signaling, which could potentially be responsible for Gt translocation. An important extension of the analysis of Gt translocation in mice lacking the GAP complex is the demonstration of a similar threshold shift to lower light intensities in mice expressing the W70A mutant of the Pγ-subunit [14]. This mutation markedly reduces transducin's ability to activate PDE6 as well as blocks the ability of Pγ to potentiate the RGS9 GAP complex in inactivating $G\alpha_{t1}$GTP [67]. Thus, the light threshold is a function of transducin inactivation, not a signaling event downstream of Gt. Furthermore, Lobanova et al. [14] have also shown that the threshold can be shifted toward higher light intensity in mice overexpressing the RGS9 GAP complex. This analysis suggests that the light threshold for transducin translocation is reached when the rate of Gt activation by R* exceeds the capacity of the RGS9 GAP complex to inactivate $G\alpha_{t1}$GTP [14].

In contrast to transducin translocation in rods, cone Gt does not move to the IS in light-adapted cones even in the absence of RGS9 [15, 68–70]. Partial translocation of cone $G\alpha_{t2}$ was observed only with high intensity light [71]. Interestingly, when expressed in transgenic rods, $G\alpha_{t2}$ was able to translocate in a light-dependent fashion [15, 71]. A recent study has addressed this important difference between rod and cone transducins [15]. The cone Gt, comprised of $G\alpha_{t2}$ and $G\beta_3\gamma_8$, was still bound to the membrane as a heterotrimer upon activation with GTPγS. Yet, the activation of cone Gt coupled with the addition of Gα and Gβγ dissociating peptide caused release of $G\alpha_{t2}$ and $G\beta_3\gamma_8$ from the membrane and enabled their translocation in cones [15]. Thus, the study results clearly favor the diffusion model by demonstrating subunit dissociation-dependent and energy-independent translocation of Gt [15].

Although the diffusion model for the OS→IS movement of Gt is supported by the abundance of evidence, few studies point to a potential role of cytoskeletal elements in the process [36, 72]. One study in particular found that perturbation of microtubules slowed the movement of transducin in the OS→IS direction [36]. Nevertheless, another recent study using similar methodology has found that in contrast to the Gt transport during dark adaptation, the light-induced movement of Gt is not critically dependent on cytoskeletal systems [37]. Moreover, the scale and the kinetics of light-driven movement of Gt may well be incompatible with the capacity of molecular motors, as the movement by diffusion appears to meet these criteria [12].

Steady-State Distribution of Transducin in Light-Adapted Rods In the dark, ~85% of Gt is localized in the OS, and saturating light causes translocation of up to 90% of the OS transducin to the inner compartments of rod cells. Thus, less than 10% of the total Gt remains in the light adapted OS, and its distribution is much more polarized than it would have been predicted for diffusing proteins. Quantification of the cytoplasmic spaces of living rods with enhanced green fluorescent proteins confirms the prediction from the rod ultrastructure that rod discs occupy ~50% of the OS volume, i.e., ~50% OS space is not accessible to diffusing proteins [73]. Under the condition that a steady-state distribution of transducin in light-adapted rods is determined by diffusion, at least one third of Gt would have remained in the OS. Alternatively, the diffusion equilibrium can be shifted by Gt binding sites in the IS to match the observed distribution. A mechanistically similar model has been proposed for light/dark-dependent translocations of arrestin. The distribution of arrestin in rods is thought to be governed by diffusion and its binding to activated phosphorylated R* in the OS and microtubules in the IS [9]. Furthermore, arrestin translocation is triggered at a critical threshold of rod signaling and is superstoichiometric to R*, suggesting that the protein release from the IS binding sites is signal-dependent [13]. Yet, the current understanding of transducin transport may provide a simple explanation for transducin distribution without evoking unknown IS binding sites. In the light, $G\alpha_{t1}$ and $G\beta_1\gamma_1$ (or $G\beta_1\gamma_1$/phosducin) diffuse relatively rapidly to the IS where in the absence of R*-dependent activation, they "sink" each other by forming a heterotrimer, i.e., $G\alpha_{t1}$ and $G\beta_1\gamma_1$ provide IS binding sites for each other. The "sinking" effect occurs because heterotrimeric Gt is transported back to the

OS much more slowly. Thus, the apparent accumulation of Gt in the IS may result from the dynamic equilibrium of the fast diffusion into IS and slow return to the OS.

Alternative Gt-binding proteins capable of sequestering abundant Gt in the IS have not been identified. LGN, a member of the family of G-protein modulators containing G-protein regulatory or GoLoco-motifs, is expressed in the IS of rod cells [61, 62]. After light-dependent translocation from the outer segments, $G\alpha_{t1}$ co-localizes and interacts with LGN in the basal part of the IS [61]. It is unlikely, however, that LGN can account for the IS accumulation of Gt in the light, as the estimated molar ratio of LGN to $G\alpha_{t1}$ in the IS is very low [61]. Recently, $G\alpha_{t1}$ was shown to interact with GAPDH and β-actin [50]. The significance of these interactions for light-dependent translocation of transducin is unclear because GAPDH is localized primarily to the OS plasma membrane [51], whereas β-actin associates with $G\alpha_{t1}$ only in the dark [50].

Concluding Note

Recent studies have yielded major advances in understanding the mechanism of light-induced translocation of transducin in rods and provided important clues to explain the lack of transducin redistribution in cones. The preponderance of evidence suggests a simple and elegant diffusion model. According to the model, the activation of transducin by photoexcited rhodopsin causes dissociation of Gt subunits, allowing them to diffuse into the inner segment as long as the Gt activation rate exceeds the capacity of the rod RGS9 GAP complex to inactivate $G\alpha_{t1}$GTP (Fig. 2). The mechanism of transducin transport in the IS→OS direction is still poorly understood (Fig. 2). Furthermore, the pathway of transducin delivery to the OS in cones, which do not have to cope with light/dark-dependent G-protein redistribution, appears to be different from that in rods. Elucidation of the IS→OS pathways and mechanisms for transducin and other peripheral membrane proteins

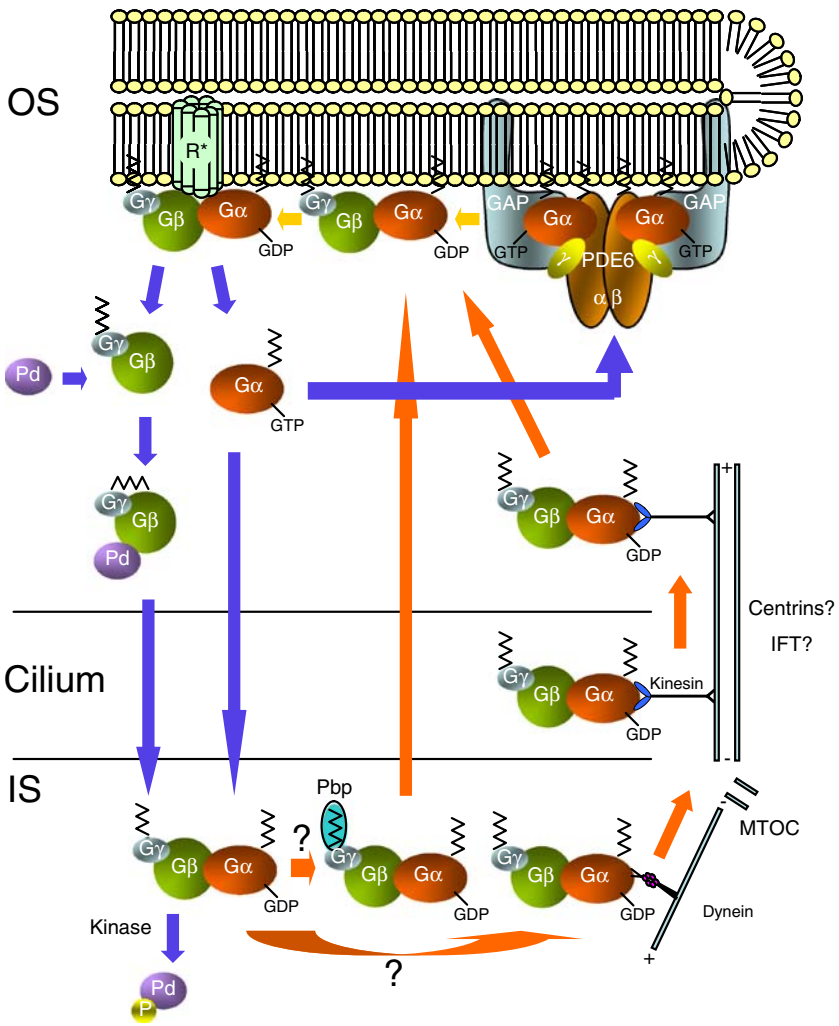

Fig. 2 Potential modes for bidirectional translocation of rod transducin. Photoexcited rhodopsin activates heterotrimeric Gt causing separation and solubilization of Gt subunits. $G\alpha_{t1}$GTP forms the activated membrane-bound complex with PDE6, which is subsequently inactivated by the RGS9 GAP complex. When the rate of activation of Gt by R* exceeds the capacity of the rod RGS9 GAP complex to inactivate $G\alpha_{t1}$GTP, $G\alpha_{t1}$GTP and $G\beta_1\gamma_1$ are able to diffuse into the IS. The diffusion of a more hydrophobic $G\beta_1\gamma_1$ is facilitated by phosducin (*Pd*). In the absence of R*-induced activation, $G\alpha_{t1}$ hydrolyzes GTP and associates with $G\beta_1\gamma_1$ in the IS. Formation of the heterotrimer serves as the trigger for Gt return to the OS in the dark and may require phosphorylation and dissociation of Pd. The mechanism for the return of Gt to the OS in the dark is not known, but may involve diffusion facilitated by a prenyl-binding protein (*Pbp*) or a molecular motor-driven transport

targeted to the OS remains an important challenge for future investigations.

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References

1. Burns ME, Arshavsky VY (2005) Beyond counting photons: trials and trends in vertebrate visual transduction. Neuron 48:387–401
2. Lamb TD, Pugh EN Jr (2006) Phototransduction, dark adaptation, and rhodopsin regeneration the proctor lecture. Invest Ophthalmol Vis Sci 47:5137–5152
3. Fu Y, Yau KW (2007) Phototransduction in mouse rods and cones. Pflugers Arch 454:805–819
4. Strauss O (2005) The retinal pigment epithelium in visual function. Physiol Rev 85:845–881
5. Brann MR, Cohen LV (1987) Diurnal expression of transducin mRNA and translocation of transducin in rods of rat retina. Science 235:585–587
6. Philp NJ, Chang W, Long K (1987) Light-stimulated protein movement in rod photoreceptor cells of the rat retina. FEBS Lett 225:127–132
7. Whelan JP, McGinnis JF (1988) Light-dependent subcellular movement of photoreceptor proteins. J Neurosci Res 20:263–270
8. Sokolov M, Lyubarsky AL, Strissel KJ, Savchenko AB, Govardovskii VI, Pugh EN Jr, Arshavsky VY (2002) Massive light-driven translocation of transducin between the two major compartments of rod cells: a novel mechanism of light adaptation. Neuron 34: 95–106
9. Nair KS, Hanson SM, Mendez A, Gurevich EV, Kennedy MJ, Shestopalov VI, Vishnivetskiy SA, Chen J, Hurley JB, Gurevich VV, Slepak VZ (2005) Light-dependent redistribution of arrestin in vertebrate rods is an energy-independent process governed by protein-protein interactions. Neuron 46:555–567
10. Strissel KJ, Lishko PV, Trieu LH, Kennedy MJ, Hurley JB, Arshavsky VY (2005) Recoverin undergoes light-dependent intracellular translocation in rod photoreceptors. J Biol Chem 280:29250–29255
11. Fain GL (2006) Why photoreceptors die (and why they don't). Bioessays 28:344–354
12. Calvert PD, Strissel KJ, Schiesser WE, Pugh EN Jr, Arshavsky VY (2006) Light-driven translocation of signaling proteins in vertebrate photoreceptors. Trends Cell Biol 16:560–568
13. Calvert PD, Strissel KJ, Schiesser WE, Pugh EN, Arshavsky VY (2006) Arrestin translocation is induced at a critical threshold of visual signaling and is superstoichiometric to bleached rhodopsin. J Neurosci 26:1146–1153
14. Lobanova ES, Finkelstein S, Song H, Tsang SH, Chen CK, Sokolov M, Skiba NP, Arshavsky VY (2007) Transducin translocation in rods is triggered by saturation of the GTPase-activating complex. J Neurosci 27:1151–1160
15. Rosenzweig DH, Nair KS, Wei J, Wang Q, Garwin G, Saari JC, Chen CK, Smrcka AV, Swaroop A, Lem J, Hurley JB, Slepak VZ (2007) Subunit dissociation and diffusion determine the subcellular localization of rod and cone transducins. J Neurosci 27:5484–5494
16. Kerov V, Rubin WW, Natochin M, Melling N, Burns ME, Artemyev NO (2007) N-terminal fatty acylation of transducin profoundly influences its localization and the kinetics of photoresponse in rods. J Neurosci 27:10270–10277
17. Wedegaertner PB, Wilson PT, Bourne HR (1995) Lipid modifications of trimeric G proteins. J Biol Chem 270:503–506
18. Chen CA, Manning DR (2001) Regulation of G proteins by covalent modification. Oncogene 20:1643–1652
19. Karan S, Zhang H, Li S, Frederick JM, Baehr W (2008) A model for transport of membrane-associated phototransduction polypeptides in rod and cone photoreceptor inner segments. Vis Res 48:442–452
20. Neubert TA, Johnson RS, Hurley JB, Walsh KA (1992) The rod transducin a subunit amino terminus is heterogeneously fatty acylated. J Biol Chem 267:18274–18277
21. Bhatnagar RS, Schall OF, Jackson-Machelski E, Sikorski JA, Devadas B, Gokel GW, Gordon JI (1997) Titration calorimetric analysis of AcylCoA recognition by myristoylCoA:protein *N*-myristoyltransferase. Biochemistry 36:6700–6708
22. Bigay J, Faurobert E, Franco M, Chabre M (1994) Roles of lipid modifications of transducin subunits in their GDP-dependent association and membrane binding. Biochemistry 33:14081–14090
23. Resh MD (1999) Fatty acylation of proteins: new insights into membrane targeting of myristoylated and palmitoylated proteins. Biochim Biophys Acta 1451:1–16
24. Kosloff M, Elia N, Selinger Z (2002) Structural homology discloses a bifunctional structural motif at the N-termini of Ga proteins. Biochemistry 41:14518–14523
25. Lai RK, Perez-Sala D, Cañada FJ, Rando RR (1990) The g subunit of transducin is farnesylated. Proc Natl Acad Sci U S A 87:7673–7677
26. Fukada Y, Takao T, Ohguro H, Yoshizawa T, Akino T, Shimonishi Y (1990) Farnesylated g-subunit of photoreceptor G protein indispensable for GTP-binding. Nature 346:658–660
27. Higgins JB, Casey PJ (1996) The role of prenylation in G-protein assembly and function. Cell Signal 8:433–437
28. Marrari Y, Crouthamel M, Irannejad R, Wedegaertner PB (2007) Assembly and trafficking of heterotrimeric G proteins. Biochemistry 46:7665–767
29. Fu HW, Casey PJ (1999) Enzymology and biology of CaaX protein prenylation. Recent Prog Horm Res 54:315–342
30. Gelb MH, Brunsveld L, Hrycyna CA, Michaelis S, Tamanoi F, Voorhis WCV, Waldmann H (2006) Therapeutic intervention based on protein prenylation and associated modifications. Nat Chem Biol 2:518–528
31. Michaelson D, Ahearn I, Bergo M, Young S, Philips M (2002) Membrane trafficking of heterotrimeric G proteins via the endoplasmic reticulum and Golgi. Mol Biol Cell 13:3294–3302
32. Takida S, Wedegaertner PB (2003) Heterotrimer formation, together with isoprenylation, is required for plasma membrane targeting of Gbg. J Biol Chem 278:17284–17290
33. Zhang H, Huang W, Zhang H, Zhu X, Craft CM, Baehr W, Chen C-K (2003) Light-dependent redistribution of visual arrestins and transducin subunits in mice with defective phototransduction. Mol Vis 9:231–237
34. Kerov V, Chen D, Moussaif M, Chen YJ, Chen CK, Artemyev NO (2005) Transducin activation state controls its light-dependent translocation in rod photoreceptors. J Biol Chem 280:41069–41076
35. Natochin M, Barren B, Artemyev NO (2006) Dominant negative mutants of transducin-a that block activated receptor. Biochemistry 45:6488–6494
36. Peterson JJ, Orisme W, Fellows J, McDowell JH, Shelamer CL, Dugger DR, Smith WC (2005) A role for cytoskeletal elements in the light-driven translocation of proteins in rod photoreceptors. Invest Ophthalmol Vis Sci 46:3988–3998
37. Reidel B, Giebl A, Wolfrum U (2006) Arrestin and transducin translocations associated with the dark adaptation of rod photoreceptor cells are fully dependent on the cytoskeleton [abstract]. Invest Ophthalmol Vis Sci 47:ARVO E-Abstract 5528

38. Willardson BM, Howlett AC (2007) Function of phosducin-like proteins in G protein signaling and chaperone-assisted protein folding. Cell Signal 19:2417–2427
39. Gaudet R, Bohm A, Sigler PB (1996) Crystal structure at 2.4 angstroms resolution of the complex of transducin bg and its regulator, phosducin. Cell 87:577–588
40. Loew A, Ho YK, Blundell T, Bax B (1998) Phosducin induces a structural change in transducin bg. Structure 6:1007–1019
41. Sokolov M, Strissel KJ, Leskov IB, Michaud NA, Govardovskii VI, Arshavsky VY (2004) Phosducin facilitates light-driven transducin translocation in rod photoreceptors. Evidence from the phosducin knockout mouse. J Biol Chem 279:19149–19156
42. Krispel CM, Sokolov M, Chen Y, Song H, Herrmann R, Arshavsky VY, Burns ME (2007) Phosducin regulates the expression of transducin bg subunits in rod photoreceptors and does not contribute to phototransduction adaptation. J Gen Physiol 130:303–312
43. Obin M, Lee BY, Meinke G, Bohm A, Lee RH, Gaudet R, Hopp JA, Arshavsky VY, Willardson BM, Taylor A (2002) Ubiquitylation of the transducin bg subunit complex. Regulation by phosducin. J Biol Chem 277:44566–44575
44. Song H, Belcastro M, Young EJ, Sokolov M (2007) Compartment-specific phosphorylation of phosducin in rods underlies adaptation to various levels of illumination. J Biol Chem 282:23613–23621
45. Gillespie PG, Prusti RK, Apel ED, Beavo JA (1989) A soluble form of bovine rod photoreceptor phosphodiesterase has a novel 15-kDa subunit. J Biol Chem 264:12187–12193
46. Florio SK, Prusti RK, Beavo JA (1996) Solubilization of membrane-bound rod phosphodiesterase by the rod phosphodiesterase recombinant delta subunit. J Biol Chem 271:24036–24047
47. Norton AW, Hosier S, Terew JM, Li N, Dhingra A, Vardi N, Baehr W, Cote RH (2005) Evaluation of the 17-kDa prenyl-binding protein as a regulatory protein for phototransduction in retinal photoreceptors. J Biol Chem 280:1248–1256
48. Zhang H, Li S, Doan T, Rieke F, Detwiler PB, Frederick JM, Baehr W (2007) Deletion of PrBP/d impedes transport of GRK1 and PDE6 catalytic subunits to photoreceptor outer segments. Proc Natl Acad Sci USA 104:8857–8862
49. Matsuoka K, Orci L, Amherdt M, Bednarek SY, Hamamoto S, Schekman R, Yeung T (1998) COPII-coated vesicle formation reconstituted with purified coat proteins and chemically defined liposomes. Cell 93:263–275
50. Chen J, Wu M, Sezate SA, Matsumoto H, Ramsey M, McGinnis JF (2008) Interaction of glyceraldehyde-3-phosphate dehydrogenase in the light-induced rod a-transducin translocation. J Neurochem 104:1280–1292
51. Hsu SC, Molday RS (1990) Glyceraldehyde-3-phosphate dehydrogenase is a major protein associated with the plasma membrane of retinal photoreceptor outer segments. J Biol Chem 265:13308–13313
52. Roepman R, Wolfrum U (2007) Protein networks and complexes in photoreceptor cilia. Subcell Biochem 43:209–235
53. Giessl A, Trojan P, Rausch S, Pulvermüller A, Wolfrum U (2006) Centrins, gatekeepers for the light-dependent translocation of transducin through the photoreceptor cell connecting cilium. Vis Res 46:4502–4509
54. Scholey JM (2003) Intraflagellar transport. Annu Rev Cell Dev Biol 19:423–443
55. Pazour GJ, Baker SA, Deane JA, Cole DG, Dickert BL, Rosenbaum JL, Witman GB, Besharse JC (2002) The intraflagellar transport protein, IFT88, is essential for vertebrate photoreceptor assembly and maintenance. J Cell Biol 157:103–113
56. Luby-Phelps K, Fogerty J, Baker SA, Pazour GJ, Besharse JC (2008) Spatial distribution of intraflagellar transport proteins in vertebrate photoreceptors. Vis Res 48:413–423
57. Marszalek JR, Liu X, Roberts EA, Chui D, Marth JD, Williams DS, Goldstein LS (2000) Genetic evidence for selective transport of opsin and arrestin by kinesin-II in mammalian photoreceptors. Cell 102:175–187
58. Baehr W, Karan S, Maeda T, Luo D, Li S, Bronson JD, Watt CB, Yau K, Frederick JM, Palczewski K (2007) The function of guanylate cyclase 1 and guanylate cyclase 2 in rod and cone photoreceptors. J Biol Chem 282:8837–8847
59. Alves ID, Salgado GFJ, Salamon Z, Brown MF, Tollin G, Hruby VJ (2005) Phosphatidylethanolamine enhances rhodopsin photoactivation and transducin binding in a solid supported lipid bilayer as determined using plasmon-waveguide resonance spectroscopy. Biophys J 88:198–210
60. Fanelli F, Dell'Orco D (2005) Rhodopsin activation follows precoupling with transducin: inferences from computational analysis. Biochemistry 44:14695–14700
61. Kerov VS, Natochin M, Artemyev NO (2005) Interaction of transducin-a with LGN, a G-protein modulator expressed in photoreceptor cells. Mol Cell Neurosci 28:485–495
62. Nair KS, Mendez A, Blumer JB, Rosenzweig DH, Slepak VZ (2005) The presence of a Leu-Gly-Asn repeat-enriched protein (LGN), a putative binding partner of transducin, in ROD photoreceptors. Invest Ophthalmol Vis Sci 46:383–389
63. Kassai H, Aiba A, Nakao K, Nakamura K, Katsuki M, Xiong WH, Yau KW, Imai H, Shichida Y, Satomi Y, Takao T, Okano T, Fukada Y (2005) Farnesylation of retinal transducin underlies its translocation during light adaptation. Neuron 47:529–539
64. Mendez A, Lem J, Simon M, Chen J (2003) Light-dependent translocation of arrestin in the absence of rhodopsin phosphorylation and transducin signaling. J Neurosci 23:3124–3129
65. Catty P, Pfister C, Bruckert F, Deterre P (1992) The cGMP phosphodiesterase-transducin complex of retinal rods. Membrane binding and subunits interactions. J Biol Chem 267:19489–19493
66. Clerc A, Bennett N (1992) Activated cGMP phosphodiesterase of retinal rods. A complex with transducin a subunit. J Biol Chem 267:6620–6627
67. Tsang SH, Burns ME, Calvert PD, Gouras P, Baylor DA, Goff SP, Arshavsky VY (1998) Role for the target enzyme in deactivation of photoreceptor G protein in vivo. Science 282:117–121
68. Kennedy MJ, Dunn FA, Hurley JB (2004) Visual pigment phosphorylation but not transducin translocation can contribute to light adaptation in zebrafish cones. Neuron 41:915–928
69. Elias RV, Sezate SS, Cao W, McGinnis JF (2004) Temporal kinetics of the light/dark translocation and compartmentation of arrestin and a-transducin in mouse photoreceptor cells. Mol Vis 10:672–681
70. Coleman JE, Semple-Rowland SL (2005) GC1 deletion prevents light-dependent arrestin translocation in mouse cone photoreceptor cells. Invest Ophthalmol Vis Sci 46:12–16
71. Chen J, Wu M, Sezate SA, McGinnis JF (2007) Light threshold-controlled cone a-transducin translocation. Invest Ophthalmol Vis Sci 48:3350–3355
72. McGinnis JF, Matsumoto B, Whelan JP, Cao W (2002) Cytoskeleton participation in subcellular trafficking of signal transduction proteins in rod photoreceptor cells. J Neurosci Res 67:290–297
73. Peet JA, Bragin A, Calvert PD, Nikonov SS, Mani S, Zhao X, Besharse JC, Pierce EA, Knox BE, Pugh EN Jr (2004) Quantification of the cytoplasmic spaces of living cells with EGFP reveals arrestin-EGFP to be in disequilibrium in dark adapted rod photoreceptors. J Cell Sci 117:3049–3059