Abstract. The Drosophila retinal degeneration B (rdgB) gene encodes an integral membrane protein involved in phototransduction and prevention of retinal degeneration. RdgB represents a nonclassical phosphatidylinositol transfer protein (PITP) as all other known PITPs are soluble polypeptides. Our data demonstrate roles for RdgB in proper termination of the phototransduction light response and dark recovery of the photoreceptor cells. Expression of RdgB’s PITP domain as a soluble protein (RdgB-PITP) in rdgB mutant flies is sufficient to completely restore the wildtype electrophysiological light response and prevent the degeneration. However, introduction of the T59E mutation, which does not affect RdgB-PITP’s phosphatidylinositol (PI) and phosphatidycholine (PC) transfer in vitro, into the soluble (RdgB-PITP-T59E) or full-length (RdgB-T59E) proteins eliminated rescue of retinal degeneration in rdgB flies, while the light response was partially maintained. Substitution of the rat brain PITPα, a classical PI transfer protein, for RdgB’s PITP domain (PITPα or PITPα-RdgB chimeric protein) neither restored the light response nor maintained retinal integrity when expressed in rdgB flies. Therefore, the complete repertoire of essential RdgB functions resides in RdgB’s PITP domain, but other PITPs possessing PI and/or PC transfer activity in vitro cannot supplant RdgB function in vivo. Expression of either RdgB-T59E or PITPα-RdgB in rdgB flies produced a dominant retinal degeneration phenotype. Whereas RdgB-T59E functioned in a dominant manner to significantly reduce steady-state levels of rhodopsin, PITPα-RdgB was defective in the ability to recover from prolonged light stimulation and caused photoreceptor degeneration through an unknown mechanism. This in vivo analysis of PITP function in a metazoan system provides further insights into the links between PITP dysfunction and an inherited disease in a higher eukaryote.

The Drosophila retinal degeneration B protein (RdgB) plays a critical role in the fly photoreceptor cell. The rdgB mutant phenotype is characterized by retinal degeneration whose onset, while discernible in dark-reared flies, is greatly accelerated by raising the flies in light (Harris and Stark, 1977; Stark et al., 1983). Typically, rdgB mutant flies begin to exhibit the morphological hallmarks of photoreceptor cell degeneration several days after eclosion (Harris and Stark, 1977; Stark et al., 1983). In addition, these mutant flies exhibit an abnormal light response, as recorded by the rapid deterioration of the electroretinogram (ERG), shortly after the fly’s initial exposure to light. This ERG defect is manifested before any obvious physical signs of retinal degeneration (Harris and Stark, 1977), which suggests that the defect in the light response may precipitate the course of retinal degeneration.

In the photoreceptor cell, RdgB localizes to both the axon and the subrhabdomeric cisternae (SRC) (Vihitel et al., 1993; Suzuki and Hiosawa, 1994). The SRC is an extension of the endoplasmic reticulum that functions both as an intracellular Ca\(^{2+}\) store and a compartment through which rhodopsin traffics en route to the rhabdomere (Walz, 1982; Matsumoto-Suzuki et al., 1989; Suzuki and Hiosawa, 1991). Thus, RdgB is the first identified protein required for visual transduction that is not localized in the photoreceptor rhabdomere. Genetic epistasis analyses suggest RdgB functions downstream of both rhodopsin and...
phospholipase C (PLC) in the visual transduction cascade as both the ninaE (encoding the opsin expressed in photoreceptor cells R1-6 [O’Tousa et al., 1985; Zuker et al., 1985]) and norpA (encoding phospholipase C [Bloomquist et al., 1993]) mutations suppress the rdgB-dependent, light-enhanced retinal degeneration (Harris and Stark, 1977; Stark et al., 1983). Consistent with this view, constitutive activation of the Drosophila G protein transducin analogue (DGlq), either by application of nonhydrolyzable GTP analogues or by expression of a constitutively activated Gα subunit (DGlq1), effects a rapid degeneration of rdgB retinas in the absence of light (Rubinstein et al., 1989; Lee et al., 1994). RdgB apparently functions downstream of the inac-encoded protein kinase C (PKC) because: (a) application of phorbol ester to rdgB mutant retinas, which presumably activates the inac-encoded PKC, stimulates retinal degeneration in the absence of light (Minke et al., 1990); and (b) the rdgB retinal degeneration is weakly suppressed by the inac mutation (Smith et al., 1991). Thus, the available evidence identifies an execution point for RdgB downstream of PKC in the visual transduction cascade.

RdgB is a 116-kD membrane polypeptide with six potential transmembrane domains (Vihtelic et al., 1991). Additionally, the amino-terminal 281 RdgB residues share 42% amino acid identity with the rat brain phosphatidylinositol (PI) transfer protein α isoform (PITPα) (Vihtelic et al., 1993). Whereas PITPs are operationally defined by their ability to catalyze the transfer of either PI or phosphatidylethanolamine (PC) monomers between membrane bilayers in vitro (Bankaitis et al., 1990; Cleves et al., 1991; Wirtz, 1991), how the phospholipid transfer activity pertains to in vivo function is less clear. The yeast PITP (Sec14p) uses its PI and PC binding activities in two independent, yet complementary, ways that serve to preserve a Golgi pool of diacylglycerol that is critical for the biogenesis of Golgi-derived secretory vesicles (Kearns et al., 1997). Reconstitution studies suggest that mammalian PITPs play important roles in PLC-mediated inositol signaling, ATP-dependent, Ca²⁺-activated secretion, and constitutive secretion from the trans-Golgi network (Hay and Martin, 1993, 1995; Thomas et al., 1993, 1995; Ohashi et al., 1995). However, because the PITP requirement for these processes is generally satisfied by any PITP (even those lacking any primary sequence identity), the physiological relevance of these PITP involvements remains to be determined (Skinner et al., 1993; Cunningham et al., 1995; Ohashi et al., 1995; Alb et al., 1996). The recent finding that the mouse vibrator mutation represents a hypomorphic mutation in the pitpn gene, which encodes PITPα, indicates that PITP function is important to neuronal function (Hamilton et al., 1997). RdgB’s PITP domain (when expressed as a soluble protein in Escherichia coli) is able to effect intermembrane transfer of PI in vitro (Vihtelic et al., 1993). Unlike all previously characterized PITPs, which are 32–35-kD soluble proteins (Bankaitis et al., 1989; Cleves et al., 1991; Wirtz, 1991), RdgB is a large integral membrane protein. In spite of postulated in vivo activities for PITPs, the function of RdgB in the photoreceptor cell remains unknown. Recently, vertebrate orthologues of the rdgB gene were identified in mice, bovines, and humans (Chang et al., 1997). Expression of the mouse rdgB cDNA in rdgB null mutant flies resulted in the elimination of the retinal degeneration and complete restoration of the wild-type ERG light response (Chang et al., 1997). Thus, the Drosophila RdgB protein defines a new class of functionally equivalent transmembrane PITPs.

In this work, we analyzed RdgB’s involvement in the Drosophila phototransduction cascade and the mechanism by which it prevents the onset of retinal degeneration. This represents the first in vivo analysis of the transmembrane PITP class, and we report several novel and unanticipated aspects of RdgB function. We demonstrate that the complete repertoire of RdgB functions essential for normal phototransduction reside in the PITP domain. Expression of this domain as a soluble polypeptide fully complements the rdgB null allele. Yet, other PITPs that possess PI and/or PC transfer activities in vitro cannot substitute for RdgB in the photoreceptor cell. Whereas the recessive rdgB null mutation demonstrates an essential role for RdgB in proper termination of the ERG light response and dark recovery of the photoreceptor cell, one novel dominant rdgB mutation affects the maintenance of steady-state rhodopsin levels in photoreceptor cells. Another dominant rdgB mutation induces retinal degeneration and compromises the rapid regeneration of a wild-type ERG light-response amplitude subsequent to multiple or prolonged light exposure. Taken together, these data indicate an underlying complexity to the mechanism of RdgB function and its role in the photoreceptor cell that is not easily reconciled with a simple role in potentiating signal transduction via phosphoinositide-driven signaling pathways.

**Materials and Methods**

**In Vitro Mutagenesis of the rdgB cDNA**

The pTV vector contains a wild-type rdgB cDNA (consisting of 427 bp of 5’ untranslated sequence, the entire rdgB open reading frame, and 555 bp of 3’ untranslated sequence) downstream of a 317-bp ninaE promoter fragment, which is sufficient for gene expression in photoreceptors R1-6 (Misser and Rubin, 1987). Single-stranded pTV DNA was prepared and in vitro mutagenized with the mutagenic primers 5'-GCTCCGAGAAGCTTTCGCTGCTG-3' (Kunkel et al., 1987), which introduced a HindIII site (underlined sequence) at nucleotides 827–832 (Vihtelic et al., 1991), which is the 3' boundary of the PITP domain to create pTVh1. Introduction of the HindIII site generated two conservative changes in RdgB, D276E, and V278F. An Xhol site located between the ninaE promoter and the rdgB cDNA allowed removal of the PITP domain in pTVh1 by Xhol/HindIII digestion. The mutagenized PITP fragments (see below) were subcloned into pTVh1 lacking the Xhol/HindIII fragment. The soluble rat PITPs was PCR-amplified from a rat PITPs cDNA with primers 5'-CTCCGAGAAGCTTTCGCTGCTG-3' and 5'-AAGCTTCC TTTACGCAAGTCAGTG-3', and cloned with Xhol and HindIII sites (underlined), respectively. The PCR product was subcloned into the pCR2.1 vector (Invitrogen, Carlsbad, CA), excised by Xhol/HindIII digestion, and ligated into pTVh1 lacking the Xhol/HindIII fragment to yield the chimeric PITPs-RdgB construct. The rdgB-pitp construct was made by digestion of pTVh1 with HindIII and HpaI, followed by filling in the ends with Klenow fragment and blunt end ligation. This created a stop codon immediately after residue 277 in the PITP domain. The pitpa and rdgB-pitp-TsE constructs were made in the same manner as the rdgB-pitp construct, using pitpa-rdgB and rdgB-TsE constructs as the starting DNAs, respectively.

To mutagenize codon 59 of rdgB, pTVh1 was digested with Xhol and HindIII to liberate the 1.2-kb restriction fragment carrying the entire codon sequence of RdgB-PITP domain. This fragment was subcloned into pBluescript SK (Strategene, La Jolla, CA) to yield pSKPT. Single-stranded DNA was subsequently prepared and mutagenized using the primer 5'-GGTATATTTCAATACGCAAAGAAATCTTATCAGT.
GG-3' to effect the TσA missense substitution (mutagenized codon underlined) (Kunkel et al., 1987). The TσE substitution was constructed using a mutagenic primer of the exact same sequence with the exception that codon 59 was altered to GAA. The authenticity of each mutagenesis reaction was confirmed by sequencing each 1.2-kb restriction fragment cassette. The 1.2-kb mutagenized Xhol/HindIII fragments were subcloned into pTVh1 lacking the wild-type Xhol/HindIII region to introduce the Tσ9 mutations into the full-length RdgB. The mutagenized cassettes were also dualistically amplified with the forward and reverse primers 5'-GGTTAATTTGTCGATCAAGGAGTACC-3' (rdgB initiator codon in italics) and 5'-AGCGAATTCAGCTTTTTGCTGCTGGCCG-3', and clamped with EcoRI and HindIII at the 5' and 3' ends of each product, respectively. These PCR products were subsequently digested with EcoRI and HindIII and the 0.9-kb restriction fragments, containing the entire PIP open reading frame, were individually subcloned into the T7 RNA polymerase/promoter vector pT7-5 (Tabor, S., Harvard Medical School, Cambridge, MA). The authenticity of each construct was confirmed by nucleotide sequencing and the constructs were subsequently transformed into the E. coli strain BL21(DE3) (Novagen, Inc., Madison, WI) for expression of RdgB-PITP proteins.

**Nucleotide Sequence Analysis**

Nucleotide sequencing was accomplished by the dyeodeoxy chain-termination method (Sanger et al., 1977) using either single- or double-stranded plasmid DNA as a template and the Sequenase version 2.0 sequencing kit (Amersham Corp., Arlington Heights, IL).

**Expression of the Soluble RdgB-PITP in E. coli**

Wild-type and mutant versions of RdgB-PITP were expressed as soluble polypeptides in E. coli. Briefly, 1 liter of SuperBroth (Miller, 1972), containing ampicillin (50 μg/ml) was inoculated with a 1:100 dilution of an overnight E. coli culture carrying the desired PITP expression plasmid and grown to an OD600 of 0.3 at 37°C with shaking. Isopropyl β-D-thiogalacto-pyranoside (IPTG) was added (final concentration 1 mM) and the cultures were incubated for an additional 3 h. Cells were harvested by centrifugation, washed in cold lysis buffer (50 mM NaPO4 [pH 7.1], 300 mM NaCl, 1 mM NaN3, 0.2 mM PMSF, and 5 mM EDTA), and pellets were resuspended in 10 ml of cold lysis buffer containing 1.2 volume of 0.1 mm glass beads (BioSpec Products, Inc., Bartlesville, OK). Samples were vigorously vortexed seven times in 1 min bursts with cooling on ice between each burst. The lysates were clarified by centrifugation for 5 min at 2,000 g, 20 min at 20,000 g, and finally 60 min at 100,000 g to yield the final cytosolic fraction. Total protein concentration of the cytosol fraction was determined by the bichinonic acid (BCA) (Pierce Chemical Co., Rockford, IL).

**Phospholipid Transfer Assays**

E. coli cytosolic preparations (1 mg total protein per reaction) were individually assayed for PI and PC transfer in vitro as previously described (Aitken et al., 1990; Skinner et al., 1993). Sphingomyelin (SM) transfer assays were performed exactly as PC transfer assays, with the exception that [N-methyl-3H]SM (56 mCi:mmol; Amersham Corp.) was used as transfer substrate (0.09 μM per assay). Quantitative ELISAs were used to normalise the RdgB content in each E. coli cytosolic fraction using a direct sandwich assay with polyclonal mouse anti-RdgB serum directed against RdgB-PITP. Secondary goat anti-mouse antibodies conjugated to horseradish peroxidase (Bio-Rad Laboratories, Hercules, CA) were used for development of signal in the presence of o-phenylenediamine. After quenching, A405 was measured with an EL311fx automated microplate reader (BioTek Instruments, Inc., Winooski, VT).

**Germine Transformation**

pTVh1 plasmids containing mutant and wild-type rdgB CDNA were digested with Xba1 and Kpn1 to liberate a single restriction fragment containing the nirAE promoter and rdgB cDNA. These fragments were individually subcloned into pCaSpeR-4 (Ashburner, 1989), the resulting constructs were purified on a CsCl density gradient, and cojected with Δ2-3 helper DNA into w1118 embryos using standard techniques (Ashburner, 1989b). Multiple independent lines were isolated that expressed each transgene. These independent lines were assayed to determine that the phenotypes described are due to expression of the transgene and not a fortuitous P element insertion into a particular genomic region. The transgenic lines (in a rdgB-null mutant background) were tested for the level of protein expression from the transgenes by immunoblots. The expression level of the various RdgB constructs (from one copy of a given transgene) relative to RdgB in wild-type flies is shown in Table I.

**Immunoblot Analyses**

Two newly eclosed (>3-h old), dark-raised flies were decapitated in room light and homogenized in 10 μl extraction buffer (2.3% SDS, 10% glycerol, 62.5 mM Tris Cl [pH 6.8], and 0.1% bromophenol blue). The homogenate was incubated at 37°C for 1 h and centrifuged briefly (Ozaki et al., 1993). The supernatant was resolved on a 12.5% polyacrylamide-SDS gel (Laemmli, 1970). Proteins were transferred to nitrocellulose using a transfer apparatus (Bio-Rad Trans-Blot SD Semi-Dry Transfer Cell; Bio-Rad Laboratories) at 15 V for 30 min, the membrane was blocked at least 2 h, and the membrane was finally washed for 20 min in TBBTS (0.05% Tween-20 in TBS). The membranes were incubated overnight in either 1:1,000 dilution of anti–rhodopsin polyclonal antiserum, 1:1,000 dilution of anti–Copol polyclonal antiserum (both provided by J.E. O’Toole, University of Notre Dame, Notre Dame, IN), 1:500 dilution of affinity-purified anti–Dgq polyclonal antiserum (Lee et al., 1994), 1:1,000 dilution of anti–Trp polyclonal antiserum (provided by C. Montell, Johns Hopkins University, Baltimore, MD), 1:3,000 dilution of anti–Gbe ascites (provided by C. Zuker, University of California at San Diego, San Diego, CA), or anti–RdgB monoclonal supernatant. The membranes were washed three times (10 min per wash) with TBBTS, and incubated an additional 2 h in the presence of goat anti–rabbit or goat anti–mouse alkaline phosphatase–conjugated secondary antibody (Sigma Chemical Co., St. Louis, MO) diluted 1:15,000 and 1:3,000, respectively, in 2% Blotto. The membranes were washed twice for 5 min with TBBTS. A final 5 min wash with 0.1 M Tris (pH 9.5) preceded colorimetric detection, which was performed according to manufacturer’s protocol (Bio-Rad Laboratories).

**Electrophysiology**

Newly eclosed, dark-reared flies were prepared for ERG analysis in dim red light. After positioning the recording electrode into the eye and the reference electrode into the back of the head, the fly was dark adapted for 1 h before recording the initial response to white light as previously described (Zars and Hyde, 1996). All rdgB flies with and without transgenes were vermillion (v) eyed, whereas all rdgB+ flies with and without transgenes were w+. No significant differences were observed in the ERG light-response amplitudes between the w+ and v rdgB+ flies. Prolonged depolarizing afterpotentials (PDA) were examined from either w rdgB+, rdgB+; cn bw, rdgB+; cn bw P[rdgB-TsE]; rdgB+; cn bw P[pita-rdgB]; or w; nirAE/T flies. The cinnabar (cn) and brown (bw) mutations yield a phenotypically white eye and produced the same PDA in a rdgB+ background as the white (w) mutation.

**Characterization and Histology of Photoreceptor Degeneration**

Flies were initially analyzed for retinal degeneration by inspecting the deep pseudopupil; a virtual image produced from the rhabdomeres of ~20 adjacent ommatidia (Franceschini, 1972). Integrity of both the rhabdomeres and of the ommatidial array is required for production of the deep pseudopupil. All the flies examined were in a w+ (wild-type eye color) background, which ensured that all the flies had a consistent eye color, and therefore received equivalent light exposure, regardless of the expression of the different transgenes. The flies were raised in either constant light or under a 12 h light/dark cycle. To establish a time course of degeneration, 3 or 4 replicates of 30–80 flies were examined daily for the deep pseudopupil. At least 100 flies of each genotype were examined for each time point. Prolonged degeneration was also examined by light microscopy of retinal tissue sections. Either white (w) or vermillion (v) control and experimental flies were raised in the desired light conditions for the appropriate period and then decapitated; the heads were bisected, fixed, and embedded in Polybed 812 (Polysciences Inc., Warrington, PA) as previously described (Lee et al., 1994). 1-μm sections were cut and stained with methylene blue azure II.
Results

RdgB’s PITP Domain Is Sufficient to Rescue the rdgB Mutant Retinal Degeneration and Light-Response Phenotypes

The rdgB2 null mutation causes a light-enhanced retinal degeneration characterized by the reduction and loss of rhabdomeres, photoreceptor cell death, and the appearance of holes in the retinal tissue (Fig. 1 A). Before any histological signs of degeneration, the rdgB2 mutant loses its ERG light response (Fig. 1 A). By contrast, a wild-type retina possesses a well-ordered ommatidial arrangement containing large rhabdomeres and produces an ERG light response of some 25 mV (Fig. 1 B). We ectopically expressed a wild-type rdgB cDNA in rdgB2 flies. After 6 d in a 12-h light/dark cycle, these transgenic flies still maintained a wild-type ERG light response and exhibited little evidence of retinal degeneration (Fig. 1 C). Indeed, the only detectable histological abnormality was loss of the central R7 and R8 rhabdomeres in some ommatidia; an expected result given that RdgB expression was restricted to photoreceptors R1-6 by the ninaE promoter. Surprisingly, RdgB-PITP expression completely protected the R1-6 photoreceptor cells from degeneration, even at 17 d after eclosion (Fig. 1 D). The ERG light response of these transgenic flies was also wild-type (Fig. 1 D). The preservation of the R1-6 cells and the ERG light response was still apparent 30 d after eclosion (data not shown).

To examine the functional importance of RdgB’s PITP domain, we introduced a translational termination codon at position 277 in the wild-type rdgB cDNA. This resulted in RdgB’s PITP domain being translated as a soluble protein (RdgB-PITP). We germline transformed this construct into flies and expressed it in rdgB2 flies. After 6 d in a 12-h light/dark cycle, these transgenic flies still maintained a wild-type ERG light response and exhibited little evidence of retinal degeneration (Fig. 1 C). Indeed, the only detectable histological abnormality was loss of the central R7 and R8 rhabdomeres in some ommatidia; an expected result given that RdgB expression was restricted to photoreceptors R1-6 by the ninaE promoter. Surprisingly, RdgB-PITP expression completely protected the R1-6 photoreceptor cells from degeneration, even at 17 d after eclosion (Fig. 1 D). The ERG light response of these transgenic flies was also wild-type (Fig. 1 D). The preservation of the R1-6 cells and the ERG light response was still apparent 30 d after eclosion (data not shown). These collective data demonstrate that RdgB-PITP, which represents only 27% of the full-length RdgB primary sequence, was sufficient for complete rescue of both the retinal degeneration and electrophysiological defects associated with rdgB2 null alleles.

A Threonine-59 Missense Mutation Inactivates PI, but Not PC, Transfer Activity of RdgB-PITP

We previously demonstrated that RdgB-PITP exhibits PI-transfer activity (Vihitelc et al., 1993). To assess the range of phospholipid transfer capability, and to determine whether RdgB-PITP exhibited phospholipid transfer properties more similar to mammalian PITPα (which transfers PI and PC) or PITPβ (which transfers PI, PC, and SM), we expressed RdgB-PITP as a soluble polypeptide in E. coli (Fig. 2 A, lane 1) and assayed its ability to mobilize PI, PC, and SM between membrane bilayers in vitro. RdgB-PITP catalyzed robust transfer of both PI and PC in vitro (Fig. 2 B), with 5.3 ± 0.1% and 6.4 ± 1.2% of total radiolabeled PI and PC substrate transferred, respectively. However, we did not detect SM transfer activity with RdgB-PITP (Fig. 2 B). Thus, RdgB-PITP exhibited biochemical properties more closely resembling those of PITPα than PITPβ.

We extended this functional comparison between RdgB-
PITP and PITPα to analyzing the biochemical effects of specific missense mutations. Previously, we demonstrated that the PI transfer activity of mammalian PITPα is sensitive to amino acid substitutions involving T59, a residue that is conserved amongst all presently known metazoan PITPs and defines a consensus PKC phosphorylation site in PITPs and RdgB (Alb et al., 1995). The T59E alteration selectively abolishes PI transfer activity in PITPα without affecting PC transfer activity, while the T59A substitution reduces the specific activity for PI transfer some twofold relative to wild-type (Alb et al., 1995). We expressed the T59E and T59A mutant forms of RdgB-PITP in E. coli (Fig. 2A) and assayed them for PI and PC transfer activities. However, the biochemical consequences associated with these individual mutations were, in each case, opposite from those anticipated from the PITPα data. The T59E RdgB-PITP transferred 5.7 ± 0.1% and 6.9 ± 1.7% of total radiolabeled PI and PC substrate, respectively. The relative specific activities for PI and PC transfer were 102 ± 2.3% and 97 ± 6.8% of wild-type RdgB-PITP, respectively (Fig. 2B). By contrast, the T59A RdgB-PITP failed to exhibit any detectable PI transfer activity, even though PC transfer activity was essentially unaffected, with 6.6 ± 0.8% of total radiolabeled PC substrate transferred (95 ± 2.1% of wild-type RdgB-PITP, Fig. 2B). Thus, the T59A mutation caused the loss of PI transfer activity without affecting PC transfer capability. These data indicated that, while the PI transfer activities of both RdgB-PITP and mammalian PITPα were selectively sensitive to substitutions at T59, the spectrum of substitutions for T59 that were permissive for PI transfer clearly differed between RdgB-PITP and mammalian PITPα.

Expression of the T59E Mutant in rdgB2 Flies Partially Rescues the ERG Defect without Suppressing the Retinal Degeneration

To dissect the functional contributions of PI and PC transfer activity to RdgB function in vivo, we introduced the T59A substitution in the full-length RdgB protein and T59E into both RdgB-PITP and RdgB. We expressed these T59 mutant proteins in rdgB2 null flies and assessed their ability to restore a wild-type ERG light response and suppress retinal degeneration. Three independent germ-line transformed P[rdbg-T59A] mutant lines failed to exhibit detectable rescue of either mutant phenotype. A combination of immunoblotting and reverse transcriptase PCR amplification experiments (using poly[A] mRNA as PCR template) demonstrated that the T59A mutation, while not deleterious to the stability of the RdgB-PITP domain when expressed in E. coli (Fig. 2), nevertheless produced an unstable full-length RdgB in flies (data not shown). This destabilization precluded us from examining the effect of the T59A mutation on RdgB function in vivo.

Incorporation of the T59E mutation, however, did not affect the stable expression of either the full-length RdgB (RdgB-T59E) or RdgB-PITP (RdgB-PITP-T59E) (Table I). We compared the ERG light responses between newly

| Transgene-encoded protein | Percent of wild-type expression |
|---------------------------|---------------------------------|
| RdgB                      | 134.0 ± 8.0%                    |
| RdgB-T59E                 | 121.0 ± 15.0%                   |
| RdgB-PITP                 | 24.1 ± 3.9%                     |
| RdgB-PITP-T59E            | 42.4 ± 5.8%                     |
| PITPα-RdgB                | 116.4 ± 13.2%                   |

Head protein extracts were produced from rdgB2 flies expressing one copy of each transgene. Triplicate immunoblots of head extracts were performed using anti-RdgB antisera. Expression levels of RdgB and RdgB variants were quantitated by scanning laser densitometry and compared to the level of RdgB protein in wild-type flies. Because transgene expression was limited to the R1-6 photoreceptor cells, whereas the wild-type control expressed RdgB in the retina, antennae, and various regions in the brain (Vihletic et al., 1993), the actual levels of transgene-encoded proteins in the retina (relative to wild type) may be higher than indicated.
flies expressing T59E-containing proteins was significantly faster than rdgB2 flies, it was still two to three times slower than wild type (Fig. 3).

We also measured the effect of light saturation on the dark recovery of these flies. After 5 min of saturating light, wild-type flies yielded a normal ERG light-response amplitude with <30 s of dark recovery (Fig. 3). By contrast, the rdgB2 ERG amplitude was essentially eliminated by previous light treatment. Even after 5 min of dark recovery, the rdgB2 flies lacked any significant light response to subsequent light stimuli (Fig. 3). The rdgB2 flies required nearly 35 min of dark treatment to regenerate the wild-type ERG light-response amplitude (data not shown). This defective dark recovery was not a result of failure to return to baseline, as rdgB2 flies displayed responses that decayed to baseline before 35 min (data not shown).

Significantly, rdgB2 flies expressing either RdgB-T59E or RdgB-PITP-T59E elicited a small ERG light response after 30 s of dark treatment and one-third of the original ERG amplitude after 5 min of dark recovery (Fig. 3). A wild-type ERG response was restored to these flies after 6–12 min of dark treatment (not shown). Thus, both the RdgB-PITP-T59E and RdgB-T59E proteins effected a substantial, but nonetheless incomplete, rescue of both the ERG light-response termination and prolonged dark recovery times characteristic of rdgB2 flies.

The partial restoration of the ERG phenotype in rdgB2 flies expressing either T59E mutant protein was not translated into any significant protection from retinal degeneration. Retinal sections from 6-d-old rdgB2; P[rdgB-pitp]; P[rdgB-pitp-T59E], rdgB2; P[rdgB-T59E], rdgB2; P[rdgB-T59E], and rdgB2; P[rdgB-pitp-rdgB] flies raised in a 12 h light/dark cycle were examined by light microscopy (Fig. 4). All three genotypes displayed dark staining, photoreceptor cell bodies, small or missing rhabdomeres, and holes in the retina. Whereas the rdgB2; P[rdgB-pitp-T59E] retinas were phenotypically similar to the rdgB2 retinas, the rdgB2; P[rdgB-pitp-T59E] flies exhibited a more severe form of degeneration, with a greater number of holes and ommatidial disorganization. Because the levels of RdgB-T59E and RdgB-PITP-T59E exceeded the level of RdgB-PITP protein needed for suppressing retinal degeneration in rdgB2 flies (Table I), the inability to prevent degeneration was not a result of insufficient protein levels. As the T59E substitution did not measurably reduce the PI and PC transfer activities of the soluble RdgB-PITP in vitro (Fig. 2B), the failure of the T59E-containing proteins to fully rescue rdgB mutant phenotypes suggests that either the phospholipid transfer activities of RdgB-PITP are more sensitive to perturbations in vivo than in vitro, or that the phospholipid transfer activity is not sufficient to completely fulfill RdgB-PITP’s function in vivo.

**PITPα Cannot Substitute for the PITP Domain of RdgB In Vivo**

To further examine the role of PI and/or PC transfer in prevention of rdgB mutant phenotypes, we expressed in rdgB2 flies either the soluble rat brain PITPα or a full-length chimeric RdgB molecule containing the wild-type rat brain PITPα in place of the RdgB PITP domain (PITPα-RdgB). PITPα shares a 42% primary sequence identity with the RdgB-PITP domain (Vihitelc et al., 1993),
and exhibits the same phospholipid-transfer substrate spectrum as RdgB-PITP (Fig. 2 B). If the ability to bind and/or transfer PI and PC are the sole essential RdgB functions, then PITPa and/or PITPa-RdgB should fully rescue rdgB2 mutant phenotypes.

Whereas immunoblot analyses confirmed the stable expression of PITPa and PITPa-RdgB in rdgB2 flies (data not shown), neither protein restored a wild-type light response to rdgB2 flies (Fig. 3). The rdgB2; P[pitpa-rdgB] flies exhibited an ERG response to subsequent light stimulation that was similar to rdgB2 flies and required nearly 35 min of dark recovery to regenerate the initial light-response amplitude. However, the initial ERG light-response amplitude for rdgB2; P[pitpa-rdgB] flies was significantly smaller than rdgB2 flies (45 ± 12.5% of the maximal rdgB2 ERG amplitude, Fig. 3). These data suggest that the PITPa-RdgB protein caused an additional adverse effect on the photoreceptors and did not simply fail to fulfill the requirement for RdgB. The ERG light-response amplitude and dark recovery time for rdgB2; P[pitpa] flies were nearly identical to rdgB2 flies. Furthermore, rdgB2; P[pitpa-rdgB] flies exhibited the morphological hallmarks of rdgB-mediated retinal degeneration (Fig. 4), while rdgB2; P[pitpa] flies exhibited even smaller and fewer number of rhabdomeres. The failure of PITPa and PITPa-RdgB to even partially suppress either the rdgB2 ERG defects or the retinal degeneration further separates RdgB from the classical PITPs and suggests that the RdgB-PITP domain executes a phototransduction-relevant function that mammalian PITPa cannot.

Dominant Retinal Degeneration Phenotypes in Full-Length RdgB Mutants

We expressed all the above rdgB transgenes in a rdgB+ background to determine if they possessed a dominant mutant phenotype. We anticipated that the transgenes that previously failed to rescue the rdgB2 mutant phenotypes would behave as inactive forms of RdgB and would, therefore, exhibit a fully recessive character. Alternatively, these RdgB variants could disrupt potential protein–protein interactions or compete with the wild-type RdgB for a particular molecule, in which case, they would exhibit a dominant phenotype. We assayed for retinal degeneration by the loss of the deep pseudopupil and further characterized all of the flies using the ERG. We found that rdgB2 flies expressing any of the three soluble proteins (RdgB-PITP, RdgB-PITP-T59E, and PITPa; Fig. 3) resulted in a light-enhanced and dose-dependent loss of the ERG light response for 30 d after eclosion (data not shown). Because the RdgB-PITP-T59E and PITPa proteins lacked a dominant degeneration or ERG phenotype, the failure to completely rescue the rdgB2 mutant phenotypes must be due to an inability to function like RdgB-PITP, rather than causing degeneration through a novel mechanism.

Surprisingly, the rdgB-T59E transgene, which partially restored the ERG light response in rdgB2 flies, produced a dominant retinal degeneration. Expression of RdgB-T59E resulted in a light-enhanced and dose-dependent loss of the deep pseudopupil in rdgB2 flies. Degeneration of wild-type flies expressing one copy of P[rdgB-T59E] was first observed 4 d after eclosion under constant light conditions and at 13 d in a 12-h light/dark cycle (Fig. 5). The deep pseudopupil loss became increasingly prevalent so that essentially all flies in the population had experienced degeneration by 17 d after eclosion in constant light and by 26 d in a 12-h light/dark cycle. Retinal degeneration was never observed, under any circumstances, in wild-type fly controls (not shown). The severity of this dominant retinal degeneration phenotype was proportional to the ratio of P[rdgB-T59E]/rdgB+. Both the onset and rate of degeneration were accelerated in transgenic flies raised in constant light when the P[rdgB-T59E]/rdgB+ ratio was 2:1 rather

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than 1:1 (Fig. 5). Indeed, rdgB\(^+\) flies that possessed two copies of P[rdgB-T59E] lost their deep pseudopupil at essentially the same rate as did rdgB\(^2\) mutant flies raised in identical conditions.

Expression of the PITPa-RdgB protein in rdgB\(^2\) flies resulted in a decreased ERG amplitude relative to that observed in rdgB\(^2\) flies alone, suggesting that the chimeric protein was further detrimental to the rdgB\(^2\) photoreceptor (Fig. 3). We found that PITPa-RdgB expression in a wild-type background produced a dose-dependent dominant loss of the deep pseudopupil (Fig. 5). Flies with two copies of the pitpa-rdgB transgene first exhibited deep pseudopupil loss at 2 d after eclosion, while the onset of degeneration was not observed until 8 d after eclosion in flies expressing 1 pitpa-rdgB transgene (Fig. 5). Because expression of multiple rdgB-pitp-T59E and pitpa transgenes produced no dominant effects (data not shown), the RdgB-T59E- and PITPa-RdgB-associated dominant phenotypes seemingly required the carboxy-terminal region of RdgB.

**Unique Histological Abnormalities Associated with Dominant-Negative RdgB-T59E and PITPa-RdgB Alleles**

We compared the histology of the dominantly degenerating rdgB-T59E and PITPa-RdgB retinas with that of the wild-type and RdgB\(^2\) photoreceptors. Photoreceptors R1-6 (Leonard et al., 1995) of RdgB-retinal degenerations. White-eyed versions (cn bw) of wild-type (A), rdgB\(^2\) (B), rdgB\(^+\); P[rdgB-T59E] (C), and rdgB\(^+\); P[pitpa-rdgB] (D) flies were raised in a 12-h light/dark cycle for 6 d after eclosion. The rdgB\(^2\) retinal sections exhibited the reduction and loss of rhabdomeres, formation of holes (long arrows), and condensation of the photoreceptor cell bodies (black arrowheads). The rdgB\(^+\); P[rdgB-T59E] flies revealed a reduction in the size of the R1-6 outer rhabdomeres, with few holes appearing in the retina. The R1-6 cell bodies appear to be nearly the same in both the young and old retinas, which is roughly similar to the wild-type cell bodies. The rdgB\(^+\); P[pitpa-rdgB] flies showed signs of degeneration that more closely matched the rdgB\(^2\) mutant flies, with holes (long arrows) appearing in the retinal sections. Additionally, the microvillar rhabdomeres are beginning to unpack (white arrowheads). Bar, 10 \(\mu\)m.

![Figure 5](image)

**Figure 5.** Time course of the dominant RdgB-T59E and PITPa-RdgB retinal degenerations. The time course of retinal degeneration was determined based upon the loss of the deep pseudopupil. The fraction of flies that retained a wild-type deep pseudopupil was determined daily for 30 d after eclosion. Flies raised in constant light include rdgB\(^2\) (filled triangles), rdgB\(^+\) flies containing two copies of the P[rdgB-T59E] transgene (open squares), rdgB\(^+\) flies containing one copy of the P[rdgB-T59E] transgene (filled squares), rdgB\(^+\) flies containing two copies of the P[pitpa-rdgB] transgene (open circles), and rdgB\(^+\) flies containing one copy of the P[pitpa-rdgB] transgene (filled circles). The standard deviation is shown as vertical lines. Each point represents the average of four trials (n > 25 flies per trial). The standard deviation is shown as vertical lines.

![Figure 6](image)

**Figure 6.** Histology of the dominant RdgB-T59E and PITPa-RdgB retinal degenerations. White-eyed versions (cn bw) of wild-type (A), rdgB\(^2\) (B), rdgB\(^+\); P[rdgB-T59E] (C), and rdgB\(^+\); P[pitpa-rdgB] (D) flies were raised in a 12-h light/dark cycle for 6 d after eclosion. The rdgB\(^2\) retinal sections exhibited the reduction and loss of rhabdomeres, formation of holes (long arrows), and condensation of the photoreceptor cell bodies (black arrowheads). The rdgB\(^+\); P[rdgB-T59E] flies revealed a reduction in the size of the R1-6 outer rhabdomeres, with few holes appearing in the retina. The R1-6 cell bodies appear to be nearly the same in both the young and old retinas, which is roughly similar to the wild-type cell bodies. The rdgB\(^+\); P[pitpa-rdgB] flies showed signs of degeneration that more closely matched the rdgB\(^2\) mutant flies, with holes (long arrows) appearing in the retinal sections. Additionally, the microvillar rhabdomeres are beginning to unpack (white arrowheads). Bar, 10 \(\mu\)m.
ard et al., 1992; Kumar and Ready, 1995). The dominant pitpa-rdgB degeneration morphology was more similar to the rdgB phenotype, with the most striking defects being the numerous perforations in the retina and the reduction in R1-6 rhabdomere size relative to R7 (Fig. 6 D). Additionally, the R1-6 microvillar rhabdomeres began to exhibit signs of unpacking (Fig. 6 D) that we had not previously observed in any rdgB mutants. Thus, while the dominant rdgB-T59E mutant phenotype approximated the ninaE hypomorphic phenotype, the dominant pitpa-rdgB phenotype was morphologically more like the rdgB mutant retina with some additional mutant characteristics.

**Unique Electrophysiological Abnormalities Associated with Dominant-negative RdgB-T59E and PITPα-RdgB Alleles**

We examined whether the rdgB; P[rdgB-T59E] and/or the rdgB; P[pitpa-rdgB] flies exhibited an electrophysiological defect. All the flies that were tested were newly eclosed and subjected to ERG analysis after a 1-h dark adaptation period. Whereas the light-response amplitudes of rdgB; P[rdgB-T59E] flies were reduced by ~40% of wild type (Fig. 7, B and A, respectively), the rdgB; P[pitpa-rdgB] flies were essentially wild type (Fig. 7 C). Wild-type flies expressing multiple copies of either P[rdgB-pitp] (Fig. 7 D) or P[rdgB+] (data not shown), failed to mimic the reduced amplitude observed for rdgB; P[rdgB-T59E] flies, demonstrating the specificity of the T59E mutation. Moreover, extension of these ERG analyses to different light intensities failed to produce the wild-type light-response amplitudes in rdgB; P[rdgB-T59E] flies (Fig. 7 E). These data demonstrated that expression of RdgB-T59E in a rdgB+ background resulted in a reduced photosensitivity, while PITPα-RdgB failed to effect the light-response amplitude.

Because the reduced photosensitivity (like the small rhabdomeres) was consistent with reduced functional rhodopsin, we compared the ERG light responses between white-eyed (cn bw) versions of wild-type (A), ninaE+ (which fails to express any of the R1-6 opsin), (B), rdgB; P[rdgB-T59E] (C), and rdgB; P[pitpa-rdgB] flies (D) were tested for the ERG light response using 5 s of either orange (a) or blue light (b) stimulation. Wild-type flies exhibit both a PDA and inactivation of the R1-6 light response by blue light. The ninaE flies possess neither the PDA nor the blue light inactivation. The rdgB; P[rdgB-T59E] flies also fail to exhibit a PDA and R1-6 inactivation by blue light. The rdgB; P[pitpa-rdgB] flies appear to possess a PDA, but they failed to rapidly return to baseline after the subsequent orange light stimulus. A 5-mV scale is shown at the bottom.
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Figure 9. The dominant RdgB-T59E protein preferentially affects rhodopsin protein levels. Immunoblot analyses were performed on head extracts from <1-d-old dark-raised flies of the following genotypes: rdgB− (wild-type), ninaE17, rdgB+; [rdgB-T59E], rdgB−; P[rdgB-T59E], rdgB−; P[piipa-rdgB], and rdgB+; P[rdgB−]. Two head equivalents per sample were electrophoresed, transferred to nitrocellulose, and incubated with either anti-NinaE polyclonal or anti-Trp polyclonal antisera. Triplicate blots were used to generate the average percent of wild-type protein and standard deviation.

Figure 10. The dominant PITPα-RdgB protein prevents the rapid recovery of the light-response amplitude after prolonged saturating-light treatment. A 2-s ERG light response was recorded from wild-type, rdgB−; P[rdgB-T59E], rdgB−; P[piipa-rdgB], and rdgB−; P[rdgB−] flies, followed by 20 min of saturating light, 30 s of dark recovery, and another 2-s ERG light-response recording. The difference was determined between the first and second light-response recordings. Five flies of each genotype were recorded with the average difference in the light-response amplitude and standard deviation shown. The average initial light-response amplitudes were: wild-type (28.2), rdgB−; P[rdgB-T59E] (19.8), rdgB−; P[piipa-rdgB] (26.8), and rdgB−; P[rdgB−] (22.6 mV). The increased light-response amplitude of rdgB−; P[rdgB-T59E] flies in this figure, relative to Fig. 7, is due to the use of white-eyed flies in this data (cn bw background) and wild-type eye colored flies in Fig. 7. The other three genotypes contained some screening pigment.

type levels, Fig. 9). Also, rdgB2 null mutants failed to show reduced steady-state levels of rhodopsin (Fig. 9), which demonstrates that functional RdgB is not required for producing the wild-type rhodopsin levels. We also found that rdgB+; P[rdgB-T59E] flies elicited near wild-type levels of the trp-encoded Cα2 channel (Fig. 9), the dgg-encoded Go subunit, the gbe-encoded Gβ subunit, the rdgC-encoded serine/threonine phosphatase, and the ninaC-encoded unconventional myosins, suggesting that rhodopsin is selectively sensitive to expression of RdgB-T59E (data not shown). Therefore, the dominant retinal degeneration phenotype, the reduced photoreceptor sensitivity, loss of the PDA, and the gross reduction in mature rhodopsin levels in rdgB+; P[rdgB-T59E] flies all resulted from expression of RdgB-T59E and its interaction with some other protein and/or organelle.

The rdgB+; P[piipa-rdgB] flies appeared to have a PDA (Fig. 8 D), which was consistent with those flies expressing wild-type levels of rhodopsin (Fig. 9). However, the light response in rdgB+; P[piipa-rdgB] flies remained inactivated after the conversion of metarhodopsin to rhodopsin by the orange light stimulus (Fig. 8 D). To further examine the effect of PITPα-RdgB on the dark recovery, ERGs to a 2-s light stimulus were recorded from flies, before and 30 s after a 20-min saturating light stimulus. The rdgB+; P[piipa-rdgB] flies exhibited a significant difference between the initial and final ERG amplitudes (13.0 mV, Fig. 10), which was 49% of the initial amplitude. Only minor amplitude differences were observed for wild-type (2.6 mV, 9% of initial amplitude), rdgB+; P[rdgB-T59E] (3.8 mV, 19% of initial amplitude), and rdgB−; P[rdgB−] (3.1 mV, 14% of initial amplitude) flies (Fig. 10). The rdgB+; P[piipa] flies, which lacked the dominant degeneration phenotype, were similar to the wild-type controls showing a difference of only 3.1 mV (14% of the initial amplitude) under the same regimen. These data indicated that PITPα-RdgB expression negatively affected the recovery phase of the light response in an otherwise wild-type photoreceptor cell. It is unclear if there is a direct relationship between this electrophysiological defect and the retinal degeneration.

Discussion

In this manuscript, we detail a functional analysis of RdgB participation in the Drosophila phototransduction cascade and a description of the physiological function of a meta-
RdgB is Required for Proper Termination of the Light Response and Dark Recovery of the Photoreceptor Cell

Newly eclosed, dark-adapted rdgB null mutant flies displayed ERG light responses that had wild-type amplitudes, but were defective in photoreceptor cell repolarization after termination of the light stimulus (Fig. 3). This indicates that the RdgB PITP domain is not essential for establishing an initial PIP₂ pool in the rhabdomere, though its requirement for regeneration of this pool remains a formal possibility. We do not presently favor the idea that RdgB functions in regeneration of such a phosphoinositide pool because RdgB levels do not correlate closely with the gain of the light response. The amplitude size of the light response is controlled by levels of PI as observed by flies overexpressing the rate-limiting enzyme for PI biosynthesis, eye-CDP-diacylglycerol synthase (CDS), producing greater ERG amplitudes in response to light stimulation (Wu et al., 1995). Correspondingly, mutants that are defective in eye-CDS activity exhibit smaller light-response amplitudes (Wu et al., 1995).

If RdgB has a critical role in replenishing rhabdomeric PI, then light saturation treatment, and therefore consumption of rhabdomeric PI by its conversion into PLC substrate, would reduce or eliminate subsequent light responses in rdgB mutant flies. Indeed, rdgBª and all rdgB² mutant flies containing nonrescuing transgenes produced wild-type amplitude responses 6 to 35 min after light saturation treatment, as compared to 30 s for wild-type flies (Fig. 3). Clearly, the analyses in rdgBª null flies are consistent with a role for RdgB in restoring the cell to a state competent for subsequent light stimulation. However, if RdgB activity is critical in replenishing rhabdomeric PI, then overexpression of RdgB, as observed for eye-CDS, should increase the amplitude of light responses in rdgBª flies. Wild-type flies expressing multiple copies of [rdgB-\text{pitp}] (Fig. 7 D) or [rdgBª] (data not shown) failed to produce ERG light-response amplitudes that were significantly larger than wild type. Additionally, we did not detect any significant differences in the ERG light-response amplitude of rdgBª or rdgB²; [rdgBª] flies that were recorded over a background light (data not shown). We expected the background light would deplete the PIP₂ stores, which would result in either slower kinetics or a smaller light-response amplitude in rdgBª flies that would be compensated for by the increased copy number of RdgB. Since overexpression of RdgB did not affect the gain of the cascade, either protein-mediated PI transfer is not limiting in phototransduction activation or RdgB does not function in this respect. Additionally, the lack of rescue by RdgB-PITP-T59E and PITPα suggests that the RdgB-PITP possesses activities separate from those characterized biochemically. Our collective data fail to support the speculation that RdgB functions in the transport of PI to specific rhabdomeric pools for phototransduction-driven consumption (Hurley, 1995; Zuker, 1996).

It remains unresolved as to how RdgB promotes termination of the light response and rapid dark recovery of the photoreceptor cell, though it is clear that all of these functions reside within the RdgB-PITP domain. This domain contains multiple consensus PKC phosphorylation sequences. One site is T59, which when mutated to T59E compromises an RdgB activity in vivo, without affecting PI transfer in vitro. As RdgB’s placement in phototransduction is downstream of PKC, and since PKC is required for the wild-type rapid inactivation of the photoresponse following light cessation, the RdgB-dependent inactivation of the light response may be regulated by a phosphorylation/dephosphorylation cycle (Ranganathan et al., 1991; Smith et al., 1991; Hardie et al., 1993). If RdgB is regulated at T59 by phosphorylation in vivo, then the failure of PITPα to prevent rdgB phenotypes could result from PKC phosphorylation. Unlike RdgB-PITP, the T59E mutation essentially eliminated PI transfer in PITPα in vitro (Alb et al., 1995). In the fly, the phosphorylation of PITPα may lock the protein in a PI transfer incompetent state. The failure of the PI transfer competent RdgB-PITP-T59E protein to rescue may have resulted from loss of regulation at T59 pertinent to another RdgB-PITP activity. Thus, the failure of the RdgB-PITP-T59E and PITPα to rescue rdgBª...
mutant flies does not unambiguously rule out a role for RdgB-associated PI and/or PC transfer activity in vivo. Rather, the data indicate that other activities present in RdgB-PITP are required for wild-type RdgB function.

**RdgB Is Not Simply an Integral Membrane PITP**

RdgB-PITP catalyzes the efficient transfer of both PI and PC between membrane bilayers in vitro, but is unable to catalyze intermembrane transfer of SM (Fig. 2B). In this regard, RdgB-PITP is biochemically analogous to mammalian PITPa, and not to PITPB. Because RdgB-PITP represents the sole RdgB domain essential for normal function of the fly visual cycle (Fig. 1D), it is of primary interest to determine how the PI and/or PC transfer activities of RdgB-PITP contribute to function. Although our initial efforts to selectively inactivate the PI transfer activity of RdgB (using the T59A mutation) and assess function in vivo were unsuccessful, our data with RdgB-PITP-T59E and PITPa suggest that RdgB-PITP does not simply function to transfer phospholipids in the photoreceptor cell. Neither stable expression of RdgB-PITP-T59E nor PITPa, both of which are fully active for PI and/or PC transfer in vitro, could completely rescue the rdgB2 retinal degeneration phenotype (Fig. 4), contrary to expectations that catalysis of PI and/or PC transfer was the sole function of RdgB-PITP. It remains entirely possible that phospholipid binding serves as a molecular switch through which a second effector activity of RdgB-PITP is regulated, as has been proposed for Sec14p (McGee et al., 1994; Skinner et al., 1995; Kearns et al., 1997). It is similarly possible that the PI and/or PC bound states of RdgB-PITP may regulate an activity in the photoreceptor cell that is not responsive to mammalian PITPa presented in the RdgB context.

Although RdgB-T59E and PITPa-RdgB were both unable to phenotypically rescue rdgB2-associated retinal degeneration, these polypeptides nonetheless exhibited significant phenotypic differences at the level of the ERG light response. Expression of the PITPa-RdgB chimera in rdgB2 flies failed to affect any detectable alleviation of the rdgB2 light-response termination and dark-recovery defects, while also significantly reducing the amplitude of the light response relative to rdgB2 (Fig. 3). This suggests that the PITPa-RdgB chimera was interacting with either a molecule and/or organelle to further reduce the photoreceptor’s ability to respond to light. On the other hand, RdgB-T59E expression significantly (but not completely) improved proper termination of the light response and shortened the dark recovery time required for regeneration of a normal light response following a saturating light stimulus to rdgB2 flies (Fig. 3). Thus, while RdgB-T59E exhibited partial function in the ERG assays, the data further suggest that either: (a) the retinal degeneration phenotype is a more sensitive indicator of RdgB-PITP perturbation than the ERG light response, or (b) that the aberrant ERGs recorded are not intimately related to the mechanism of retinal degeneration.

**Dominant Mutant Forms of the RdgB-PITP Domain Reveal Unexpected and Diverse Degeneration Phenotypes**

Expression of either RdgB-T59E or PITPa-RdgB in rdgB+ flies exerted powerful effects that were manifested in dominant retinal degeneration phenotypes and proved informative with regard to previously unappreciated aspects of RdgB function. In the case of RdgB-T59E, the associated dominant effects operated through the reduction of rhodopsin protein levels in photoreceptors R1-6; the primary evidence involved direct measurements of steady-state rhodopsin levels in mutant flies (Fig. 9). Additional observations include: (a) the reduced photosensitivity of rdgB+: P[rdgB-T59E] flies (Figs. 7, B and E); (b) the aberrant ERG responses of rdgB+: P[rdgB-T59E] flies that bore the signature of R1-6 opsin insufficiency (Fig. 8 C); and (c) the observation that rdgB+: P[rdgB-T59E] retinas exhibited degeneration morphologies closely resembling those associated with hypomorphic ninaE alleles (Fig. 6 C). However, this dominant mutation did not exert a general effect on the biogenesis and/or transport of rhodomorphic proteins because the levels of the trp-encoded Ca2+ channel (Fig. 9), Gq, Gβe, NimaC p174, and the rdgC-encoded serine/threonine phosphatase were unaffected (data not shown). Because RdgB is not required for rhodopsin expression, as young rdgB2 null mutants exhibit near wild-type steady-state levels of rhodopsin on immunoblots (Fig. 9), the specificity and mechanism of rhodopsin depletion by RdgB-T59E is unclear.

The dominant retinal degeneration phenotype associated with expression of the PITPa-RdgB chimera molecule was distinct from that of RdgB-T59E. Retinal sections of rdgB2: P[pitpa-rdgB] flies bore morphological hallmarks of degeneration (Fig. 6) that more closely resembled those associated with rdgB2 rather than ninaE, which is consistent with the wild-type steady-state rhodopsin levels in rdgB2: P[pitpa-rdgB] flies (Fig. 9). Additionally, the rdgB2: P[pitpa-rdgB] flies exhibited an ERG dark-recovery defect that was similar, but much more subtle, to rdgB2 mutants (Fig. 10). However, to detect the loss of the ERG light-response amplitude in rdgB2: P[pitpa-rdgB] flies, the flies were exposed to a very prolonged saturating light stimulus (20 min), rather than the 5 min used in Fig. 3. Under these conditions, the rdgB2: P[pitpa-rdgB] flies exhibited a 49% reduction in the light-response amplitude compared to only a 9–19% reduction in three other genotypes. The rdgB2: P[pitpa-rdgB] flies also exhibited a very slow rate of light-response inactivation to the second orange light stimulus (Fig. 8 D). Thus, PITPa-RdgB appeared to antagonize RdgB activity in the photoreceptor cell.

Taken together, the data indicate that RdgB-T59E strongly interferes with rhodopsin biogenesis, while PITPa-RdgB potently interferes with the activity of wild-type RdgB. Both of these dominant phenotypes suggest that RdgB physically interacts with at least one other component of the *Drosophila* phototransduction cascade. The failure to observe any dominant phenotypes associated with either RdgB-PITP-T59E or PITPa suggests that the integral membrane nature of these proteins must be critical for these molecular interactions. Because RdgB-T59E and PITPa-RdgB have dramatically different effects in the cell, RdgB’s large carboxy-terminus (which is common to both proteins) likely places the mutant protein in the proper spatial environment and the different attached PITP domains confer the phenotypes. While the identities of RdgB-interacting proteins remain unknown, the recent identification of novel
mutations that suppress rdgB defects may provide relevant clues (Paetka, D., V. Elagin, and D.R. Hyde, unpublished data). In addition, these findings provide the first demonstration that dominant mutant forms of PITP molecules can be generated and that these can yield informative phenotypes when expressed in a eukaryotic cell.

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