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Isolation and Characterization of Drosophila *retinal degeneration B* Suppressors

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ABSTRACT

The Drosophila retinal degeneration B protein (RdgB) is a novel integral membrane phosphatidylinositol transfer protein required for photoreceptor cell viability and light response. We isolated one intragenic suppressor (*rdgB*^{su100}) and four autosomal suppressors of the hypomorphic *rdgB*^{KS222} retinal degeneration phenotype. The *rdgB*^{su100} suppressor dramatically slowed *rdgB*^{KS222}'s photoreceptor degeneration without significantly improving the electroretinogram (ERG) light response. One autosomal recessive suppressor [*su(rdgB)69*] significantly slowed *rdgB*^{KS222} retinal degeneration and restored the ERG light response near to that of the wild type. Unlike all the previously characterized *rdgB* suppressors, the four new autosomal suppressors do not affect the ERG light response in *rdgB*$^{+}$ flies. Only *Su(rdgB)116* exhibited a mutant phenotype in a *rdgB*$^{+}$ background, which was smaller R1-6 rhabdomeres. We also examined the extent to which two previously identified visual transduction mutations suppressed *rdgB* retinal degeneration. Absence of one of the light-activated calcium channels (*trp*^{CM}) slowed the onset of *rdgB*-dependent degeneration. However, loss of protein kinase C (*inaC*^{209}), which blocks photoreceptor cell deactivation, desensitization, and light adaptation, failed to suppress *rdgB* degeneration under normal light conditions. This demonstrates that TRP activity, but not INAC, is required for rapid *rdgB*-dependent degeneration.

THE Drosophila *retinal degeneration B* (*rdgB*) mutant exhibits light-enhanced retinal degeneration and an abnormal light response. The degeneration, which begins at the photoreceptor cell's synaptic terminal, is histologically apparent 3–4 days after eclosion (Hotta and Benzer 1970; Harris and Stark 1977; Stark and Carlson 1982). However, *rdgB*'s electroretinogram (ERG) light response is defective within hours after eclosion and completely lost within the first day (Harris and Stark 1977; Milligan *et al.* 1997). This suggests that the degeneration is a likely consequence of the photoreceptor's abnormal light response physiology.

Previous genetic and biochemical data suggest that the RdgB protein functions subsequent to protein kinase C (PKC) in the visual transduction cascade. Mutations in either the *ninaE*-encoded R1-6 opsin (O'Tousa *et al.* 1985; Zuker *et al.* 1985) or the *norpA*-encoded phospholipase C (Bloomquist *et al.* 1988) suppress the *rdgB* retinal degeneration phenotype (Harris and Stark 1977; Stark and Sapp 1989). Additionally, a constitutively active $DG_q\alpha$ mutation stimulates rapid *rdgB* retinal degeneration in the dark (Lee *et al.* 1994). The *inaC* mutation also weakly suppresses *rdgB*-dependent retinal degeneration (Smith *et al.* 1991). The *inaC* gene encodes a retinal-specific PKC that is required for photoreceptor deactivation, desensitization, and light adaptation (Smith *et al.* 1991; Hardie *et al.* 1993). Consistent with this result, application of a phorbol ester also induces rapid *rdgB*-dependent retinal degeneration in the dark, presumably by stimulating PKC (Minke *et al.* 1990). Furthermore, mutation of a putative PKC phosphorylation site in RdgB (threonine 59 to glutamic acid) dramatically reduces RdgB activity *in vivo* (Milligan *et al.* 1997). PKC and all other known phototransduction components, excluding the ryanodine receptor (Arnon *et al.* 1997), are localized to the rhabdomere (reviewed in Hyde *et al.* l995; O'Tousa 1997). However, RdgB was immunolocalized to the subrhabdomeric cisternae (SRC), an extension of the endoplasmic reticulum that lies adjacent to the rhabdomere (Vihtelic *et al.* 1993; Suzuki and Hirosawa 1994). It is presently unclear how the visual transduction cascade regulates RdgB in the spatially distinct SRC.

RdgB's photoreceptor cell function is unknown. The RdgB protein contains six putative transmembrane domains with both the N and C termini in the cytosol between the SRC and rhabdomere (Vihtelic *et al.* 1991, 1993). The N terminus possesses two distinct domains. One domain binds Ca^{2+} *in vitro* (Vihtelic *et al.* 1993). The presence of this domain and the finding that voltage-gated calcium channel blockers inhibit *rdgB*-mediated retinal degeneration (Sahly *et al.* 1992) suggest that Ca^{2+} is involved in RdgB function. The second domain is composed of the N-terminal 276 amino acids, which are >40% identical with the rat brain phosphatidylinositol transfer protein (PITPα; Vihtelic *et al.* 1993). Unlike RdgB, all previously characterized PITPs are 30- to 35-kD soluble proteins (Bankaitis *et al.* 1989; Cleves *et al.* 1991; Wirtz 1991). RdgB's N terminus,

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expressed as a soluble protein (RdgB-PITP), possesses phosphatidylinositol transfer activity *in vitro* (Vihtelic *et al.* 1993; Milligan *et al.* 1997). Thus, RdgB defines a new class of integral membrane PITPs. Expression of this soluble RdgB-PITP is sufficient to suppress both the retinal degeneration and ERG light response phenotypes in $rdgB^2$ null mutants (Milligan *et al.* 1997). However, the phospholipid transfer activity is not RdgB's critical function *in vivo* (Milligan *et al.* 1997). Recently, mouse and human *rdgB* homologs that contain over 40% amino acid identity with Drosophila RdgB were identified (Chang *et al.* 1997; Guo and Yu 1997). Expression of the mouse *rdgB* cDNA suppressed the *rdgB*-dependent degeneration and ERG mutant phenotypes in flies (Chang *et al.* 1997). This functionally equivalent vertebrate RdgB homolog suggests that the entire RdgB molecule is important to a basic function in both invertebrate and vertebrate photoreceptors.

To further elucidate RdgB's role in the photoreceptor cell, we identified five new suppressors of the *rdgB*-mediated retinal degeneration phenotype. One intragenic suppressor, which possessed two missense mutations in the first putative intralumenal loop of RdgB, slowed the rapid $rdgB^{KS222}$ photoreceptor degeneration without significantly altering the defective light response. We also isolated two dominant and two recessive autosomal suppressors. One suppressor [*su(rdgB)69*] significantly slowed $rdgB^{KS222}$ retinal degeneration and restored the *rdgB* ERG light response to nearly that of the wild type. We further examined the genetic relationship of the visual transduction cascade and *rdgB*-mediated retinal degeneration. We found that the *trp* mutation, but not *inaC*, significantly slowed *rdgB* degeneration. This suggests that the light-induced Ca^{2+} entry into the photoreceptor cell stimulates *rdgB* degeneration, while PKC activity is not absolutely required to activate the *rdgB*-dependent degeneration.

MATERIALS AND METHODS

Scoring retinal degeneration: Wild-type and vermilion eye-colored flies of various genotypes were collected daily and raised under either constant light or a 12-hr light:dark cycle. The flies were scored daily for the presence of a deep pseudopupil, which is a virtual image of the rhabdomeres from several adjacent ommatidia (Franceschini 1972). The percent of flies that retained their deep pseudopupil (dpp^+) for a given day was calculated. At least three replicates of 25–150 flies, with a minimum of 100 flies total, were analyzed for each genotype to determine the average percent of dpp^+ flies and standard deviation for each day. White-eyed flies ($inaC^{209}$)were illuminated with blue light to score the presence or absence of a dark pseudopupil (O'Tousa 1997).

Retinal degeneration was also examined by both light and electron microscopy of retinal tissue sections. Flies were raised in a 12-hr light:dark cycle and then decapitated. The heads were bisected, fixed, and embedded in Polybed 812 as described previously (Lee *et al.* 1994). For light microscopy, 2-μm sections were stained with 1% methylene blue and 1% azure II. For electron microscopy, 0.5- to 0.8-μm sections were stained with 4.9% uranyl acetate for 5 min, rinsed in methanol, incubated in 1% lead citrate for 2 min, and rinsed in 0.1 m NaOH, followed by a water rinse.

Electrophysiology: ERGs were performed as described (Larrivee *et al.* 1981; Blake *et al.* 1991; Zars and Hyde 1996). One- to two-day-old flies raised in a 12-hr light:dark cycle were prepared under dim red light and dark adapted 4 min before stimulation with white light (1.2×10^{-3} W/cm^2). Average light response amplitudes were calculated from recordings of at least five different flies, with representative light response recordings shown.

PCR amplification and DNA sequencing: The *rdgB* gene was PCR amplified from wild-type (Oregon-R), $rdgB^{KS222}$, and $rdgB^{su100}$ genomic DNAs in four overlapping clones using Taq DNA Polymerase (Fisher Biotech, Pittsburgh, PA), and primers based on the *rdgB* sequence (Vihtelic *et al.* 1991). To minimize PCR errors, three independent PCR reactions were performed on each *rdgB* genomic fragment from all three genotypes. The PCR products were cloned into pCR2.1 (Invitrogen, Carlsbad, CA) and sequenced. Reverse transcription PCR (RT-PCR) was performed on mRNA isolated from $rdgB^{KS222}$ and $rdgB^{su100}$ flies to confirm the presence of the Gln147term mutation in the mRNA. Poly(A)$^+$ mRNA was isolated from 50 $rdgB^{KS222}$ and $rdgB^{su100}$ fly heads using the QuickPrep Micro mRNA purification kit (Pharmacia Biotech, Piscataway, NJ). First-strand cDNA and the PCR reaction were carried out sequentially using reverse transcriptase, oligo(dT) primers, and the SuperScript Preamplification system (GIBCO BRL, Gaithersburg, MD). The *rdgB* PCR primer sequences were 5′ GAGTCGC GAGGAGAGCCAT GGCG 3′ and 5′ TGCTTGGGATCCTCCT CCTTCAC 3′, which correspond to nucleotides 84–106 and 460–482, respectively (Vihtelic *et al.* 1991). The PCR products were cloned into the pCR2.1 vector.

DNA sequencing was performed by the dideoxy chain-termination method (Sanger *et al.* 1977) using either single-stranded or double-stranded plasmid template DNA with the Sequenase version 2.0 sequencing kit (Amersham, Arlington Heights, IL). While sequencing the wild-type *rdgB* cDNAs and genomic clones, we identified three nucleotide differences relative to the original published sequence (Vihtelic *et al.* 1991). First, an additional C is present at position 3161. Second, an additional GC is inserted at position 3470. Third, the G at position 3472 is not present. Numbering of the *rdgB* nucleotides corresponds to the numbering found in Vihtelic *et al.* (1991). These changes increase the open reading frame an additional 584 bp to a TGA codon at position 3751-3753, which encodes a putative protein of 1250 amino acids and now agrees with the rdgB sequence of Rubboli *et al.* (1997).

Generation of suppressor mutants: Suppressors were generated using an F_3 free recombination mutagenesis scheme (Ashburner 1989). Male $rdgB^{KS222}$ flies were starved for ~6 hr, fed 25 mm EMS (Sigma, St. Louis, MO) in a 0.1% sucrose solution overnight, and mated *en masse* to $rdgB^{KS222}$; *SM1*/*Gla* virgin females. $rdgB^{KS222}$; *SM1* or $rdgB^{KS222}$; *Gla* F_1 males and virgin females were pair mated, and the resulting F_2 offspring were mated *inter se*. The F_3 progeny were raised 5–8 days in a 12-hr light:dark cycle before deep pseudopupil analysis (Franceschini 1972). Under these conditions, all $rdgB^{KS222}$ flies lacked the deep pseudopupil by 3 days after eclosion.

Mapping of suppressor mutations: F_3 flies possessing a deep pseudopupil were individually mated to $rdgB^{KS222}$; *SM1*/*Sco*; *TM2*/*Sb* flies. F_1 $rdgB^{KS222}$; *SM1*; *TM2* virgin females and $rdgB^{KS222}$; *Sco*; *Sb* males were mated. The F_2 progeny were raised 5–8 days in a 12-hr light:dark cycle and scored for the presence or absence of the deep pseudopupil. Segregation of the dpp^+ phenotype from the dominantly marked second and third chromosomes assigned the mutation to a chromosome and determined the dominant-recessive nature of the suppressors.

Complementation: All *X* chromosome suppressors were tested for complementation with *norpA* by mating male suppressor flies to virgin female $norpA^{P41}$ (Lindsley and Zimm 1990) flies and analyzing the ERG of the female progeny. Third chromosome suppressors were tested for complementation with *ninaE* by mating male suppressor flies to virgin female w^{1118}; $ninaE^{I17}$ flies (O'Tousa *et al.* 1985) and analyzing the male progeny for the presence of a dark pseudopupil (O'Tousa 1997). The *su(rdgB)69* and *Su(rdgB)116* mutations are not *trp* and *dgq* alleles, respectively, because they complemented the corresponding ERG and retinal degeneration phenotypes.

Recombination mapping: Each of the suppressor stocks was individually crossed to a stock that is homozygous for one of the following multiply marked chromosomes (in a $rdgB^{KS222}$ background): *y cv v* $rdgB^{+}$ *f, al b cn sp,* and *ru h th st cu sr e ca* (*X*, second and third chromosomes, respectively). The resulting F_1 heterozygous females were crossed to $rdgB^{KS222}$ stocks to score for the presence of the suppressor mutation by deep pseudopupil. Males both possessing and lacking a deep pseudopupil were individually mated to the multiply marked chromosomal stocks to score the presence or absence of all the recessive markers.

Immunoblots: Immunoblots to detect RdgB protein expression were performed essentially as described (Lee *et al.* 1994). Heads from two newly eclosed (<8 hr old), dark-raised flies were homogenized in 10 μl extraction buffer (2.3% SDS, 10% glycerol, 62.5 mm Tris-HCl, pH 6.8, 1 mm EGTA, and 0.01% bromophenol blue). The homogenate was incubated at 37° for 1 hr, centrifuged briefly, and resolved on a 5% polyacrylamide-SDS gel (Laemmli 1970). Proteins were transferred to nitrocellulose with a semidry transfer apparatus (Bio-Rad, Hercules, CA) at 17 V for 40 min. The membrane was blocked for 2 hr in 5% nonfat dry milk in TBS (20 mm Tris-HCl, pH 7.5, 500 mm NaCl), followed by washing twice for 20 min in TTBS (0.05% Tween 20 in TBS). The membranes were incubated overnight at room temperature in a 1:10 dilution of anti-RdgB monoclonal supernatant. The membranes were washed three times (10 min each) with TTBS and incubated for 2 hr with goat anti-mouse alkaline phosphatase-conjugated secondary antibody (Sigma) diluted 1:3,000 in 2% nonfat dry milk in TBS. The membranes were washed twice for 5 min and once for 15 min with TTBS. A final 5 min wash with 0.1 m Tris-HCl, pH 9.5, preceded colorimetric detection (Bio-Rad). RdgB protein levels were determined by scanning three independent immunoblots on a Pharmacia/LKB Ultrascan laser densitometer.

RESULTS

Isolation of novel *rdgB* suppressors: The Drosophila RdgB protein is a novel integral membrane PITP (Vihtelic *et al.* 1993; Milligan *et al.* 1997). To examine RdgB's role in light response and photoreceptor cell viability, we performed an F_3 free recombination screen (Ashburner 1989) to identify suppressors of the $rdgB^{KS222}$-mediated retinal degeneration. The $rdgB^{KS222}$ allele, which is a hypomorphic mutation based on its phenotype relative to other *rdgB* alleles (Harris and Stark 1977; Vihtelic *et al.* 1993), expresses ~20% of wild-type-sized RdgB protein relative to the wild type (Figure 1A). We identified 12 suppressors from 3204 independent F_3 families (Table 1). As expected from previous work (Harris and Stark 1977; Stark and Sapp 1989), both *norpA* (three alleles) and *ninaE* (one allele) mutations were identified (Table 1). The remaining six recessive and two dominant mutations represent previously unidentified *rdgB* suppressors. One recessive suppressor ($rdgB^{su100}$) is *X* linked. The seven autosomal suppressors comprise four complementation groups with one recessive and one dominant locus on both the second and third chromosomes.

The $rdgB^{su100}$ is an intragenic suppressor of $rdgB^{KS222}$: The $rdgB^{su100}$ mutation was intriguing for two reasons: first, it was an *X*-linked suppressor that was not a *norpA* allele and second, recombination mapping placed it very close to the $rdgB^{KS222}$ mutation. In fact, we failed to separate the $rdgB^{KS222}$ and $rdgB^{su100}$ mutations among 3845 recombinants. However, $rdgB^{su100}$ behaved as an unusual *rdgB* allele because it was a recessive rather than a dominant suppressor.

To determine the molecular nature of this mutation, we sequenced the $rdgB^{KS222}$ and $rdgB^{su100}$ alleles. While the $rdgB^{KS222}$ allele contains a single nonsense mutation (Gln147TAG) within the PITP domain (Figure 1B), im-

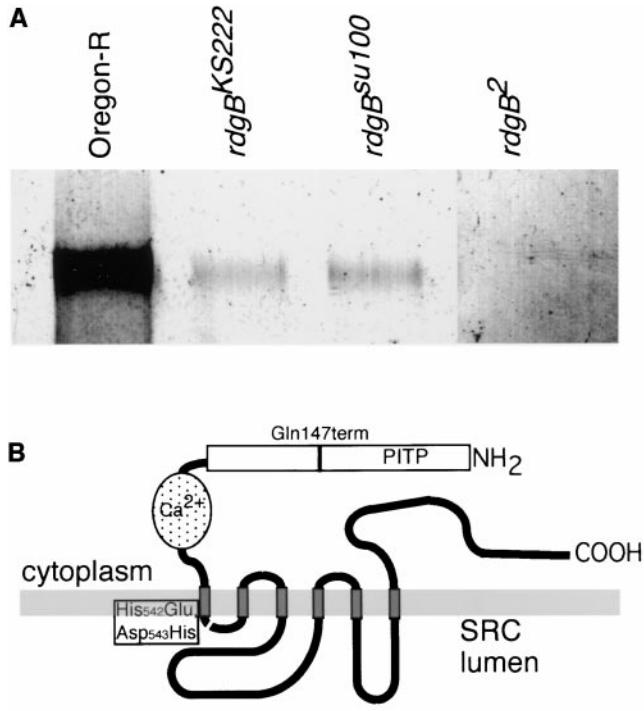

Figure 1.—Molecular characterization of the $rdgB^{KS222}$ and $rdgB^{su100}$ mutants. (A) Protein extracts from (lane 1) 2-day-old Oregon-R (wild type), (lane 2) 2-day-old $rdgB^{KS222}$, (lane 3) 2-day-old $rdgB^{su100}$, and (lane 4) 2-day-old $rdgB^{2}$ (a null allele) flies were tested by immunoblots with anti-RdgB monoclonal antibody. Equivalent levels of protein, ~15% of wild type, were found in the $rdgB^{su100}$ and $rdgB^{KS222}$ flies at 2 days after eclosion. No RdgB protein was detected in $rdgB^{2}$ extracts. (B) A schematic of the RdgB protein and its putative domains. The N and C termini, the PITP domain, a region that binds Ca^{2+} *in vitro* (Ca^{2+}), and the six putative hydrophobic membrane-spanning domains are shown (Vihtelic *et al.* 1991, 1993). The cytoplasmic and lumenal sides of the SRC are labeled. The $rdgB^{KS222}$ nonsense mutation at Gln147 (small black bar) and the two $rdgB^{su100}$ missense mutations (His542-Glu and Asp543His) in the first lumenal loop (small white bar) are shown.

TABLE 1

Suppressors of the *rdgB*^{KS222} retinal degeneration phenotype

Suppressor	Chromosome	Map location	$t_{1/2}$ of dpp$^-$	Allele
norpA^{su68}	*X*		ND	*norpA*
norpA^{su76}	*X*		ND	*norpA*
norpA^{su99}	*X*		ND	*norpA*
ninaE^{su71}	3rd		ND	*ninaE*
rdgB^{su100}	*X*	1-43	5	*rdgB*
su(rdgB)69	3rd	3-101	>32	
su(rdgB)82	2nd	2-82	13	
Su(rdgB)83	3rd	3-57	22	
su(rdgB)102	3rd	3-101	ND	*su(rdgB)69*
su(rdgB)103	3rd	3-101	ND	*su(rdgB)69*
su(rdgB)104	3rd	3-101	ND	*su(rdgB)69*
Su(rdgB)116	2nd	2-65	21	

The map locations were determined by recombination mapping and scoring a minimum of 270 recombinant progeny. The map location of the *su(rdgB)69*, *su(rdgB)102*, *su(rdgB)103*, and *su(rdgB)104* mutations, which are alleles based on complementation analysis and recombination mapping, was calculated from the combined data of these four alleles. Map distances are accurate within eight map units. Retinal degeneration in an *rdgB*^{KS222} background was recorded as the $t_{1/2}$ for dpp$^-$, which is the number of days until half of the flies (minimum of 190 flies per genotype), which were raised in 12-hr light:dark cycle, lacked a wild-type deep pseudopupil. The $t_{1/2}$ for *rdgB*^{KS222} deep pseudopupil loss under identical conditions is 1–2 days. The $t_{1/2}$ was not determined (ND) for *norpA*, *ninaE*, nor the three *su(rdgB)69* alleles.

munoblots reveal that *rdgB*^{KS222} expresses low levels of wild-type-sized RdgB protein rather than a truncated protein (Figure 1A). This is not a cross-reacting protein because we failed to detect a similar protein in the *rdgB*^{2} null mutant (Figure 1A). We performed RT-PCR on *rdgB*^{KS222} mRNA to determine if alternative splicing removed the termination codon to yield the full-length RdgB protein. The sequences of 27 clones generated from five independent RT-PCR reactions were identical to the wild-type *rdgB* sequence (Vihtelic *et al.* 1991), except for the presence of the nonsense mutation. Translation through the nonsense mutation, rather than alternative splicing, must yield the full-length RdgB protein in *rdgB*^{KS222} flies. Both *rdgB*^{KS222} and the *rdgB*^{su100} flies (generated from *rdgB*^{KS222}) expressed nearly identical levels of RdgB protein on immunoblots (21.5 ± 3.1 and 23.4 ± 2.2%, respectively; Figure 1A). Therefore, the suppression is not through increased amounts of full-length RdgB protein. The *rdgB*^{su100} allele contained the expected *rdgB*^{KS222} nonsense mutation and two additional second-site mutations (His542Glu and Asp543His, Figure 1B) in RdgB's first putative lumenal loop (Vihtelic *et al.* 1993). It is likely that these two missense mutations partially restore RdgB activity by interacting with the *rdgB*^{KS222} mutation in the PITP domain, which was previously shown to be critical and sufficient for RdgB activity *in vivo* (Milligan *et al.* 1997).

The *rdgB*^{su100} mutation delayed the *rdgB*^{KS222} deep pseudopupil loss by only a few days (Figure 2B), which was the weakest effect by any of the five suppressors that we isolated. Light microscope sections of 10-day-old *rdgB*^{su100} flies raised in a 12-hr light:dark cycle that lacked a deep pseudopupil revealed some disorganized ommatidia and small and/or missing R1-6 rhabdomeres that were not apparent in the wild type (Figure 2, E and C, respectively). However, these *rdgB*^{su100} retinas lacked the massive degeneration observed in identically raised *rdgB*^{KS222} flies (Figure 2D). Electron microscopy confirmed that *rdgB*^{su100} R1-6 photoreceptors were abnormally shaped and had reduced rhabdomeres at 10 days relative to the wild type (Figure 2, H and F, respectively), but they lacked the complete R1-6 cell and rhabdomere loss observed in *rdgB*^{KS222} flies (Figure 2G). The rapid *rdgB*^{su100} deep pseudopupil loss is clearly caused by the disruption of the precisely reiterated R1-6 rhabdomeric trapezoid within and between adjacent ommatidia. While the *rdgB*^{su100} mutation did not dramatically restore the ERG light response amplitude (9 and 6 mV for *rdgB*^{su100} and *rdgB*^{KS222}, respectively; Figure 1A), it did possess an off transient that is absent in *rdgB*^{KS222} ERGs. The presence of the off transient indicates that these photoreceptors may possess either an improved light response physiology or better synaptic connections than *rdgB*^{KS222} photoreceptors.

Characterization of the autosomal *su(rdgB)69*: The *su(rdgB)69* complementation group consists of four alleles (Table 1) with nearly identical phenotypes. The *su(rdgB)69* mutation significantly slowed the loss of the *rdgB*^{KS222} deep pseudopupil under both 12-hr light:dark and constant light regimens (>50% of the flies retained a deep pseudopupil at >30 and 5 days, respectively; Figure 3B). The only obvious histological abnormality in *rdgB*^{KS222}; *su(rdgB)69* flies raised in 12-hr light:dark for 10 days was the reduced size of the R1-6 rhabdomeres

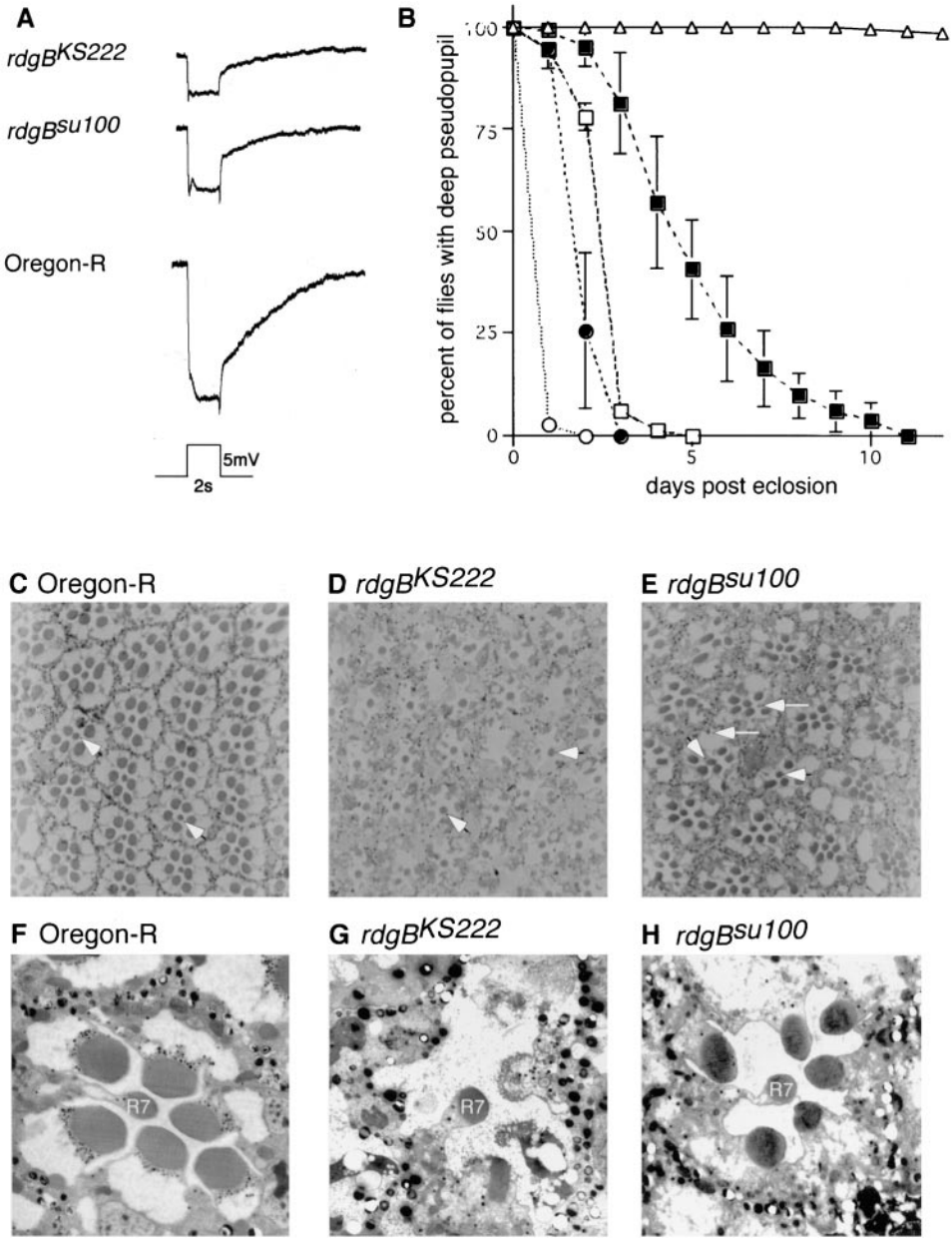

Figure 2.—Phenotype of the *rdgB*^{su100} intragenic suppressor. (A) The ERG light response of 1- to 2-day-old *rdgB*^{KS222}, *rdgB*^{su100}, and Oregon-R (wild-type) flies, raised in 12-hr light:dark cycle, are shown. Flies were dark adapted for 4 min before a 2-sec light stimulus, as indicated by the raised bar below the ERGs. Scales of 5 mV and 2 sec are shown. The *rdgB*^{su100} mutations are in a *rdgB*^{KS222} mutant background. (B) Flies were raised in either constant light [*rdgB*^{KS222} (open circles), *rdgB*^{su100} (open squares), Oregon-R (open triangles)] or a 12-hr light:dark cycle [*rdgB*^{KS222} (solid circles), *rdgB*^{su100} (solid squares)] and analyzed daily for the presence of a deep pseudopupil (dpp$^+$). The percent of dpp$^+$ flies on each day is plotted against their age. (C–E) Light microscopy of 10-day-old Oregon-R (C), *rdgB*^{KS222} (D), and *rdgB*^{su100} (E) flies raised in a 12-hr light:dark cycle. The size of the *rdgB*^{su100} R1-6 rhabdomeres are reduced relative to the R7 rhabdomere (arrowhead), and some of the ommatidia are misshapen (arrow). The *rdgB*^{KS222} (D) and *rdgB*^{su100} (E) retinas lacked a deep pseudopupil before histology. (F–H) Wild-type (F), *rdgB*^{KS222} (G), and *rdgB*^{su100} (H) flies were raised in a 12-hr light:dark cycle for 10 days, and retinal sections were examined by electron microscopy. The R7 rhabdomeres are labeled.

relative to R7 (Figure 3E). This is in stark contrast to the massive loss of ommatidial organization and photoreceptor cells in 10-day-old *rdgB*^{KS222} mutant retinas (Figure 3D). Even at 20 days, the *rdgB*^{KS222}; *su(rdgB)69* retina lacked only a few rhabdomeres per ommatidium (Figure 3F). The *su(rdgB)69* mutation also effectively improved the *rdgB*^{KS222} ERG light response amplitude from 6 to 15 mV, relative to the 24 mV for similarly aged wild-type flies (Figure 3A).

Because *su(rdgB)69* strongly suppressed the *rdgB*^{KS222} retinal degeneration phenotype, we examined if it also suppressed the *rdgB*^{2} null retinal degeneration phenotype. The *su(rdgB)69* allele significantly slowed the time course of the *rdgB*^{2}-dependent deep pseudopupil loss, although not as dramatically as with *rdgB*^{KS222} (Figure 3B). Surprisingly, the ERG light response of newly eclosed *rdgB*^{2}; *su(rdgB)69* flies was nearly identical to that of *rdgB*^{2} flies (data not shown).

Characterization of the autosomal *su(rdgB)82*: The weakest of the four autosomal suppressors, *su(rdgB)82*, is located on the right arm of the second chromosome (Table 1). This suppressor slowed the *rdgB*^{KS222} retinal degeneration such that 50% of the *rdgB*^{KS222}; *su(rdgB)82* flies retained a deep pseudopupil at days 3 and 13 under constant light and 12-hr light:dark regimens, respectively (Figure 4B). Retinal sections of 10-day-old *rdgB*^{KS222}; *su(rdgB)82* flies (raised in 12-hr light:dark) revealed great variability in the extent of degeneration. Flies retaining a deep pseudopupil showed excellent ommatidial arrangement, few missing rhabdomeres, and highly variable R1-6 rhabdomere sizes (Figure 4E). Retinal sections of sibling flies that lacked a deep pseudopupil were indis-

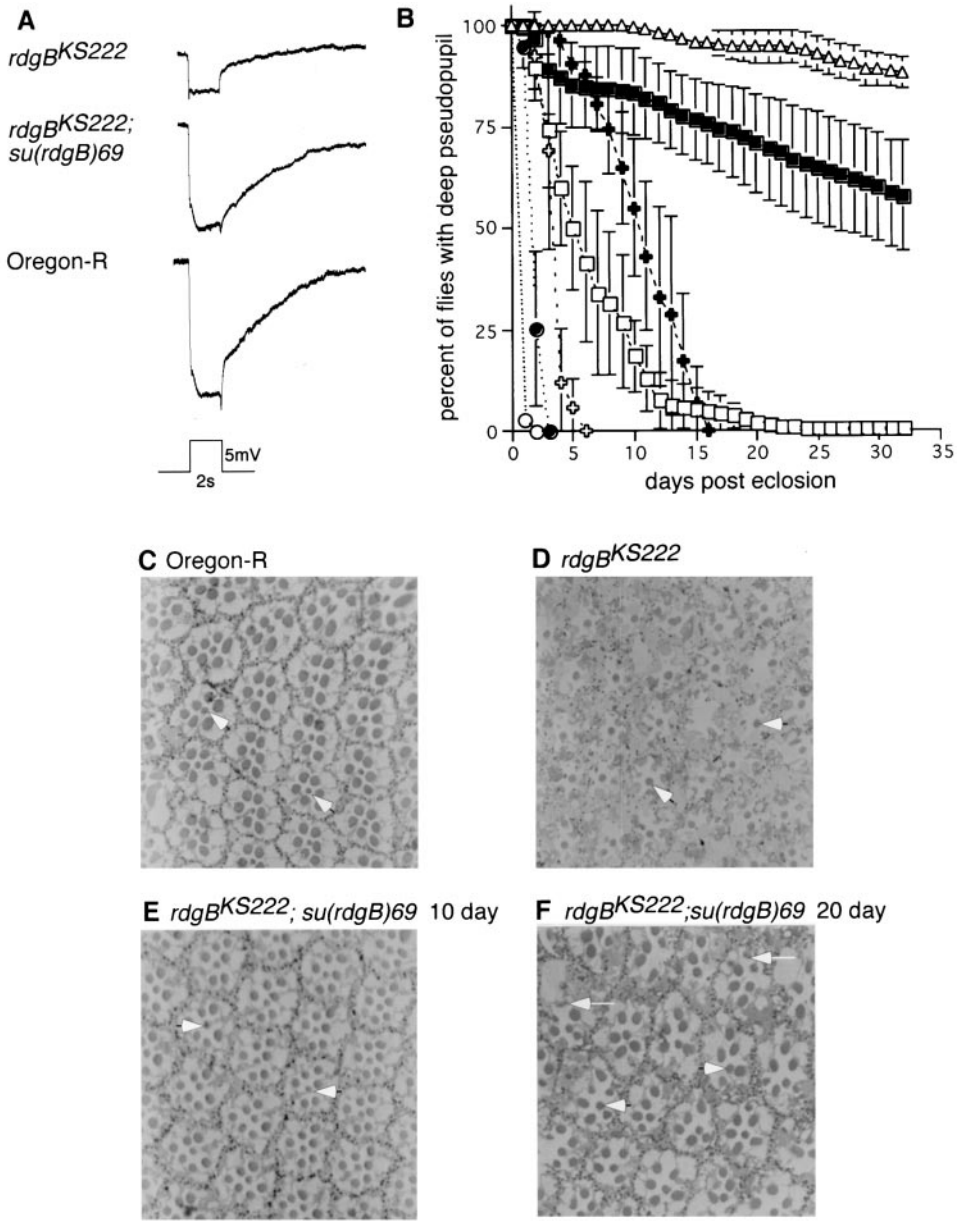

Figure 3.—Suppression of *rdgB*^{KS222} by *su(rdgB)69*. (A) The ERG light response of 1- to 2-day-old *rdgB*^{KS222}, *rdgB*^{KS222}; *su(rdgB)69*, and Oregon-R (wild-type) flies raised in a 12-hr light:dark cycle were recorded as in Figure 2. Scales of 5 mV and 2 sec are shown. (B) Vermilion-eyed [*rdgB*^{2} and *rdgB*^{2}; *su(rdgB)69*] or wild-type eye-colored (all others) flies were raised either in constant light [*rdgB*^{KS222} (open circles), *rdgB*^{KS222}; *su(rdgB)69* (open squares), Oregon-R (open triangles)] or in a 12-hr light:dark cycle [*rdgB*^{KS222} (solid circles), *rdgB*^{KS222}; *su(rdgB)69* (solid squares), *rdgB*^{2} (open cross), *rdgB*^{2}; *su(rdgB)69* (solid cross)] and analyzed daily for the presence of a deep pseudopupil (dpp^{+}). The percent of dpp^{+} flies on each day is plotted against their age. (C–F) Retinal sections of Oregon-R (C), *rdgB*^{KS222} (D), and *rdgB*^{KS222}; *su(rdgB) 69* (E and F) flies raised in a 12-hr light:dark cycle for either 10 days (C–E) or 20 days (F) were examined by light microscopy. The R7 rhabdomere (arrowhead) is indicated for orientation. The 20-day-old *rdgB*^{KS222}; *su(rdgB)69* retinas (F) possess several ommatidia that lack one to two rhabdomeres (white arrows). *rdgB*^{KS222}; *su(rdgB)69* flies that possessed or lacked a deep pseudopupil gave similar histological results.

tinguishable from 10-day-old *rdgB*^{KS222} mutant flies (Figure 4, F and D, respectively). While the ERG light response amplitude of *rdgB*^{KS222}; *su(rdgB)82* flies (8 mV) was not significantly different than that of *rdgB*^{KS222} flies (6 mV; Figure 4A), it possessed an off transient.

Characterization of the autosomal dominant suppressors *Su(rdgB)83* and *Su(rdgB)116*: Two dominant suppressors were isolated, *Su(rdgB)83* and *Su(rdgB)116*, which mapped to the right arms of the third and second chromosomes, respectively (Table 1). Both *Su(rdgB)83* and *Su(rdgB)116* slowed the *rdgB*^{KS222} deep pseudopupil loss to a similar rate. Half of the *rdgB*^{KS222}; *Su(rdgB)83* flies possessed a deep pseudopupil until days 4 and 22 under constant light and 12-hr light:dark conditions, respectively (Figure 5B), while half the *rdgB*^{KS222}; *Su(rdgB)116* flies retained their deep pseudopupil until day 5 in constant light or day 21 in a 12-hr light:dark regimen (Figure 5C). However, the suppressed degeneration phenotypes were histologically distinct. While 10-day-old *rdgB*^{KS222}; *Su(rdgB)83* flies raised in 12-hr light:dark showed loss of some R1-6 rhabdomeres and photoreceptor cell bodies, large variability in R1-6 rhabdomere size, and holes in the retinal tissue (Figure 5F), they were better organized and possessed larger R1-6 rhabdomeres than similarly aged *rdgB*^{KS222} retinas (Figure 5E). The *rdgB*^{KS222}; *Su(rdgB)116* R1-6 rhabdomeres were uniformly smaller than those from the wild type, but very few R1-6 rhabdomeres or photoreceptors were missing, and the overall ommatidial arrangement was well intact (Figure 5G). However, the superior ommatidial organization of *rdgB*^{KS222}; *Su(rdgB)116* relative to *rdgB*^{KS222}; *Su(rdgB)83* did not correlate with an improved light response. The ERG light response amplitudes of *rdgB*^{KS222}; *Su(rdgB)83* flies and *rdgB*^{KS222}; *Su(rdgB)116* flies were ~16 and 12 mV, respectively (Figure 5A).

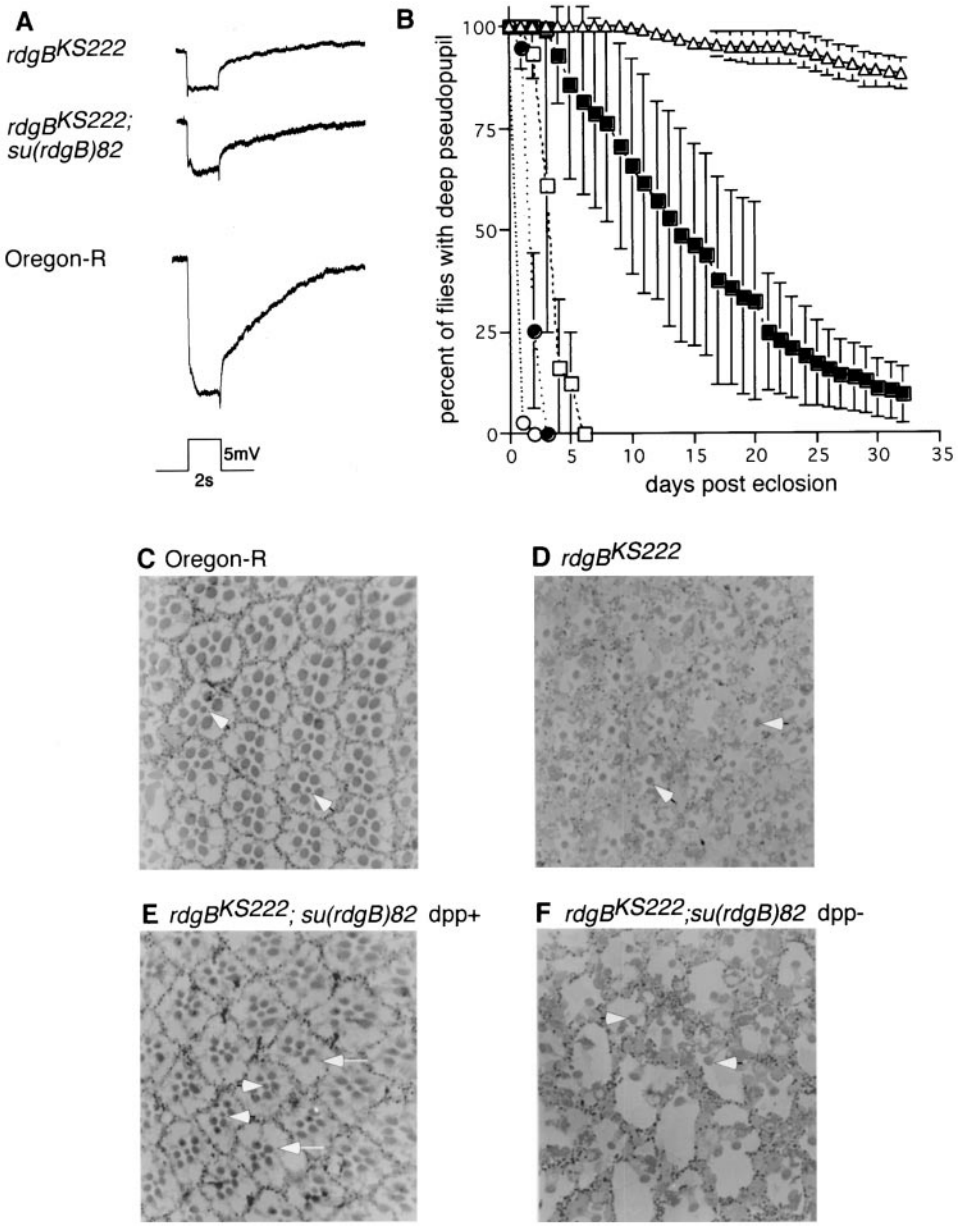

Figure 4.—Suppression of *rdgB*^{KS222} by *su(rdgB)82*. (A) The ERG light responses of 1- to 2-day-old *rdgB*^{KS222}, *rdgB*^{KS222}; *su(rdgB)82*, and Oregon-R (wild-type) flies raised in a 12-hr light:dark cycle were recorded as in Figure 2. Scales of 5 mV and 2 sec are shown. (B) Flies were raised in either constant light [*rdgB*^{KS222} (open circles), *rdgB*^{KS222}; *su(rdgB)82* (open squares), Oregon-R (open triangles)] or in a 12-hr light:dark cycle [*rdgB*^{KS222} (solid circles), *rdgB*^{KS222}; *su(rdgB)82* (solid squares)] and analyzed daily for the presence of a deep pseudopupil (dpp$^+$). The percent of dpp$^+$ flies on each day is plotted against their age. (C–F) Oregon-R (C), *rdgB*^{KS222} (D), and *rdgB*^{KS222}; *su(rdgB)-82* (E and F) flies were raised in a 12-hr light:dark cycle for 10 days, and retinal sections were examined by light microscopy. The dpp$^+$ *rdgB*^{KS222}; *su (rdgB)82* retina (E) possesses several ommatidia with swollen cell bodies or lacking a single rhabdomere (white arrows), as well as R1-6 rhabdomeres that vary in size relative to R7 (arrowheads).

Phenotypes of the suppressors in a wild-type (*rdgB*$^+$) background: We examined each autosomal suppressor in a *rdgB*$^+$ background for either a deep pseudopupil phenotype or an aberrant ERG light response. All the suppressors possessed wild-type ERG light responses (data not shown). The only detectable abnormality associated with any of the suppressors was a light-enhanced deep pseudopupil loss for *Su(rdgB)116* flies (Figure 6A). The histology of *Su(rdgB)116*/+ retinas (Figure 6C) was very similar to *rdgB*^{KS222}; *Su(rdgB)116*/+ (Figure 5G), with small R1-6 rhabdomeres, very few missing rhabdomeres, and few swollen cell bodies in the retinal sections. Even at 20 days, *Su(rdgB)116*/+ retinas (Figure 6D) exhibited relatively few abnormalities in the photoreceptor cells and ommatidial organization. Because the *rdgB*^{KS222}; *Su(rdgB)116*/+ deep pseudopupil loss was significantly faster than that of *Su(rdgB)116*/+ (Figures 5C and 6A, respectively), the *Su(rdgB)116* mutation must not completely suppress the *rdgB*^{KS222} degeneration phenotype.

The Drosophila *trp* mutation, but not *inaC*, suppresses *rdgB* retinal degeneration: Because previous experiments indicated that the *rdgB*-mediated retinal degeneration was dependent on stimulation of PKC in the visual transduction cascade, we examined the ability of two visual transduction mutations (*inaC* and *trp*) to suppress *rdgB* degeneration. The *inaC* gene encodes a retinal-specific PKC (INAC; Smith *et al.* 1991), while the *trp* gene encodes one of the light-activated calcium channels (TRP; Hardie and Minke 1992).

During a 12-hr light:dark cycle, *inaC*^{209} flies maintained their deep pseudopupil for at least 15 days (Figure 7). However, *rdgB*^{ota1}; *inaC*^{209} and *rdgB*^{KS222}; *inaC*^{209} deep pseu-

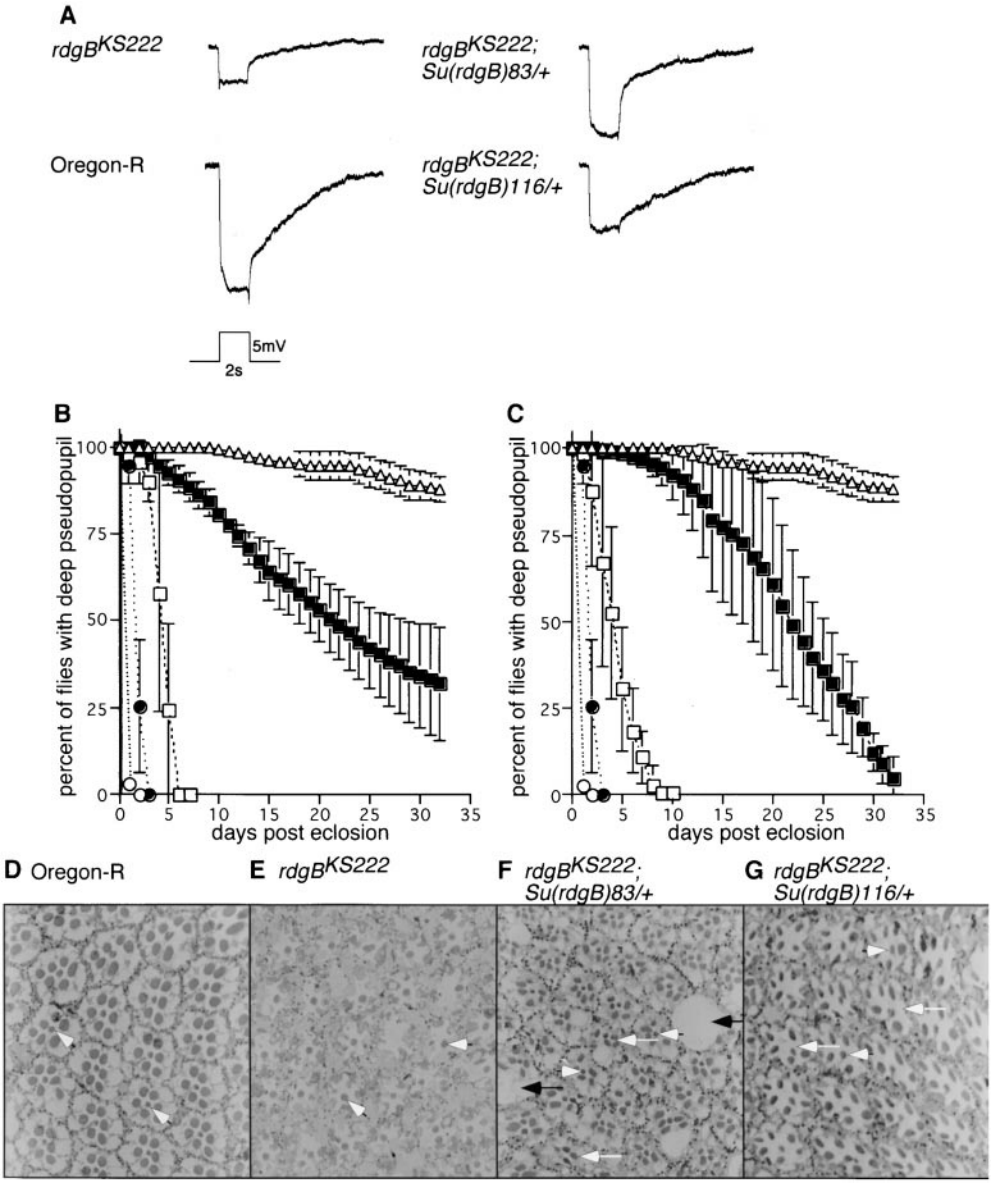

Figure 5.—Dominant suppression of *rdgB*^{KS222} by *Su(rdgB)83* and *Su(rdgB)116*. (A) The ERG light responses of 1- to 2-day-old *rdgB*^{KS222}, Oregon-R (wild-type), *rdgB*^{KS222}; *Su(rdgB)83*/+, and *rdgB*^{KS222}; *Su(rdgB)116*/+ flies raised in a 12-hr light:dark cycle were recorded as in Figure 2. Scales of 5 mV and 2 sec are shown. (B and C) Flies were raised in either constant light [*rdgB*^{KS222} (open circles), *rdgB*^{KS222}; *Su(rdgB)83*/+ (open squares, B), *rdgB*^{KS222}; *Su(rdgB)116*/+ (open squares, C), Oregon-R (solid triangles)] or in a 12-hr light: dark cycle [*rdgB*^{KS222} (solid circles), *rdgB*^{KS222}; *Su(rdgB)83*/+ (solid squares, B), *rdgB*^{KS222}; *Su(rdgB)116*/+ (solid squares, C)] and analyzed daily for the presence of a deep pseudopupil (dpp^{+}). The percent of dpp^{+} flies on each day is plotted against their age. (D–G) Oregon-R (D), *rdgB*^{KS222} (E), *rdgB*^{KS222}; *Su(rdgB)83*/+ (F), and *rdgB*^{KS222}; *Su(rdgB)116*/+ (G) flies were raised in a 12-hr light:dark cycle for 10 days, and retinal sections were examined by light microscopy. Ommatidia lacking rhabdomeres and/or cell bodies (white arrows) and large holes in the section (black arrows) are marked. The R7 rhabdomere (arrowhead) is shown for orientation. *rdgB*^{KS222}; *Su(rdgB)116*/+ flies that either retained or lacked a deep pseudopupil gave similar histological results.

dopupil loss was not significantly different from that of *rdgB*^{ota1} and *rdgB*^{KS222} flies, respectively (Figure 7). Retinal sections confirmed that *inaC*^{209} failed to suppress the photoreceptor degeneration and ommatidial disorganization apparent in 6-day-old *rdgB*^{ota1} and *rdgB*^{KS222} flies (Figure 8).

The *trp*^{CM} flies also maintained their deep pseudopupil for at least 15 days (Figure 7). The *rdgB*^{ota1}; *trp*^{CM} flies began losing their deep pseudopupil 7 days after eclosion in a 12-hr light:dark cycle, 6 days later than *rdgB*^{ota1} flies (Figure 7). Thus, *trp*^{CM} slowed the initiation of *rdgB*^{ota1} retinal degeneration without completely preventing deep pseudopupil loss. While *trp*^{CM} failed to delay initiation of *rdgB*^{KS222} retinal degeneration, it significantly slowed the rate of deep pseudopupil loss (Figure 7). Retinal sections confirmed that *trp*^{CM} dramatically suppressed photoreceptor degeneration and ommatidial disorganization in both *rdgB*^{ota1} and *rdgB*^{KS222} flies (Figure 8). Consistent with the deep pseudopupil loss results, the *rdgB*^{KS222}; *trp*^{CM} retina possessed a higher degree of ommatidial disorganization and significantly more R1-6 rhabdomere loss than the *rdgB*^{ota1}; *trp*^{CM} retina (Figure 8, H and E, respectively). Thus, TRP activity, but not INAC, is required for *rdgB*-mediated retinal degeneration.

DISCUSSION

The Drosophila RdgB is a novel integral membrane PITP (Vihtelic *et al.* 1993) that is required for the viability of the photoreceptor cell and to produce a light response. While expressing only RdgB's N-terminal PITP domain as a soluble protein (RdgB-PITP) is sufficient to prevent retinal degeneration and restore the ERG light response in *rdgB*^{2} null mutants, phospholipid transfer is not the critical activity (Milligan *et al.* 1997). Furthermore, the identification of a functionally equiva-

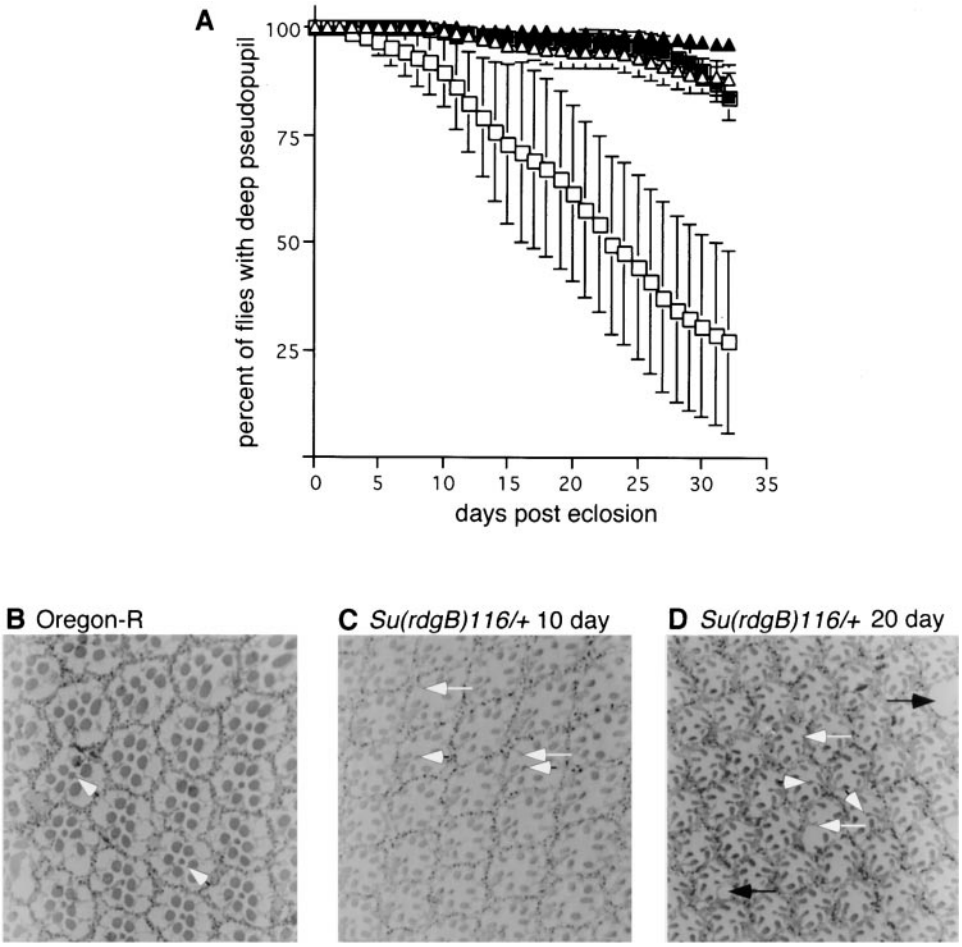

Figure 6.—The *Su(rdgB)116* mutant exhibits a dominant retinal degeneration phenotype. (A) Flies were raised in either constant light [*Su(rdgB)116*/+ (open squares), Oregon-R (open triangles)] or in a 12-hr light:dark cycle [*Su(rdgB)116*/+ (solid squares), Oregon-R (solid triangles)] and analyzed daily for the presence of a deep pseudopupil (dpp^+). The percent of dpp^+ flies on each day is plotted against their age. (B–D) Oregon-R (B) and *Su(rdgB)116*/+ (C and D) flies were raised in a 12-hr light:dark cycle for either 10 days (B and C) or 20 days (D), and retinal sections were examined by light microscopy. The 10-day-old *Su(rdgB)116*/+ retinas possess small R1-6 rhabdomeres, relative to R7 (arrowheads), and several ommatidia lack a single rhabdomere (white arrow, C). Swollen cell bodies (black arrows) are also present by 20 days after eclosion (D). *Su(rdgB)116*/+ flies that either retained or lacked a deep pseudopupil gave similar histological results.

lent vertebrate RdgB ortholog makes elucidating RdgB's function more relevant (Chang *et al.* 1997; Guo and Yu 1997). Identifying suppressors of *rdgB*-dependent degeneration revealed four new features about RdgB and its mutant phenotypes. First, an intragenic $rdgB^{KS222}$ suppressor ($rdgB^{su100}$) suggested that RdgB's putative intralumenal loops could play a role in RdgB's activity. Second, three different suppressors [$rdgB^{su100}$, *su(rdgB)69*, and *su(rdgB)82*] revealed that the *rdgB*-dependent degeneration may not be strictly caused by a defective light response. Third, while the *inaC*-encoded PKC may affect RdgB activity, INAC is not required to stimulate *rdgB*-dependent retinal degeneration. Fourth, *trp*-encoded Ca^{2+} channel activity is required for rapid *rdgB*-dependent retinal degeneration. While the four autosomal mutations exhibited a range of suppression, only *Su(rdgB)116* exhibited any detectable mutant phenotype in an $rdgB^+$ background. This suggests that the other three suppressors affect redundant functions, or that the mutant phenotypes were too subtle to detect.

Genetic and molecular characterization of $rdgB^{KS222}$ and $rdgB^{su100}$ revealed several interesting features about the RdgB protein. The $rdgB^{KS222}$ mutation is a nonsense mutation (TAG) at position 147 within the PITP domain. Because we failed to detect a truncated $RdgB^{KS222}$ protein on immunoblots and RT-PCR confirmed the presence of the nonsense mutation in the mRNA, the truncated protein must be relatively unstable. More surprisingly, we detected low levels of wild-type-sized $RdgB^{KS222}$ protein on immunoblots, which suggests that translation proceeds through this nonsense mutation. The ability of Drosophila to translate through nonsense mutations, particularly UAG, has been described for the *kelch*, *elav*, and *synapsin* genes (Xue and Cooley 1993; Samson *et al.* 1995; Klagges *et al.* 1996). While the $rdgB^{KS222}$ mutant phenotypes could result from reduced levels of RdgB protein, immunoblots reveal that the mechanism of $rdgB^{su100}$ suppression is not caused by increased steady-state levels of RdgB protein relative to $rdgB^{KS222}$ (Figure 1A). Because $rdgB^{su100}$ is a recessive suppressor, the $RdgB^{su100}$ protein likely possesses more activity than the $RdgB^{KS222}$ protein and less than $RdgB^+$. Confirmation of this hypothesis must await a biochemical assay for RdgB. While RdgB's N-terminal PITP domain is sufficient and essential for *in vivo* function (Milligan *et al.* 1997), the $rdgB^{su100}$ molecular data suggest that other regions of the protein can affect RdgB's activity *in vivo*.

While the $rdgB^{su100}$ mutations did not significantly delay deep pseudopupil loss (Figure 2B), they dramatically slowed R1-6 rhabdomere and cell body degeneration

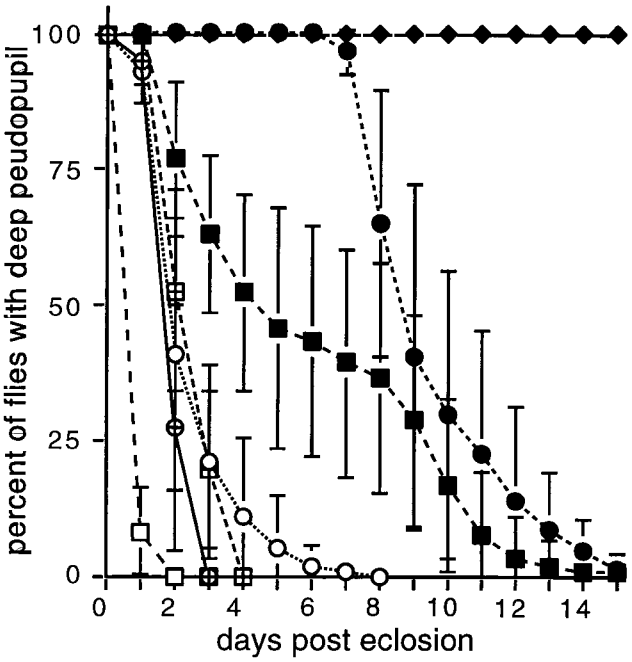

Figure 7.—Effect of trp^{CM} and $inaC^{209}$ mutations on *rdgB*-deep pseudopupil loss. Wild-type eye-colored $rdgB^{ota1}$ (hatched circles), $rdgB^{ota1}$; trp^{CM} (solid circles), $rdgB^{ota1}$; $inaC^{209}$ (open circles), $rdgB^{KS222}$ (hatched squares), $rdgB^{KS222}$; trp^{CM} (solid squares), $rdgB^{KS222}$; $inaC^{209}$ (open squares), trp^{CM} (solid diamonds), and white-eyed $inaC^{209}$ (solid diamonds) flies were raised in a 12-hr light:dark cycle and analyzed daily for the presence of a deep pseudopupil (dpp^+). The percent of dpp^+ flies on each day is plotted against their age. No deep pseudopupil loss was observed for either the $inaC^{209}$ or trp^{CM} flies.

(Figure 2, G and H). The small perturbations in the $rdgB^{su100}$ rhabdomere and ommatidial arrangement most likely resulted in deep pseudopupil loss. While the ERG light response amplitude of $rdgB^{su100}$ flies was not significantly different from that of $rdgB^{KS222}$, the $rdgB^{su100}$ flies did possess an off transient (Figure 2A) that originated postsynaptically to the photoreceptors in the lamina (reviewed in Pak 1975). All four autosomal suppressors also restored the off transient, even if the light response was not significantly different from that of $rdgB^{KS222}$. Thus, the presence of off transients may indicate an improved light response or simply the preservation of the photoreceptor cells' synaptic connections in the lamina caused by the dramatically slowed degeneration. Two models exist for the preservation of the $rdgB^{su100}$ photoreceptors without significant restoration of the ERG light response. First, the $rdgB^{KS222}$-dependent degeneration is not a direct consequence of the abnormal light response physiology, which suggests that RdgB is required for multiple and distinct photoreceptor cell functions. This is supported by *su(rdgB)82* dramatically slowing $rdgB^{KS222}$ retinal degeneration without significantly affecting the mutant ERG light response, and by *su(rdgB)69* slowing the $rdgB^2$ null deep pseudopupil loss without significantly restoring the ERG light response. To further support that *rdgB*-dependent degeneration and defective light response are not intimately linked, expressing an RdgB protein lacking the Ca^{2+}-binding domain restores a wild-type ERG light response in $rdgB^2$ flies without fully preventing retinal degeneration (R. B. Elagina, S. C. Milligan and D. R. Hyde, unpublished results). Alternatively, the ERG light response may be more sensitive to perturbations in RdgB activity than in photoreceptor viability. Thus, the $rdgB^{su100}$ mutations restored sufficient RdgB activity to suppress retinal degeneration without providing the minimal activity required for the wild-type ERG light response. However, this model fails to explain how an RdgB protein lacking the Ca^{2+}-binding domain restores a normal ERG light response in $rdgB^2$ flies without preventing degeneration.

The four autosomal suppressors exhibit three unique features that were unobserved in previous *rdgB* suppressors. First, the autosomal suppressors lack a mutant ERG light response phenotype in an $rdgB^+$ background. Because all previous suppressors disrupted the ERG light response by affecting key components of the visual transduction cascade, these new suppressors either affect a previously unrecognized aspect of RdgB function, or they affect redundant components in the light response. Second, the four autosomal suppressors most likely compensate for reduced activity in the $rdgB^{KS222}$ mutant. However, immunoblots reveal that this is not caused by increased RdgB protein levels (data not shown). Surprisingly, *su(rdgB)69* compensated for complete loss of RdgB activity in $rdgB^2$ flies to delay deep pseudopupil loss. Third, two of these mutations are the first identified dominant suppressors of *rdgB*-mediated retinal degeneration. Unlike *rdgB* suppressors that inactivate the visual transduction cascade, these dominant suppressors may stimulate components downstream of RdgB that are normally not activated in the *rdgB* mutant.

The dominant *Su(rdgB)116* mutation was the only autosomal suppressor that possessed a mutant phenotype in a $rdgB^+$ background. The *Su(rdgB)116*/+ fly exhibited shrinking rhabdomeres and no obvious photoreceptor cell loss. The dominant *Su(rdgB)116*/+ histology is more similar to the hypomorphic *ninaE* mutants than to *rdgB* (Leonard *et al.* 1992; Kumar and Ready 1995), although the *Su(rdgB)116*/+ flies lacked the characteristic *nina* mutant ERG light response (Stephenson *et al.* 1983). While the histology of the $rdgB^{KS222}$; *Su(rdgB)116*/+ double mutant was indistinguishable from the *Su(rdgB)116*/+ mutant, the time course of deep pseudopupil loss for $rdgB^{KS222}$; *Su(rdgB)116*/+ flies was significantly faster than for *Su(rdgB)116*/+ flies (Figures 5C and 6A, respectively). This suggests that the suppression of $rdgB^{KS222}$-dependent degeneration was incomplete. It is unclear if the gradual rhabdomere loss directly mediates the $rdgB^{KS222}$ suppression, or if an underlying cellular process affects both rhabdomere size and photoreceptor viability.

RdgB was postulated to function after PKC in the fly visual transduction cascade on the basis of pharmacological experiments (Minke *et al.* 1990). Later, the $inaC^{209}$ mutation was shown to suppress $rdgB^{EE170}$-mediated reti-

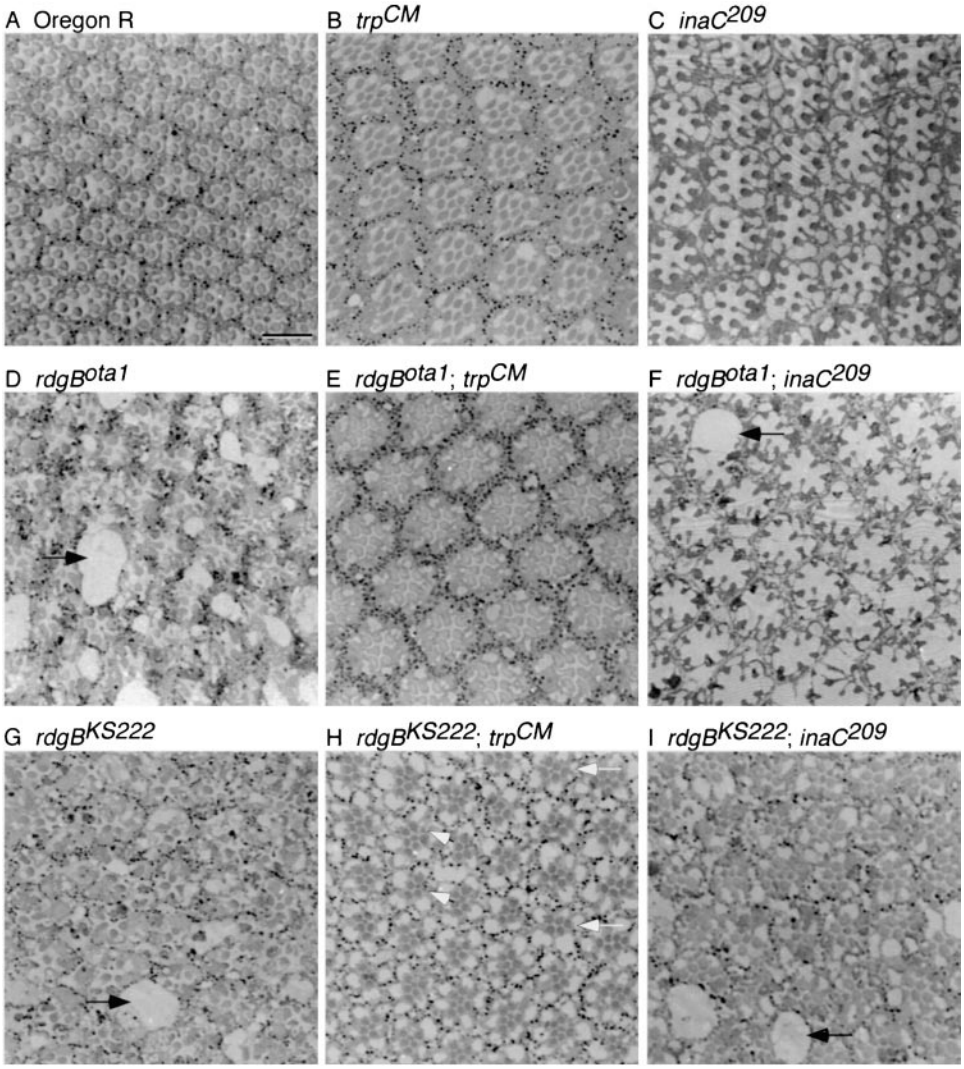

Figure 8.—Effect of $inaC^{209}$ and trp^{CM} mutations on the *rdgB*-dependent retinal degeneration. Wild-type eye-colored Oregon-R (A), trp^{CM} (B), $inaC^{209}$ (C), $rdgB^{ota1}$ (D), $rdgB^{ota1}$; trp^{CM} (E), $rdgB^{ota1}$; $inaC^{209}$ (F), $rdgB^{KS222}$ (G), $rdgB^{KS222}$; trp^{CM} (H), and $rdgB^{KS222}$; $inaC^{209}$ (I) flies were raised in a 12-hr light: dark cycle for 6 days and retinal sections were examined by light microscopy. The Oregon-R, trp^{CM}, $inaC^{209}$, $rdgB^{ota1}$; trp^{CM}, and $rdgB^{KS222}$; trp^{CM} flies possessed a deep pseudopupil, while the $rdgB^{ota1}$, $rdgB^{KS222}$, $rdgB^{ota1}$; $inaC^{209}$, and $rdgB^{KS222}$; $inaC^{209}$ flies lacked a deep pseudopupil. Large holes in the retinal sections (black arrows) are observed in D, F, G, and I. Ommatidia lacking the full complement of seven rhabdomeres (white arrows) are shown. The size of the R1-6 rhabdomeres in some ommatidia is significantly smaller than R7 (arrowheads). Bar in A, 10 μm.

nal degeneration (Smith *et al.* 1991). However, the flies were exposed to light for only 90 min, and the exact nature of the $rdgB^{EE170}$ allele is unknown. In a 12-hr light:dark cycle, $inaC^{209}$ did not suppress degeneration of either $rdgB^{KS222}$ or $rdgB^{ota1}$, which is a Pro93Ser mutation in the PITP domain (S. C. Milligan and D. R. Hyde, unpublished results). Finally, mutation of a putative PKC phosphorylation site (Thr59Glu) in RdgB's PITP domain inactivates RdgB *in vivo*, although it does not affect PI transfer activity *in vitro* (Milligan *et al.* 1997). Taken together, the *inaC*-encoded PKC may regulate some aspect of RdgB's function, although PKC is not directly upstream of RdgB and is not required for *rdgB*-dependent retinal degeneration. Consistent with earlier results (Chen and Stark 1983; Stark and Sapp 1989), the trp^{CM} mutation failed to prevent retinal degeneration in $rdgB^{KS222}$ and $rdgB^{ota1}$ flies. However, the trp^{CM} mutation significantly slowed the onset and/or rate of $rdgB^{KS222}$ and $rdgB^{ota1}$ retinal degeneration. This is consistent with Ca^{2+} channel blockers suppressing *rdgB*-mediated retinal degeneration (Sahly *et al.* 1992). The *trp* mutant has decreased light-dependent influx of Ca^{2+} into the retinal cell (Peretz *et al.* 1994), which is consistent with *rdgB* degeneration resulting from increased intracellular Ca^{2+} levels (Sahly *et al.* 1994). The increased Ca^{2+} influx in *inaC* mutants (Peretz *et al.* 1994) is also consistent with $inaC^{209}$ failing to suppress *rdgB* degeneration. Taken together, these results suggest that either the RdgB protein may modulate intracellular Ca^{2+} levels, or that RdgB may be regulated by Ca^{2+}. RdgB's localization to the subrhabdomeric cisternal membrane (Vihtelic *et al.* 1993; Suzuki and Hirosawa 1994), a putative intracellular Ca^{2+} store, and the potential Ca^{2+}-binding site in the RdgB protein is consistent with either of these two models.

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