

Isolation and Characterization of the *Drosophila* retinal degeneration B (*rdgB*) Gene

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ABSTRACT

retinal degeneration-B (rdgB) mutants of *Drosophila melanogaster* undergo rapid light-induced retinal degeneration. We conducted a molecular characterization of the *rdgB* gene to examine the nature of the gene product. Through the isolation and analysis of X-ray-induced *rdgB* alleles, the cytogenetic position of the gene was determined to be the 12C1 salivary region. Genomic DNA corresponding to this region was isolated by a chromosomal walk. The chromosomal aberrations associated with the three X-ray-induced *rdgB* alleles were shown to be within a 5-kb genomic region. A single transcription unit was affected by the alleles, identifying it as the *rdgB* gene. RNA-RNA Northern hybridization indicated the *rdgB* gene transcribed five mRNAs ranging in size from 3.9 to 9.5 kb. These mRNAs were expressed in adult heads, but not detected in bodies. Analysis of RNA isolated from wild-type and *eyes absent* heads indicated that *rdgB* mRNA expression was not restricted to the retina. DNA sequence analysis of the transcription unit revealed an open reading frame capable of encoding a 116-kD transmembrane protein. The deduced protein shows no overall homology to previously described proteins, but has sequences in common with proposed functional domains of Ca²⁺-ATPase.

PHOTORECEPTOR cells require the products of specific genes for both normal function and maintenance. The molecular analysis of genes associated with different forms of retinal degeneration provides insights into the biochemical processes required for maintenance and function of photoreceptors and other neuronal cells (CHADER, AGUIRRE and SANYAL 1988). The mouse *rd* gene, which encodes a subunit of the transducin-activated cGMP phosphodiesterase (BOWES *et al.* 1990), and the human rhodopsin gene, for which a specific missense mutation results in an autosomal dominant form of human retinitis pigmentosa (DRYJA *et al.* 1990), are examples of phototransduction components essential for maintenance of photoreceptor cells. Important phototransduction components of *Drosophila* are also identified by mutants that affect the maintenance of photoreceptors. Examples include the *ninaE* gene (O'TOUSA, LEONARD and PAK 1989), which encodes the visual pigment of photoreceptor cells R1-6 (O'TOUSA *et al.* 1985; ZUKER, COWMAN and RUBIN 1985) and *norpA* (MEYER-THOLEN *et al.* 1987; STARK, SAPP and CARLSON 1989), which encodes the phototransduction effector molecule phospholipase C (BLOOMQUIST *et al.* 1988).

The *retinal degeneration-B (rdgB)* mutation was one of the first *Drosophila* retinal degeneration mutations discovered (HEISENBERG 1971; HOTTA and BENZER 1969; PAK, GROSSFIELD and ARNOLD 1970; reviewed

by PAK 1976). Retinal degeneration in *rdgB* mutants is autonomous to the photoreceptor cells (HARRIS and STARK 1977; HOTTA and BENZER 1970). When *rdgB* flies are maintained in constant light, their outer six photoreceptor cells (R1-6) degenerate within a few days posteclosion, while *rdgB* flies maintained under dark conditions exhibit a much slower rate of degeneration (HARRIS and STARK 1977). At 3 days posteclosion, the soma of some outer retinal cells has degenerated, while all these cells show axonal degeneration (STARK *et al.* 1983). By 7 days, the photoreceptor cytoplasm is uniformly electron dense, with full liposomes and lysosome-like bodies, and the axon terminals lack synaptic vesicles and presynaptic structures. The earliest sign of degeneration is at the photoreceptor terminals, suggesting that retinal degeneration in *rdgB* flies follows from synaptic inactivation (CARLSON, STARK and CHI 1985; STARK and SAPP 1989).

The current relationship of the *rdgB* gene product to the phototransduction cascade suggests that it functions in the same pathway as, but subsequent to, phospholipase C. The rhodopsin-G-protein cascade in photoreceptors stimulates phospholipase C, which is encoded by the *norpA* gene (BLOOMQUIST *et al.* 1988). HARRIS and STARK (1977) found that *norpA* alleles could function as nonallelic suppressors of *rdgB* degeneration. The *norpA*^{full} allele suppresses *rdgB*^{KS222} in an allele-specific manner, suggesting a possible protein-protein interaction between the *rdgB* product and phospholipase C (HARRIS and STARK 1977). Recent biochemical work has further examined the role of

The sequence data presented in this article have been submitted to the EMBL/GenBank Data Libraries under the accession number X57978.

the *rdgB* gene product in the phototransduction pathway. RUBINSTEIN *et al.* (1989a) demonstrated that the *rdgB* protein acts subsequent to an activated G-protein, because application of guanosine 5'-[thio]triphosphate) GTP[γ -S] to dark-reared *rdgB* flies mimicked the light-activated, rapid degeneration of R1-6 cells. MINKE *et al.* (1990) further showed that a phorbol ester, which activates protein kinase C, caused photoreceptor degeneration in dark-reared *rdgB* flies, suggesting the *rdgB* gene product functions after protein kinase C activation. These results suggest the following cascade of events: the G-protein activation of phospholipase C produces diacyl glycerol (DAG), which activates a protein kinase C, which, in turn, directly or indirectly activates the *rdgB* protein.

The *rdgB* product may also play a role in other sensory transduction systems. WOODARD *et al.* (1989), in a genetic search for olfactory mutants, found a particular *rdgB* allele failed olfactory behavioral tests. Further work showed that some, but not all, *rdgB* alleles were defective in electroantennogram assays (C. WOODARD, E. ALCORTA and J. CARLSON, personal communication).

Our aim is to understand the *rdgB* gene product's function in both phototransduction and in other sensory transduction systems. Toward this goal, we have used a cytogenetic approach to clone the gene. In this report, we describe the cloning and initial characterization of the *rdgB* gene. From our molecular data, we hypothesize that the *rdgB* gene product may function as a calcium transporter.

MATERIALS AND METHODS

Mutagenesis and mutant characterization: New *rdgB* alleles were isolated by the following scheme. *y sc v rdgB⁺* males were irradiated with approximately 5,000 rads of γ -irradiation and crossed to *rdgB^{KS222}/rdgB^{KS222}* females. Progeny were collected and aged for 3 days under constant illumination. This regimen results in retinal degeneration of *rdgB* flies that can be scored by examination of the deep pseudopupil (FRANCESCHINI 1975). Among approximately 30,000 female progeny, rare F₁ females lacking a deep pseudopupil were selected and individually mated to *FM7*-bearing males to recover the irradiated *y sc v f X* chromosome.

For *in situ* hybridization, salivary glands were prepared using standard protocols (ASHBURNER 1989). Biotinylated probes were made by nick-translation of DNA in the presence of Bio-16-dUTP (ENZO Biochemicals), and *in situ* hybridizations carried out according to the procedure of LAVERTY and LIM (ASHBURNER 1989), using horseradish peroxidase/streptavidin supplied by FisherBiotech at a 1:250 dilution.

Cosmid and cDNA library screens: A genomic clone, λ RB2 (generously provided by MARK TANOUYE, University of California, Berkeley), which hybridizes in the proximal 12C region, was used to screen a cosmid library constructed in the cosPer vector by JOHN TAMKUN and MATT SCOTT (University of Colorado, Boulder). Standard procedures were used in screening the cosmid library and identifying cosmid c12B1 (MANIATIS, FRITSCH and SAMBROOK 1982).

A 5.0-kb *EcoRI* fragment of cosmid c12B1 was used to perform a second screen of the library and isolate overlapping cosmids extending in the distal direction. Probes were labeled by random priming (Random Primed DNA Kit, U.S. Biochemical) and [α -³²P]dATP (ICN). The 5.0-kb *EcoRI* fragment and an overlapping 3.0-kb *BamHI* fragment of c12B1 were used to probe a Drosophila head cDNA library (PALAZZOLO *et al.* 1989). Six cDNAs were isolated. Six different primers, derived from the sequence of these cDNAs, were used to construct a head cDNA extension library according to the protocols supplied by the manufacturers (U-Prime-It cDNA Synthesis Kit, Boehringer-Mannheim and Lambda Zap II, Stratagene). The extension library was probed with one of the originally isolated cDNAs; an additional 34 cDNAs were isolated.

Southern and Northern hybridizations: Genomic DNA was isolated from approximately 1 g of adult flies frozen at -20° and then ground to a fine powder in a mortar and pestle precooled in dry ice. Cells were disrupted by homogenization in 10 ml 0.1 M NaCl, 0.01 M β -mercaptoethanol, 0.01 M EDTA and 0.5% Triton X-100 at 4°. The homogenate was centrifuged at 1000 $\times g$ for 10 min and the pellet washed in the same homogenization buffer lacking Triton X-100 and recovered by centrifugation as before. Nuclei were disrupted in 5 ml 0.03 M Tris-Cl (pH 8.0), 0.1 M EDTA and 10% *N*-laurylsarcosine. The DNA was isolated by CsCl density gradient centrifugation. Two micrograms of genomic DNA were digested with the appropriate restriction enzyme and electrophoresed on a 0.7% agarose gel. The DNA was transferred to a nylon membrane (FisherBiotech) for hybridization. Probes were random primed labeled with [α -³²P]dATP. Hybridizations were carried out overnight at 65° as described by CHURCH and GILBERT (1984). Filters were washed twice (5 min each) in 2 \times SSPE (MANIATIS, FRITSCH and SAMBROOK 1982), 0.1% SDS at room temperature and then twice (45 min each) in 0.2 \times SSPE, 0.1% SDS at 65°.

RNA was isolated from Drosophila tissues, electrophoresed and transferred to nylon membranes as previously described (PALAZZOLO *et al.* 1989). RNA probes were *in vitro* transcribed from cDNA templates, hybridized and the membranes were washed as previously described (PALAZZOLO *et al.* 1989).

DNA sequencing and analysis: DNA sequencing was performed by dideoxy chain termination using templates prepared from pBluescript vectors. Reactions were carried out using the Sequenase version 2.0 system (U.S. Biochemical) according to the manufacturer's protocol. dITP sequencing protocols were used where necessary to sequence regions of structure compression. DNA and amino acid sequence analyses and searches to the GenBank and NBRF databases were performed on IBI Pestell Sequence Analysis software.

RESULTS

Cytogenetic location of the *rdgB* gene: Since all existing *rdgB* alleles are EMS-induced and not associated with a detectable cytological lesion, we generated new *rdgB* alleles that would allow precise cytological localization of the *rdgB* gene. Three new *rdgB* alleles were generated by ⁶⁰Co irradiation (*rdgB²*, *rdgB³* and *rdgB⁷*) that were viable in both hemizygous males and homozygous females. The cytology of the *rdgB⁷* allele revealed an X chromosome inversion with breakpoints in the 5C and 12C1 region (Figure 1A). The 12C1

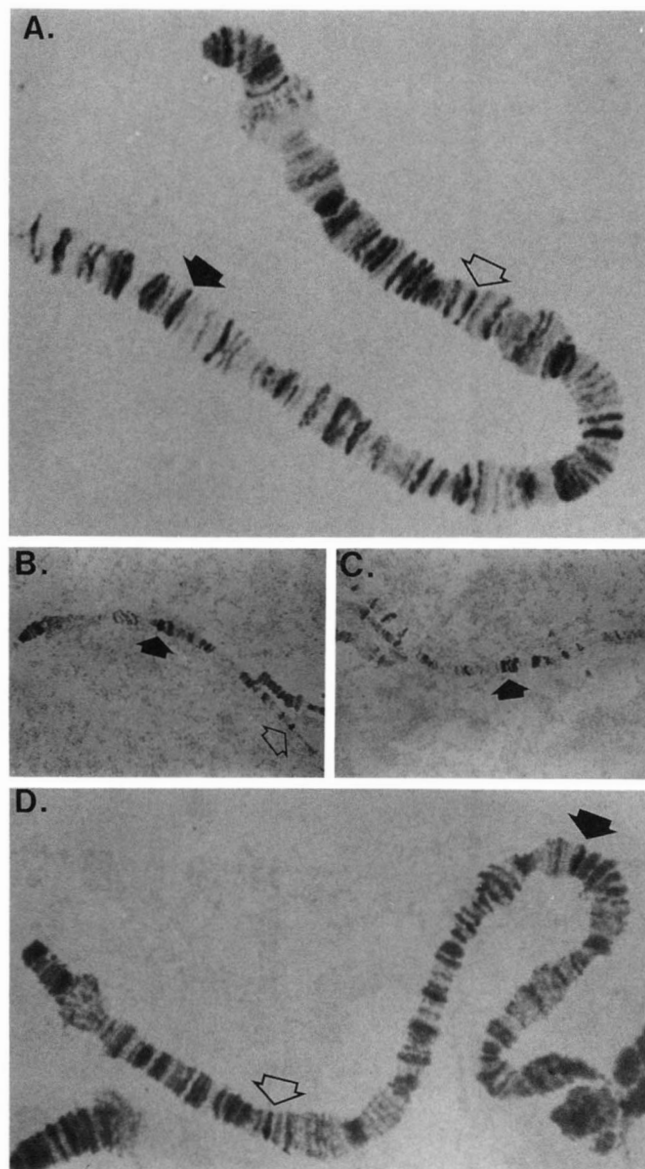


FIGURE 1.—Chromosomal *in situ* analysis of the *rdgB*⁵ inversion. (A) The inversion breakpoints of the orcein-stained *rdgB*⁵ chromosome are shown. The open arrow identifies the distal breakpoint (5C); the closed arrow marks the proximal (12C1,2) breakpoint. (B) Cosmid 12B1 DNA hybridized to the *rdgB*⁵ polytene chromosome reveals hybridization signals at the distal breakpoint (open arrow) and at the *white* gene (closed arrow). The *white* gene is labeled because *white* gene sequences are contained in the cosmid cosPer vector. (C) A more proximal view of the same X chromosome as in (B). The cosmid sequences also hybridized at the proximal breakpoint of *rdgB*⁵ (closed arrow). (D) cDNA22 hybridization shows transcription unit represented by cDNA is disrupted in the *rdgB*⁵ allele. Hybridization signals are seen at both the distal (open arrow) and proximal (closed arrow) inversion breakpoints of the *rdgB*⁵ chromosome.

breakpoint is consistent with previous genetic map location data of *rdgB* (12A-E region: HARRIS and STARK 1977; our unpublished observations). Therefore, the loss of *rdgB*⁺ function in the *rdgB*⁵ allele is likely a consequence of the 12C1 inversion breakpoint being within the *rdgB* gene.

To isolate DNA corresponding to the *rdgB*⁵ breakpoint, we performed a chromosomal walk using the genomic clone λ RB2, which hybridized *in situ* to the proximal 12C region (data not shown). Five hybridizing genomic DNAs were isolated from a cosmid library. Comparing *Eco*RI restriction maps of the isolated cosmids and λ RB2, we determined that c12B1 extended the furthest in the distal direction; a 5.0-kb *Eco*RI fragment was the most distal fragment within c12B1. Using this fragment, we rescreened the cosmid library and isolated two additional overlapping cosmids, designated c2B1 and c2B3, which extended the walk distally. The genomic restriction map and relative positions of the cosmid genomic clones are shown in Figure 2.

To correlate the cosmid DNA to the cytologic location of *rdgB*, we hybridized the cosmids *in situ* to polytene chromosomes. The most distal cosmid of the walk, c2B3, hybridized to the proximal region of 12B (data not shown). Therefore, the *rdgB* gene, at band 12C1, should reside between the λ RB2 DNA (in proximal 12C) and the most distal portion of the walk. *In situ* hybridization of cosmid c12B1 to the *rdgB*⁵ polytene chromosomes (Figure 1, B and C) showed that this cosmid contains the *rdgB*⁵ proximal inversion breakpoint. In addition, a cDNA derived from this region hybridized to both sides of the chromosomal inversion (Figure 1D), indicating this transcription unit, subsequently called the *rdgB* gene, is disrupted by the *rdgB*⁵ inversion.

Genomic localization of the *rdgB* gene: To confirm the cosmid DNA associated with the 12C1 chromosomal region contained the *rdgB* gene, we used Southern hybridization to examine the genomic DNA restriction digest patterns of the parental strain (*y sc v rdgB*⁺ *f*) and the *rdgB*², *rdgB*⁵ and *rdgB*⁷ alleles using DNA probes derived from cosmid c12B1. In every case, the restriction patterns, for the *y sc v rdgB*⁺ *f* strain and c12B1 were identical. However, each of the mutants showed altered restriction digest patterns. From these data, summarized in Figure 2, B and D, we located the alterations of the mutant alleles within a small genomic region. The *rdgB*² mutation is a deletion of about 3 kb of DNA entirely within the identified *rdgB* gene. The *rdgB*⁵ allele has a restriction polymorphism that maps to a 0.8-kb *Bam*HI fragment. The identity of this segment as the inversion's proximal breakpoint in 12C1 was confirmed by a cDNA, containing about 1 kb of DNA on either side of this 0.8-kb *Bam*HI fragment (cDNA22), that hybridized to both sides of the inversion breakpoint (see Figures 3 and 1D). *rdgB*⁷ is a deletion mutant lacking the entire region deleted in *rdgB*² and the proximal inversion breakpoint associated with *rdgB*⁵. Thus, the 5 kb of genomic DNA containing these abnormalities defines at least a portion of the *rdgB* gene.

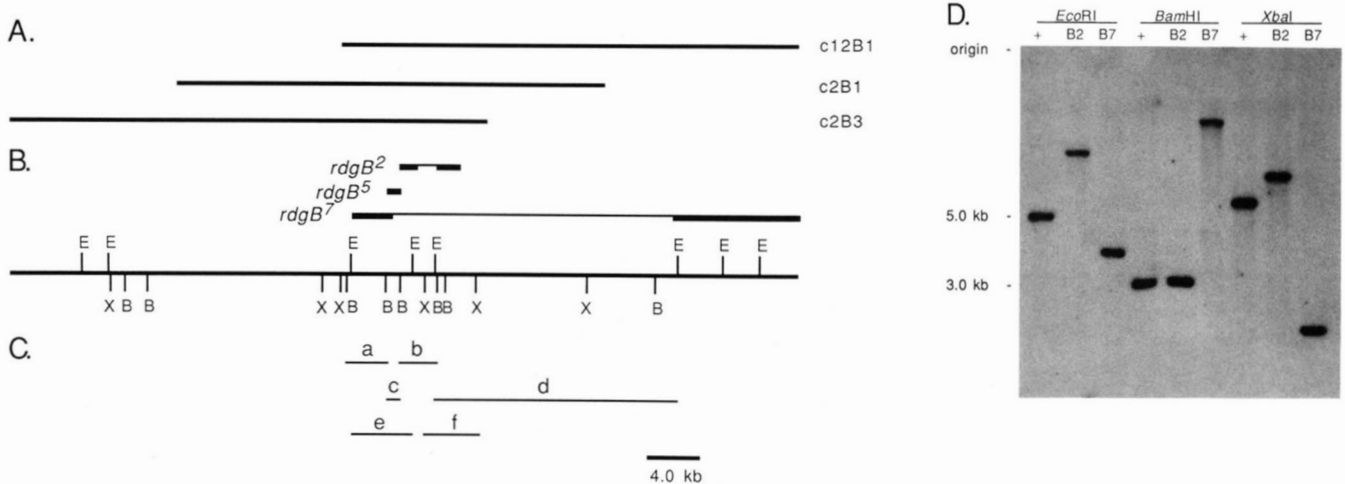


FIGURE 2.—Molecular map of the *rdgB* region. (A) Horizontal lines represent the genomic DNA contained in the cosmids c12B1, c2B1 and c2B3. Proximal is to the right. (B) A restriction map of wild-type genomic DNA that contains the *rdgB* gene. Restriction sites are labelled as follows: B, *Bam*HI; E, *Eco*RI; X, *Xba*I. Location of the defects associated with the different *rdgB* alleles is shown above the restriction map. The thin horizontal line corresponds to genomic DNA that is absent in the mutant; the thick horizontal lines represent the limits of resolution associated with mapping the breakpoints. Both *rdgB*² and *rdgB*⁷ are deletions. *rdgB*⁵ is an inversion with the proximal breakpoint in the region identified by the thick horizontal line. (C) The cosmid restriction fragments used as probes in genomic Southern analyses to locate the *rdgB* defects are shown. These are: a, 3.0-kb *Bam*HI; b, 3.0-kb *Bam*HI; c, 0.8-kb *Bam*HI; d, 20-kb *Eco*RI; e, 5.0-kb *Eco*RI; and f, 4.8-kb *Xba*I. (D) Representative genomic Southern hybridization using the distal 3.0-kb *Bam*HI fragment (fragment a) as probe. The genomic DNA was isolated from the parental *y sc v f* stock (+), *rdgB*² stock (B2) and *rdgB*⁷ stock (B7) and restricted with *Eco*RI, *Bam*HI and *Xba*I. The wild-type 5.0-kb *Eco*RI restriction fragment (fragment e) is present in wild-type flies but altered in *rdgB*² and *rdgB*⁷. The wild-type 3.0-kb *Bam*HI restriction fragment (fragment a), which overlaps the distal end of fragment e, is present in wild-type and *rdgB*² flies but altered in *rdgB*⁷ flies.

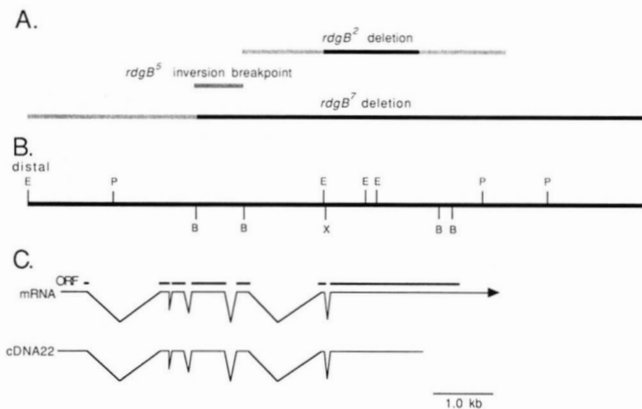


FIGURE 3.—The *rdgB* transcription unit. (A) Location of molecular defects of the three new *rdgB* alleles are shown above the restriction map. The hatched lines represent the error associated with mapping the lesions' defect, and a solid line represents genomic DNA absent in the allele. The location of the *rdgB*⁵ inversion breakpoint is shown as a hatched line. (B) Restriction map of the corresponding wild-type genomic DNA; B, *Bam*HI; E, *Eco*RI; P, *Pst*I; and X, *Xba*I. (C) A composite of several cDNAs represented as mRNA. cDNA22, which was used in the chromosomal *in situ* hybridization (Figure 1), is also shown. Horizontal lines represent the location of exons relative to the genomic DNA and *rdgB* breakpoints shown above. The open reading frame (ORF) is shown for the composite mRNA. A 1.0-kb scale is given.

The *rdgB* transcription unit: We isolated cDNAs corresponding to a transcription unit in this genomic region in two experiments. First, we screened a *Drosophila* head cDNA library (PALAZZOLO *et al.* 1989) with two genomic DNA fragments derived from cos-

mid c12B1 that contained the *rdgB*⁵ breakpoint and the *rdgB*² deletion region (see Figure 2C; probes b and e, 3.0-kb *Bam*HI and 5.0-kb *Eco*RI). Six cDNAs, ranging in size from 1.5 to 2.3 kb, mapped to the proximal region of the *rdgB*² deletion. None of the isolated cDNAs extended to the *rdgB*⁵ inversion breakpoint; the closest was approximately 2.0 kb proximal of the breakpoint. Because the cDNAs were directionally cloned and contained a poly(A)⁺ region, we were able to orient the direction of transcription and predicted that the *rdgB*⁵ breakpoint was upstream of the sequences represented in the cDNAs. Second, we constructed and screened a *rdgB*-primed head cDNA library (see MATERIALS AND METHODS) and identified 34 additional cDNA clones. The largest cDNA molecule isolated from the extension library contained DNA corresponding to the *rdgB*⁵ inversion breakpoint (see Figure 3). Figure 3C shows a representative transcript and its open reading frame in relation to the genomic restriction map and allele breakpoints. The open reading frame spans the genomic sequence that includes the entire deletion of allele *rdgB*² and the *rdgB*⁵ inversion breakpoint. In addition, the large *rdgB*⁷ deletion removes the majority of the open reading frame in the C-terminal direction. Therefore, a single transcriptional unit contains defects associated with all three *rdgB* alleles, indicating that this is the *rdgB* gene.

***rdgB* encodes a putative integral membrane pro-**

TATCCATAGAACCAACCGCTGACGACCCCGCTGTTTTCGAATTCATCATTCGACGAGCTATAGACGACGTCGGCCGCCACCCACCGCCAAATCACCCCTTCGGCCAAAAACGAAAAAAAACCCTGGACGACGACGCTCCCGCTTCACATCATCCAGCATGACCCAG	-180
CGCGGCAATCGATGATTCATTCCTCCCTAGCAGCAAGGACCAATAGAGGACGCGGAAAGGAGGAGCGCGGAAATGATGTCAGTGGTATGTCGCGGAGAGTGGCCATTCGCGAGAACGTCATAGCCGGAGGGGATGCCCGATTTCACAGCAAGGACCCCACTACCGGTGTCAAC	-1
ATG CTG ATC AAG GAG TAC CCG ATT CCG CTG CCC CTC ACC GTC GAG GAG TAC CGC ATC GCC CAG CTC TAC ATG ATT CCG AAA AAG AGT CGC GAG GAG AGC CAT GGC GAG GGC AGT GGC GTT GAG ATA ATC ATC AAT	+135
Met Leu Ile Lys Glu Tyr Arg Ile Pro Leu Pro Leu Thr Val Glu Glu Tyr Arg Ile Ala Glu Leu Tyr Met Ile Ala Lys Lys Ser Arg Glu Glu Ser His Gly Glu Gly Ser Gly Val Glu Ile Ile Asn	+45
GAG CCG TAC AAG GAT GGA CCC GGC GGT AAT GGT CAA TAC ACA AAG AAG ATC TAT CAT CTG GGC AAT CAT CTG GGC TGG ATT AAA AGT CTC TTG CCG AAA AGC GCT TTA ACC GTG GAG GAG GGC ATT GAA	+270
Glu Pro Tyr Lys Asp Gly Pro Gly Gly Asn Gly Gln Tyr Thr Lys Lys Ile Tyr His Val Gly Asp Tyr Val Lys Glu Asp Pro Lys Ser Leu Leu Pro Lys Ser Ala Leu Thr Val Glu Glu Glu Ala Met Glu	+90

TGC TAT CCG TAT ACC AGG ACT CCG TAC ACC TGT CCG TTT GTG GAG AAA TTC CTG CTG GAT ATT GAG ACA TAC TAT TAT CCG GAC AAT GGC TAT CAG GAC AAT GTC TTC CAG CTG TCC GGA AGC GAT TTG GGT AAT	+405
Cys Tyr Pro Tyr Thr Arg Thr Arg Tyr Thr Cys Pro Phe Val Glu Lys Phe Ser Leu Asp Ile Glu Thr Tyr Tyr Tyr Pro Asp Asn Gly Tyr Gln Asp Asn Val Phe Gln Leu Ser Gly Ser Asp Leu Arg Asn	+135
CGG ATC GTA GAC GTA ATT GAC ATT GTC AAG GAT CAG CTG TGG GGC GGT GAC TAT GTG AAG GAG GAG GAT CCC AAG CAC TTT GTG TCG GAC AAG AGC GGC CGT GGA CCC TTG GGC GAG GAT TGG CTG GAG GAT TAT	+540
Arg Ile Val Asp Val Ile Asp Ile Val Lys Asp Gln Leu Trp Gly Gly Asp Tyr Val Lys Glu Glu Asp Pro Lys His Phe Val Ser Asp Lys Thr Gly Arg Gly Pro Leu Ala Glu Asp Trp Leu Glu Glu Tyr	+180
TGG CCG GAA GTG AAG GGC AAA AAG CAA CCG ACA CCG GGC AAC ATG TCC CTG ATG ACC GCC TAC AAG ATC TGC CCG GTG GAT TTT CCG TAC TGG GGC ATG CAG ACA AAG CTG GAG AAG TTC ATC CAC GAT GTG GCG	+675
Trp Arg Glu Val Lys Gly Lys Lys Gln Pro Thr Pro Arg Asn Met Ser Leu Met Thr Ala Tyr Lys Ile Cys Arg Val Glu Phe Arg Tyr Trp Gly Met Gln Thr Lys Leu Glu Lys Phe Ile His Asp Val Ala	+225

CTG CCG AAG ATG ATG CTG CCG GGC CAT CCG CAG GCG TGG GCA TGG CAG GAC GAG TGG TTC GGC TTG ACC ATC GAG GAT ATA CCG GAG CTG GAG CGA CAG ACG CAA CTG CCG CTG GCC AAG AAA ATG GGC GGC GGC	+810
Leu Thr Lys Met Asp Leu Thr His Arg Glu Ala Trp Gln Asp Gln Ala Trp Gln Asp Gln Thr Phe Val Gly Leu Thr Ile Glu Asp Ile Arg Glu Thr Gln Leu Ala Leu Ala Lys Lys Met Gly Gly Gly	+270
GAG GAG TCC AGC GAC GAC AGC GTC TCG GAG CCG TAT GTC AGC AGC GCG GCC ACC GCC GCA TCC ACA ACG GGC AGC GAG CGA AAG AAG TCC GCT CCG GCT GTG CCG CCT ATT CTC ACC CAG CAG CCG CCG AGC GCC	+945
Glu Glu Cys Ser Asp Asp Ser Val Ser Glu Pro Tyr Val Ser Thr Ala Ala Thr Ala Ala Ser Thr Thr Gly Ser Glu Arg Lys Lys Ser Ala Pro Ala Val Pro Ile Val Thr Gln Gln Pro Ser Ala Glu	+315
CAG CCC AGT TCG GAT GAG GAG GCG GAG GAG GAG GAT GAC GAC GAG GAG AAG GAT GCC ATT GGC AGC GGC GAT CTG TCA GCC AAC CAA GGC GGA TCC CCG CAG CCG TCG CCG CAA AGC ATT CAA	+1080
Glu Ala Ser Ser Asp Glu Glu Gly Glu Glu Glu Asp Asp Glu Asp Glu Ser Val Val Lys Ala Ile Gly Thr Gly Val Asp Leu Ser Ala Asn Gln Gly Gly Ser Ala Gln Arg Ser Arg Ser Gln Ser Ile Gln	+360

ATG CCG CAG AAG GGC AAG TTC GGT TCA AAG GGT GCC CTT CAC TCG CCG GTG GGA TCT GCC CAT ACC TTC GAT CTC CAG GTG GCT AAC TGG CGT ATG GAG CGA TTG GAA GTG GAC TCC AAA TCC AAT TCG GAT GAG	+1215
Met Ala Gln Lys Gly Lys Phe Gly Ser Lys Gly Ala Leu His Ser Pro Val Gly Ser Ala His Ser Phe Asp Leu Gln Thr Ile Val Ala Asn Trp Arg Met Glu Arg Leu Glu Val Asp Ser Lys Ser Asn Ser Asp Glu	+405
GAA TTC TTT GAT TCG CTG GAC ACC AAT GAG ACG AAC TCG CTG GCC AAG TGG AGC TCG CTG GAG CTG CTT GGC GAG GGC GAC ACT CCG CCG CCA CAT GGC GGA CCG TCT ACT GCA CCA TCG GTG GGT GGC GGT	+1350
Pro Ser Thr Phe Asp Cys Leu Asp Thr Asn Glu Thr Asn Ser Phe Asp Lys Trp Ser Lys Thr Ser Leu Asp Glu Gly Glu Gly Asp Ser Pro Pro Pro His Gly Gly Pro Ser Ser Ala Ala Ser Val Gly Gly Arg	+450

GGC AAC TCG CCG CAA GAG GAG ACC ATA TTC AAT CAG GAC TTT CTG ATG CCG GTC GGC TCG GAG CCG GGC AAC AAG CCG GAT TTA CGT TCC TCG GCC AGC GTG GAT CCG ACT CAG GAT TCA TCG CCG CCG GGA TCG	+1485
Gly Asn Ser Arg Gln Glu Asp Ser Ile Phe Asn Gln Asp Phe Leu Met Arg Val Ala Ser Glu Arg Gly Asn Lys Arg Gln Leu Arg Ser Ser Ala Ser Val Asp Arg Ser His Asp Ser Ser Pro Pro Gly Ser	+495
CCG AGT ACA CCG TCG TGT CCC ACA ACC ATT CTG ATC GTC GTT GTC CAT GCG GGC AGC GTT TTG GAT CCG ACC AGC GAG CTG ACC AAG AAA TCC GAT GTG ACC ACA TTC CGT GGC TCC TTC GAG GCG GTT ATG	+1620
Pro Arg Thr Pro Ser Cys Pro Thr Thr Ile Leu Ile Leu Val His Ala Gly Ser Val Leu Leu Asp Ala Ala Ser Glu Leu Thr Ala Lys Ser Asp Val Thr Thr Phe Arg Tyr Ser Phe His Gly Ser Phe His Met	+540
CGA CAC GAC TAT CCG AGC CTC CTC ACC CAT GTG ACC ATC AAG ATG GTG CCG TCC CCC TGA ATA TCC ACC GAC CCG CTG GGC ATT CTC TCC AGC CTG AGT CCG TAC TCC TTT GAT GCC TCG CCG TCG CCG GCG GAT	+1755
Arg His Asp Tyr Pro Ser Leu Thr Thr His Val Thr Ile Lys Met Val Pro Cys Pro Ser Ile Cys Thr Asp Ala Leu Gly Ile Leu Ser Ser Leu Ser Pro Tyr Ser Phe Asp Ala Ser Pro Ser Ala Glu Asp	+585
ATA CCG AAT ATG GCC GAT CTC CCG ATT GGA GCT ATA CCA CTA TCT GTG GCA TCG CCA GAA TTC CAC GAG ACG GTC AAC AAG AGC GTT GCC GCT GCC AAT ATT GTC TGC CAT GAG TTT TTG AAA TCG GAG GAG	+1890
Ile Pro Asn Ile Ala Asp Val Pro Ile Gly Ala Ile Pro Leu Leu Ser Val Ala Ser Pro Glu Phe His Glu Thr Val Asn Lys Thr Val Ala Ala Ala Asn Ile Val Cys His Glu Phe Leu Lys Ser Glu Glu	+630

GGT CAC GGA TTC TCT GGC CAG ATT GTC ATG CTG GGC GAT TCG ATG GGT TCG CTG CTG GCG TAC GAG GCC CTC TGC CGA TCG AAT GGC AGC CAG CCG GGC ACG GCT TCG GGT GCC TCG AAT TCC GGC GGA GAT GCG	+2025
Gly His Gly Phe Ser Gly Gln Ile Val Met Leu Gly Asp Ser Met Gly Ser Leu Leu Ala Tyr Glu Ala Leu Cys Arg Ser Asn Gly Ser Gln Pro Gly Thr Ala Ser Gly Ala Ser Asn Ser Gly Gly Asp Ala	+675

GCC ACA AAT ATA AAT ACC CAC AAT CCG TTG AGC CCA CGT AAT TCG CGA TTG GAC GAT CAG GAG GGT TTC ATC GAA GGC GAT GAT GCC AAG CGT TTG CTA CTG GCC CCA TCG CCA CGT AGA CCG GCT TCC AGC	+2160
Ile Thr Asn Ile Asn Thr His Asn Pro Leu Ser Pro Arg Asn Ser Arg Leu Asp Asp Asp Glu Arg Phe Ile Glu Ala Asp Leu Asp Ala Lys Arg Leu Leu Val Ala Pro Ser Pro Arg Arg Arg Arg Ser	+720
TCA TCC AGC GAT TCG CGT GCC ACC AAA TTG GAC TTT GAC GTC TTC ATG TTT GCA TCG CCG CTA TCT GTG GTG CCG GCT GCA AGG AAA CTT CAC GAT GCC AAG GCC CCG GTG CCG CCG AAC TGC	+2295
Ser Ser Ser Asp Ser Arg Ala Thr Lys Leu Asp Phe Glu Val Cys Asp Phe Phe Met Phe Gly Ser Pro Leu Ser Val Val Leu Ala Ala Arg Lys Leu His Asp Ala Lys Ala Ala Leu Pro Arg Pro Asn Cys	+765
CAC GAG CTC TAC AAT CTG TTC CAT CCA ACC GAT CCG ATC GCC TCG CCG CTG GAG CCG CTT CTG AGC GGC CCG TTT TCT ATA TTG GCG CCA GTC AAT GTC CCA CCG TAC CCG AAG TAT CCG CTG GGT AAT GGA CAG	+2430
His Gln Val Tyr Asn Leu Phe His Pro Thr Asp Arg Leu Glu Ser Arg Leu Glu Pro Leu Leu Ser Ala Arg Phe Ser Ile Leu Ala Pro Val Asn Val Pro Arg Tyr Thr Lys Tyr Pro Leu Gly Asn Gly Gln	+810
CCA TTG CAT TTA TTG GAG GTC ATT CAA TCG CAT CCG CAG CCG TTT AAC GAT GGC AAT AAC CTA TTG GCT GGT CCG CGT TTG TCG GAC GCA TCC ATG CAG AGC ACG ATA TCG GGT CTG ATT GAG AAT GTC TCG CTT	+2565
Pro Leu His Leu Leu Glu Val Ile Gln Ser His Pro Gln Arg Phe Asn Asp Gly Asn Asn Leu Leu Ala Gly Arg Arg Leu Ser Asp Ala Ser Met Gln Ser Thr Ile Ser Gly Leu Ile Glu Asn Val Ser Leu	+855

AGT ACG ATC CAT GCC CTG CAA AAC TGG TGG GGC ACA AAG CCG TTG GAT TAC GCA TTA TAT TCC CCG GAG GGA TTG AAT ATT TTC CCT GCT CAC GCC TTG CCG CAC CTC TAT GCC AGC TAC TGG CAG AGT	+2700
Ser Thr Ile His Ala Leu Gln Asn Lys Trp Trp Gly Thr Lys Arg Leu Asp Tyr Ala Leu Tyr Cys Pro Glu Gly Leu Ser Asn Phe Pro Ala His Ala Leu Pro His Leu Phe His Ala Ser Tyr Trp Glu Ser	+900
CCG GAT GTC ATT GCT TTT ATT CTA CCG CAT ATT GGC AAA TTC GAG GGC ATA CCC TTT GTC GGC TCA CAG GAT GAG AAG CAC AAT GCC TTC TCT CAC CCG AAG GAG AAG TGG ATT AAG AAA CCG ACC	+2835
Pro Asp Val Ile Ala Phe Ile Leu Arg Gln Ile Gly Lys Phe Glu Gly Ile Pro Phe Val Gly Ser Asn Asp Asp Lys Asp Asn Ala Ser Phe His Pro Gly Gln Pro Arg Glu Lys Trp Ile Lys Lys Arg Thr	+945

TCG GTT AAG CTG AAA AAT GTA GCC GCC AAT CAT CCG GCC AAC GAT GTA ATC GTG CAG GAG GGC AGG GAG CAG CGA TTG AAT CCG AGA TTT ATG TAC GGA CCC CTG GAC ATG ATC ACG CTG CAC GGT GAA AAG GTG	+2970
Ser Val Lys Leu Lys Asn Val Ala Ala Asn His Arg Ala Asn Asp Val Ile Val Gln Glu Gly Arg Glu Gln Arg Leu Asn Ala Arg Phe Met Tyr Gly Pro Leu Asp Met Ile Thr Leu His Gly Lys Val	+990
GAT GTG CAC ATT ATG AAG GAT CCG CCG GCG GCG CAG TGG ACA TTC CTC AGC ACC GAG GTG ACG GAC AAG AAT GGT CCG ATT CCG TAC AGC ATT CCG GAT CAG GTA TCC CTT CCG TAT GGT ATA TAT CCG GTT AAG	+3105
Asp Val His Ile Met Lys Asp Pro Pro Ala Gly Gln Trp Thr Phe Leu Ser Thr Glu Val Thr Asp Lys Asn Gly Arg Ile Ser Tyr Ser Ile Pro Asp Gln Val Ser Leu Gly Tyr Gly Ile Tyr Pro Val Lys	+1035
ATG GTG CTC CGT GGC GAT CAC ACC TCG GTG GAT TGC TAT ATG GCG GTG GTG CCG GAT TAA CCGAATCGGTGGTTCACGATGATGGCTTATCCCGCTTCGATGTCGGGTGACAGGTAGGGATCCCAAGTGGCTGGCGGAGGTGTCGATGTTTGGC	+3264
Met Val Val Arg Gly Asp His Thr Met Ala Val Val Pro Arg	
GCACATGGCAGGAGCTGGGCTACCTGCTCATTTACATCCCGGACGACCGGATATGCGAGCAGCAACCGGTGTCTGGCTGAGCCAGCACAACCTCCCGACCGGCTGATCTGTTCCCGACGGCGCTGCCACGATCCATTGGCCCAACAGGCGGTATCTCAACAATTTGGTT	+3443
CAGAACCATGGAATCTCAATTAACCTCCCGTACCGGACGACGAGCAGGATAGTGTCTACAGCAATGTTGGCATCGCAACCGATCAAAATTTTCATCGTGGGCAAGTGGCAAGAGCTGCAGTCGAATGCCACCGTCTTACGAGTGGCTTCCCGCCACTTGGCCGCTTTCAGCGCT	+3622
GTGGTGGTTCGCTCCCGGCAAGGCGAATCCCGCATGCTCA?TCACGCGGATGCTTCAATCTCCCGGCAAGCAGCAACCGGCGGCGAGAGGCTGCAATGACAGCAGCAAGTGAATAATGCAATCCAGCAACCAATGTTTAGAGCAATGAAAACAACAACTA	+3801

FIGURE 4.—Nucleotide and deduced amino acid sequence of the *rdgB* cDNA. The first methionine in the longest open reading frame is used as the initiation codon. Six putative transmembrane domains, beginning at residues 499, 555, 586, 737, 784 and 895, are underlined. The potential N-linked glycosylation sites, located at amino acid positions 194, 414, 612, 658, 852 and 928, are indicated by three stars (***) below the Asn residues. The EEGEE putative Ca²⁺ binding domain, is found at amino acid residues 321 through 325 and marked with “&.” A homologous EF hand sequence (DXDXD; KRETSINGER 1976), found at residues 328 through 332, is marked with exclamation points (!). The potential nucleotide triphosphate binding sequence, found at amino acid positions 51 through 74, is marked by “@.”

tein: DNA sequencing of the most prevalent class of cDNA reveals an open reading frame of 3162 bp, with 358 bp of 5'-untranslated and 636 bp of 3'-untranslated sequences (Figure 4). We chose the first AUG in the longest open reading frame as the translational start codon because six of the seven preceding nucleotides matched the consensus sequence for *Drosophila* translation initiation sequences (CAVENER 1987). Comparison between the genomic and cDNA sequences revealed the exon/intron pattern with

splice junction consensus sequences positioned appropriately. Conceptual translation of the transcript produces a protein of 116 kD with a predicted pI of 5.62. A 498 amino acid N-terminal region and a 140-amino acid C-terminal tail region are joined by a 416-amino acid region containing 6 putative transmembrane domains (Figure 5). The transmembrane domains were identified as hydrophobic regions of at least 16 amino acids. The sequence contains six putative N-linked

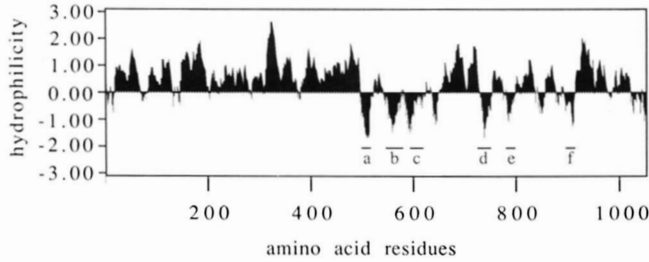


FIGURE 5.—Hydrophilicity plot for the putative *rdgB* gene product. KYTE and DOOLITTLE (1982) parameters (window size of 19 amino acids) were used. Six potential transmembrane domains are designated as a, b, c, d, e and f. These domains were identified as being at least 16 amino acids in length and flanked by a charged amino acid.

glycosylation sites, one putative ATP binding region, an EF hand domain, and a highly acidic region found in the N-terminal domain (see legend of Figure 5). The overall sequence lacks significant homology to any sequence in the GenBank and NBRF databases.

Expression of the *rdgB* gene: RNA probes, transcribed *in vitro* from several cDNAs, detected *rdgB* transcripts by Northern analysis. All probes detected five transcripts of 3.9, 4.8, 7.2, 7.9 and 9.5 kb (Figure 6). In addition, hybridization with labeled DNA fragments from cosmid c12B1 detected either the same five mRNAs or none (data not shown). This confirmed the presence of only a single transcription unit in the isolated genomic DNA. The five transcripts were identified in poly (A)⁺ mRNA prepared from fly heads but not in the poly (A)⁺ mRNA prepared from body tissue. The same five transcripts were also detected in poly (A)⁺ mRNA isolated from the heads of the *Drosophila* mutant *eyes absent* (*eya*; SVED 1986). Using a mouse polyclonal antisera generated against a *rdgB* fusion protein, we identified a single 160 kD protein by Western analysis and localized the *rdgB* protein to the photoreceptor rhabdomeres and optic lobes of the brain (data not shown).

DISCUSSION

Molecular analysis of the *rdgB* gene was carried out to elucidate the function of the *rdgB* protein and to work toward an understanding of one form of retinal degeneration. The following data establish that we have successfully cloned the *rdgB* gene. First, we generated three new X-ray-induced *rdgB* alleles and determined the *rdgB*⁵ allele is an X chromosome inversion with breakpoints in the 5C and 12C1 salivary chromosome regions. Because the 12A-E region contains the *rdgB* gene (HARRIS and STARK 1977), the breakpoint at 12C1 is likely within the *rdgB* gene. Second, we established that all three mutants contained new alterations within a 5-kb genomic region. The clustering of the different sequence anomalies identifies a genomic region containing the *rdgB* gene. Third, we isolated several cDNAs that correspond to

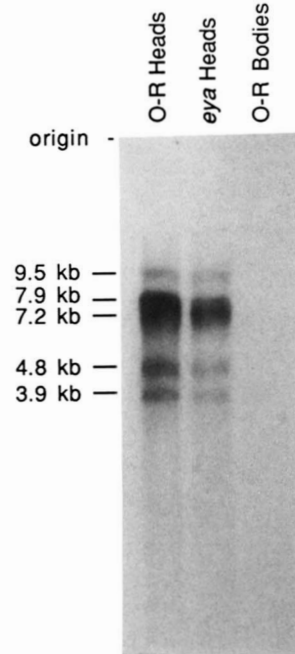


FIGURE 6.—RNA-RNA Northern analysis. cDNA22 was transcribed *in vitro*, the resulting RNA probe was hybridized to poly(A)⁺ mRNA isolated from O-R heads, the mutant *eyes absent* (*eya*) heads, and O-R bodies. The origin and the mRNA transcript sizes, in kilobases, are listed. The absence of a signal in the O-R bodies lane is not due to the absence of mRNA; hybridization with different probes detected similar amounts of mRNA in all the lanes.

a single transcriptional unit in this genomic region. This transcription unit is disrupted by all three *rdgB* mutations. Therefore, we have designated this transcriptional unit as the *rdgB* gene.

We believe that the five mRNAs transcribed from the *rdgB* gene, ranging in size from 3.9 to 9.5 kb, primarily result from alterations in the 5'- and 3'-untranslated regions and not from differential splicing within the open reading frame. DNA sequence analysis of different cDNAs revealed multiple alternative splicing events, some with small alterations within the largest open reading frame and others with large differences in the 3'-untranslated region (data not shown). Using a fusion protein that contains *rdgB* sequences common to all of the cDNAs, we generated a polyclonal antisera that detects a single 160-kD protein in *Drosophila* head extracts by Western blot analysis (data not shown). This suggests that the different mRNAs generate a similar sized protein from a common open reading frame. The size of the identified 160-kD protein is compatible with the expected primary sequence of 116 kD, especially if the six potential N-linked glycosylation sites are actually glycosylated.

We detected *rdgB* mRNA transcripts in the head, but not exclusively in retina. Polyclonal antisera raised against the *rdgB* fusion protein stains the *Drosophila* retina and neuropil of the central brain and optic lobes (lamina and medulla; data not shown). The

localization of the protein in tissues outside of the retina is consistent with the Northern hybridizations of the *eya* heads and the observation that some *rdgB* alleles affect olfaction (WOODARD *et al.* 1989; C. WOODARD, E. ALCORTA and J. CARLSON, personal communication).

Using our sequence information, we propose that the *rdgB* protein acts as a photoreceptor Ca^{2+} transporter. Although neither the *rdgB* gene nor the putative protein product share significant identity to any sequences in the Genbank and NBRF databases, hydrophobic calculations indicate the *rdgB* protein contains six potential membrane spanning domains typical for an ion channel or transporter (see CATTERALL 1988). The *rdgB* primary sequence contains regions homologous to domains of a Ca^{2+} -ATPase from rabbit muscle sarcoplasmic reticulum (MACLENNAN *et al.* 1985). The calcium-binding domain of the rabbit molecule has been ascribed to a 100 amino acid stretch which has a high concentration (17%) of glutamate. The *rdgB* protein contains a similar stretch of acidic residues on the amino terminal side of the first transmembrane domain (amino acids 242–342) with 17% glutamate and 11% aspartate residues. The sequence EEGEE, found within the acidic domain of the Ca^{2+} -ATPase, is thought to be a low affinity site of calcium binding (MACLENNAN *et al.* 1985). The EEGEE sequence is also found within the acidic region of the *rdgB* protein (residues 321 through 325). The *rdgB* protein contains a sequence homologous to an ATP binding site consensus sequence (WEINMASTER, ZOLLER and PAWSON 1986); whereas an ATP binding domain has been functionally demonstrated in the Ca^{2+} -ATPase. Although the identified domains (six hydrophobic domains, a Ca^{2+} binding domain, and ATP binding domain) of the *rdgB* protein are not positioned as they are in the Ca^{2+} -ATPase, the existence of these motifs in the sequence suggests that the *rdgB* protein may function as a photoreceptor Ca^{2+} transporter.

Some earlier observations are consistent with *rdgB* acting as a Ca^{2+} transporter. RUBINSTEIN *et al.* (1989b) showed that ERG spike potentials, an early sign of retinal degeneration in *rdgB*, can be eliminated by the Ca^{2+} chelator EGTA. This suggests that Ca^{2+} regulation may be abnormal in *rdgB* mutants, as expected if the protein acts as a Ca^{2+} transporter. We also note that a Ca^{2+} -ATPase activity has been identified in the vertebrate retinal rod outer segment discs (PUCKETT, ARONSON and GOLDIN 1985). The localization of *rdgB* protein in rhabdomeres, the analogous structure of invertebrate photoreceptors, would allow it to perform the same function. Therefore, the *rdgB* mutant phenotype, size and character of the protein, and the cellular location of the protein are consistent with *rdgB* acting as a Ca^{2+} -ATPase, even though the limited

homology with characterized proteins precludes assigning *rdgB* a definitive role. An earlier hypothesis, that *rdgB* functions as a phosphoprotein phosphatase (MINKE *et al.* 1990), is not supported by our data. We found no known phosphatase consensus sequences in the *rdgB* gene product. The topology of the *rdgB* protein as a putative integral membrane protein also makes it unlikely that it functions as a conventional phosphatase.

We are now investigating the cellular and subcellular location of the *rdgB* gene product. The spatial distribution of *rdgB* in the retina will suggest roles for the *rdgB* protein in the retina and other sensory neurons which can be tested by expressing and studying the *rdgB* protein in a heterologous system.

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