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 Ca^{2+}-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* (MEYERTHOLEN *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 $norpA^{sull}$ 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*$^{+}$ *f* 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 F_1 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/strepavidin supplied by FisherBiotech at a 1:250 dilution.

Cosmid and cDNA library screens: A genomic clone, λRB2 (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 *Eco*RI 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-^{32}P]dATP (ICN). The 5.0-kb *Eco*RI fragment and an overlapping 3.0-kb *Bam*HI 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-Mannhiem 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 × *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-^{32}P]dATP . Hybridizations were carried out overnight at 65° as described by CHURCH and GILBERT (1984). Filters were washed twice (5 min each) in 2 × SSPE (MANIATIS, FRITSCH and SAMBROOK 1982), 0.1% SDS at room temperature and then twice (45 min each) in 0.2 × 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 protcols 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 Pustell 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 ^{60}Co irradiation (*rdgB*^{2}, *rdgB*^{5} and *rdgB*^{7}) that were viable in both hemizygous males and homozygous females. The cytology of the *rdgB*^{5} allele revealed an *X* chromosome inversion with breakpoints in the 5C and 12C1 region (Figure 1A). The 12C1

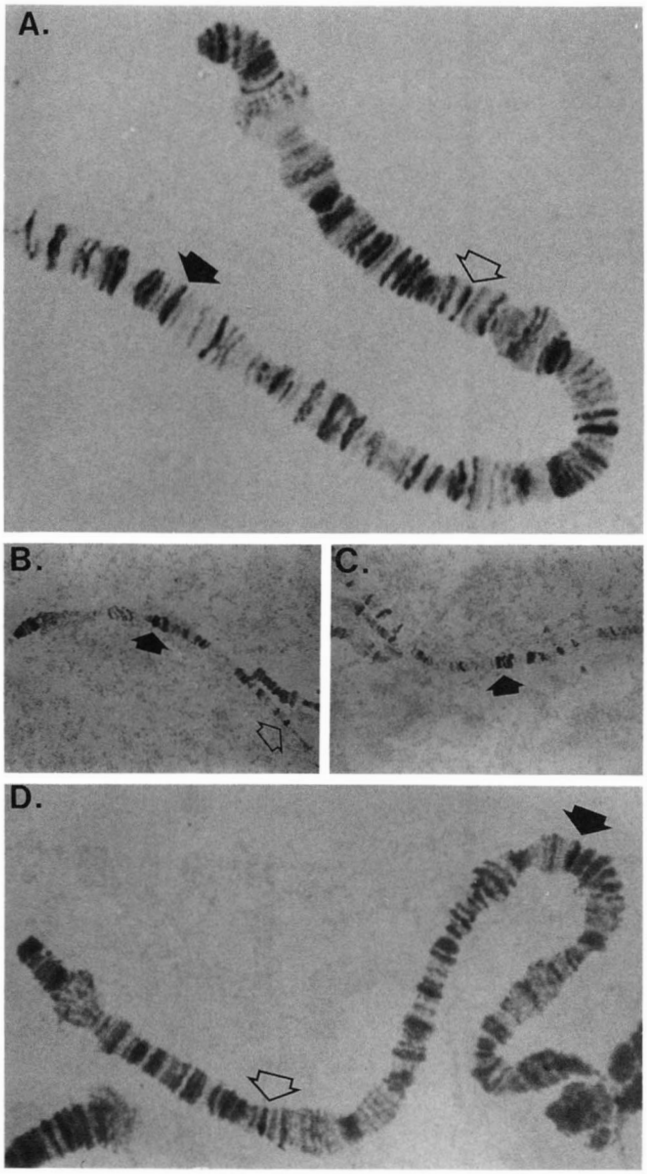

FIGURE 1.—Chromosomal *in situ* analysis of the $rdgB^5$ inversion. (A) The inversion breakpoints of the orcein-stained $rdgB^5$ chromosome are shown. The open arrow identifies the distal breakpoint (5C); the closed arrow marks the proximal (12C1,2) breakpoint. (B) Cosmid 12B1 DNA hybridized to the $rdgB^5$ 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 λRB2, 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 λRB2, 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 λRB2 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.

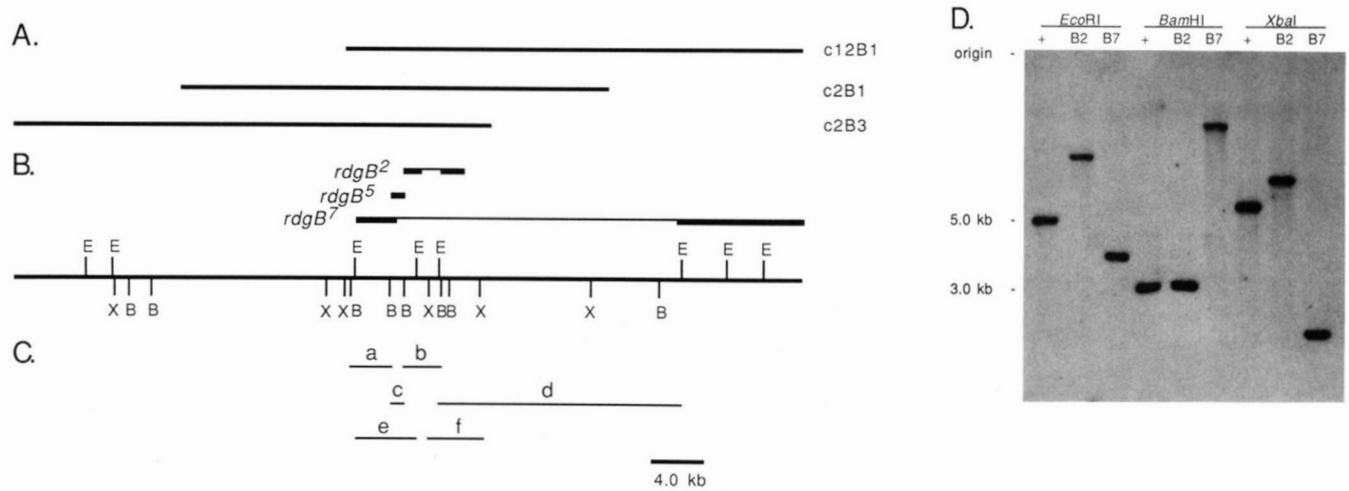

FIGURE 2.—Molecular map of the *rdgB* region. (A) Horizontal lines represent the genomic DNA contained in the cosmids c12B1, c2B1 and c2B3. Proximal is to the right. (B) A restriction map of wild-type genomic DNA that contains the *rdgB* gene. Restriction sites are labelled as follows: B, *Bam*HI; E, *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.

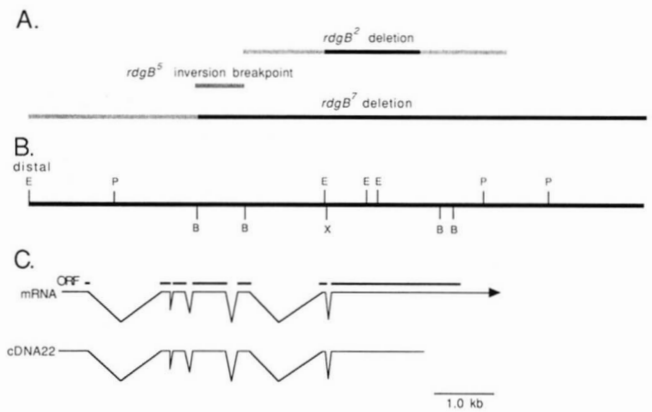

FIGURE 3.—The *rdgB* transcription unit. (A) Location of molecular defects of the three new *rdgB* alleles are shown above the restriction map. The hatched lines represent the error associated with mapping the lesions' defect, and a solid line represents genomic DNA absent in the allele. The location 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 cosmid 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-**

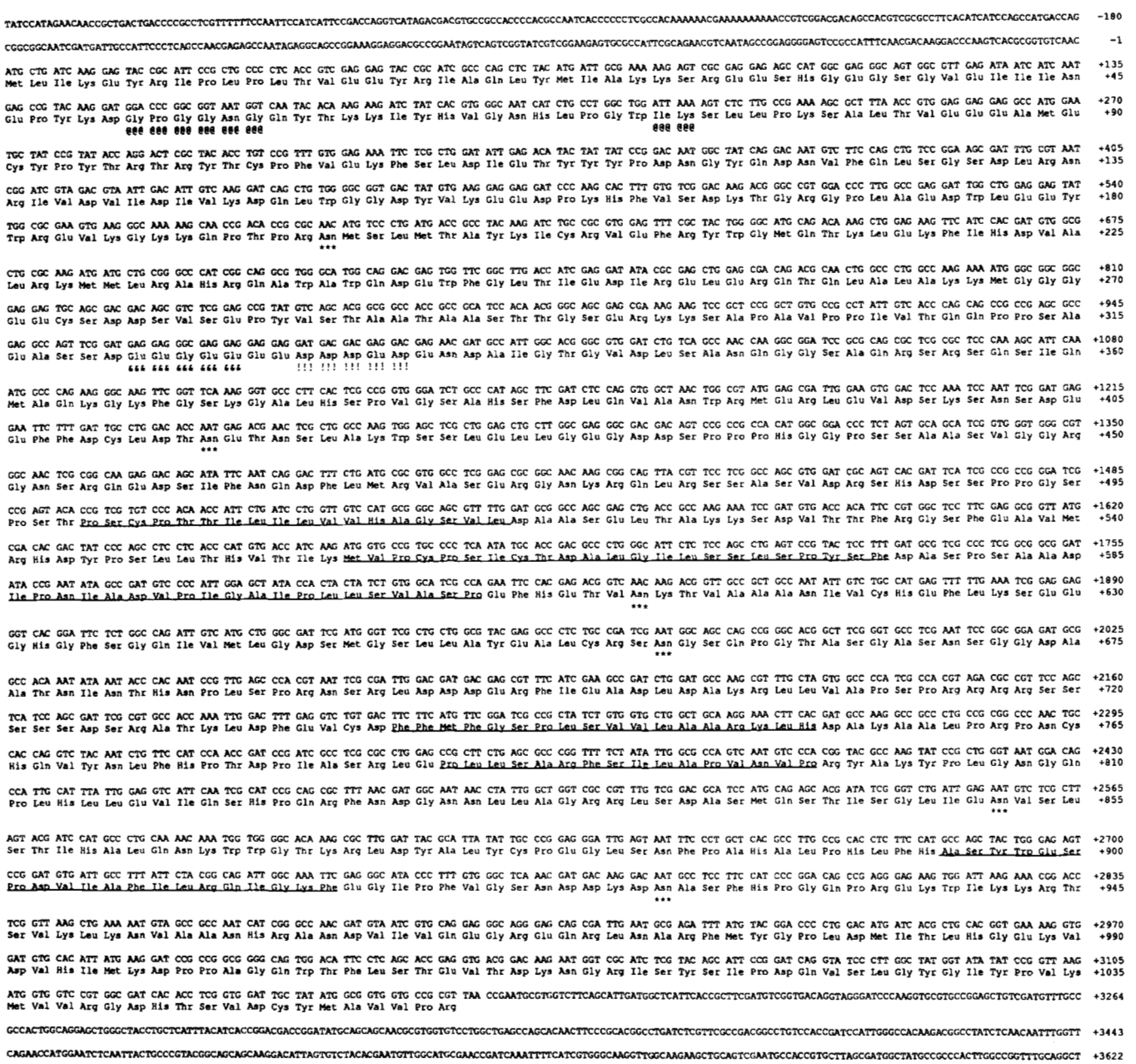

FIGURE 4.—Nucleotide and deduced amino acid sequence of the *rdgB* cDNA. The first methionine in the longest open reading frame is used as the initiation codon. Six putative transmembrane domains, beginning at residues 499, 555, 586, 737, 784 and 895, are underlined. The potential N-linked glycosylation 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 Ca^{2+} 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

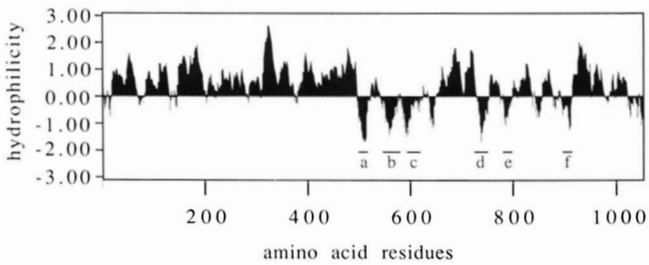

FIGURE 5.—Hydrophilicity plot for the putative *rdgB* gene product. KYTE and DOOLITTLE (1982) parameters (window size of 19 amino acids) were used. Six potential transmembrane domains are designated as a, b, c, d, e and f. These domains were identified as being at least 16 amino acids in length and flanked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

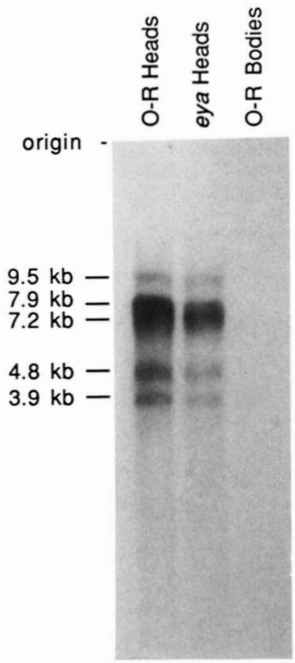

FIGURE 6.—RNA-RNA Northern analysis. cDNA22 was transcribed *in vitro*, the resulting RNA probe was hybridized to poly$(A)^+$ mRNA isolated from O-R heads, the mutant *eyes absent* (*eya*) heads, and O-R bodies. The origin and the mRNA transcript sizes, in kilobases, are listed. The absence of a signal 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.

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 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, hydropathic 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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