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Novel $G_q\alpha$ isoform is a candidate transducer of rhodopsin signaling in a *Drosophila* testes-autonomous pacemaker

(G protein/phototransduction/alternative splicing)

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ABSTRACT **DGq is the alpha subunit of the heterotrimeric GTPase (Gα), which couples rhodopsin to phospholipase C in *Drosophila* vision. We have uncovered three duplicated exons in *dgq* by scanning the GenBank data base for unrecognized coding sequences. These alternative exons encode sites involved in GTPase activity and Gβ-binding, NorpA (phospholipase C)-binding, and rhodopsin-binding. We examined the *in vivo* splicing of *dgq* in adult flies and find that, in all but the male gonads, only two isoforms are expressed. One, *dgqA*, is the original visual isoform and is expressed in eyes, ocelli, brain, and male gonads. The other, *dgqB*, has the three novel exons and is widely expressed. Remarkably, all three nonvisual B exons are highly similar (82% identity at the amino acid level) to the $G_q\alpha$ family consensus, from *Caenorhabditis elegans* to human, but all three visual A exons are divergent (61% identity). Intriguingly, we have found a third isoform, *dgqC*, which is specifically and abundantly expressed in male gonads, and shares the divergent rhodopsin-binding exon of *dgqA*. We suggest that DGqC is a candidate for the light-signal transducer of a testes-autonomous photosensory clock. This proposal is supported by the finding that rhodopsin 2 and arrestin 1, two photoreceptor-cell-specific genes, are also expressed in male gonads.**

Phototransduction in vertebrates and invertebrates is similar in that both use a heterotrimeric GTPase (G protein)-linked opsin that is activated by light-mediated retinoid isomerization (reviewed in ref. 1). Vertebrate opsins are coupled to G proteins of the "i" class (G_i) that activate cGMP-catabolizing phosphodiesterases to close cGMP-gated channels and hyperpolarize the cell. However, invertebrate opsins are linked to a $G_q\alpha$-activated phospholipase C (PLC) that mediates cell depolarization. In *Drosophila*, light-stimulated rhodopsin (Rh) activates a G protein with subunits DGq (Gα; ref. 2) and Gβe (Gβ; ref. 3). DGq then activates a PLC, NorpA (4), that produces inositol 1,4,5-trisphosphate and diacylglycerol. Inositol 1,4,5-trisphosphate in turn leads to the opening of cation channels and cell depolarization. All these gene products have been characterized as photoreceptor-cell-specific and have known mutations, as do several feedback modulators and downstream effectors.

We report that DGq has multiple isoforms resulting from the alternative splicing of three duplicated exons. One isoform, *dgqA*, corresponds to the originally described visual DGq (5). A second isoform, *dgqB*, has the three novel exons and corresponds to a cDNA recently cloned from fly antennae (6); it is widely expressed in the fly. The third isoform, *dgqC*, is only expressed in male gonads and has the visual exons at the Gβ-binding/GTPase and Rh-binding positions, but the nonvisual exon at the PLC-binding position. Since Rh2 (7) is expressed in the male gonads, we suggest that DGqC is a component of a testes-specific photosensory apparatus.

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MATERIALS AND METHODS

Gq Family Analysis. All Gαs in SwissProt and GenPept were identified using BLAST (8) and aligned with ClustalV (9). In a tree built from this alignment (PROTPARS programs from PHYLIP, J. Felsenstein, University of Washington, Seattle), DGq shares a clade with 15 additional proteins: Egl-30 (GenBank accession no. U56864, *Caenorhabditis elegans*), GBQ_LYMST (snail), GBQ_LOLFO (squid), GBQ_XENLA (*Xenopus*), GBQ_MOUSE, human Gq, (GenBank accession no. U43083), GB11_XENLA, GB11_MELGA (turkey), GB11_BOVINE, GB11_MOUSE, GB11_HUMAN, GB14_BOVINE, GB14_MOUSE, GB15_MOUSE, and GB16_HUMAN. We refer to this clade as the $G_q\alpha$ family. To construct the sequence logo of Fig. 2, the regions of all the $G_q\alpha$ family members corresponding to DGq exons 4, 7, and 8 were extracted. The letter height of a residue with frequency (f) is $f \log_2 f$ (10) using WEBLOGO (S. E. M. Brenner, Cambridge University).

Reverse Transcription PCR (RT-PCR). *Drosophila melanogaster* Oregon-R (Gelbart substrain) flies were freeze-dried (11) and microdissected. The whole heads, thoraces, and abdomens were homogenized by pulverization in liquid N_2, while the other much smaller parts were triturated in RNA extraction buffer. poly(A) RNA was isolated (MicroFastTrack kit, Invitrogen), and poly(dT) cDNA was synthesized (First-Strand kit, Pharmacia). PCR with *gapdh2* (12) primers was used to equalize the cDNA concentrations: dilution curves of all samples were compared by ethidium staining and repeated several times, until all cDNAs were approximately equilibrated (we estimate within 2-fold). Equalized cDNAs were used in hot-start (adding deoxynucleotides after reaction reaches 90°C) PCR amplifications (94°C for 20 s; 52°C for 30 s; 72°C for 40 s) for 40 cycles (Promega reagents and protocol). Products were run on 1% agarose and either were stained with ethidium bromide (see Fig. 4) or were Southern blot transferred (see Fig. 3*A*) to Zeta-Probe membranes (Bio-Rad), probed at high stringency as indicated by manufacturer, and subjected to autoradiography. Oligonucleotide probes were labeled by [α-^{32}P]dATP addition with terminal transferase. Quantitation of *dgqA*/*dgqB* ratios (Fig. 3*B*) was done by PCR essentially as above, but using more cDNA and less amplification (32 cycles). Traces of primer 3*F* were ^{32}P-labeled. PCR products were resin-purified (Prep-A-Gene, Bio-Rad), and then digested with *Mbo*II. Products were analyzed on a 5% denaturing polyacrylamide gel, which was dried and subjected to autoradiography. *dgq* oligonucleotides, by exon number (*F*, forward; *R*, reverse): 3*F*, 5′-CGTAGGGAATATCAGCTGAC-3′; 4A*F*, 5′-CTCGAATTGAACAGGCTGAT-3′; 4B*F*, 5′-TCTGAAGGATCTCGATCGTG-3′; 6*R*, 5′-GCTTTAGATTCCTCCATTCG-3′; 7A*F*, 5′-GTCCAAAACAGGATCACGCT-3′; 7B*F*, 5′-GTCCTCAGCGAGATG-

Abbreviations: Rh, rhodopsin; Arr, arrestin; PLC, phospholipase C; RT-PCR, reverse transcription–PCR.

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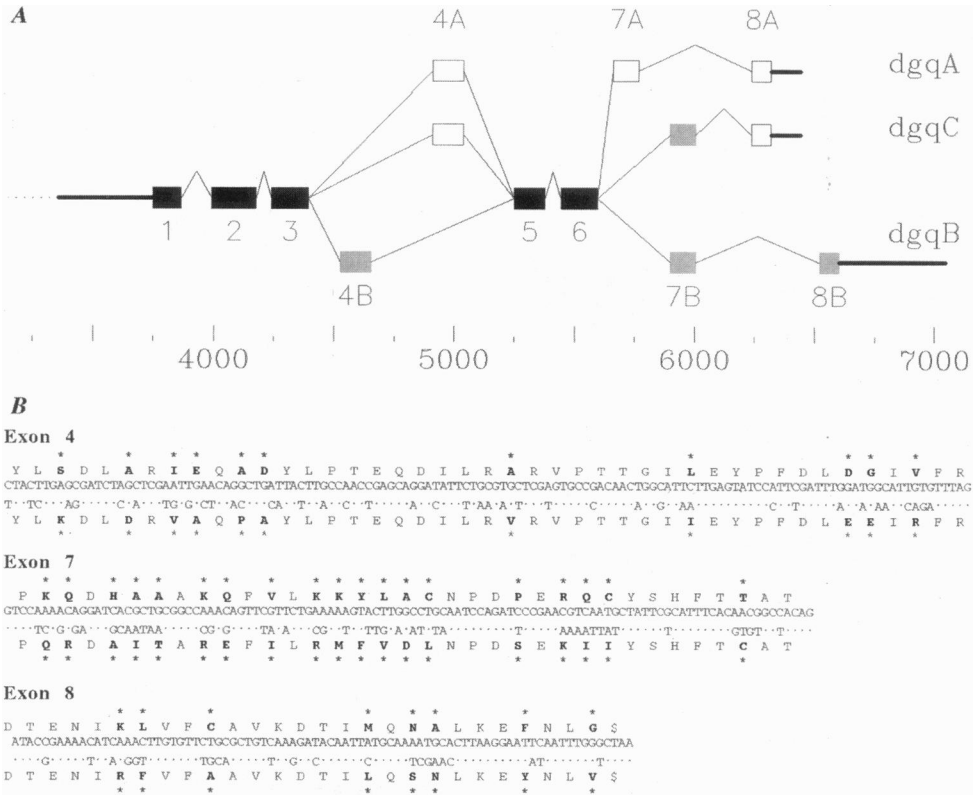

FIG. 1. (*A*) Schematic of *dgq* gene structure and mRNA splicing patterns; exon numbers taken from Lee *et al.* (5). The A exons are those from the original visual isoform (5) and the B exons are the novel exons. The nucleotide numbers begin at the 5′ terminus of an antennal *dgqB* cDNA (6), which has two untranslated exons not shown here. Horizontal shapes represent exons: thin lines are noncoding and boxes are coding. Box shading shows invariant sequence as black, visual-specific as white, and nonvisual as gray. Diagonal lines show splicing patterns. (*B*) Alignment of the *dgq* alternative exons. The nucleotide and amino acid sequence of the original A isoform (5) appears above the novel B sequence. Conserved positions in nucleotide sequence are noted by periods (.). Divergent amino acids are in boldface and marked with asterisks (*). Stop codons are marked with dollar signs ($). Only the coding sequence of the terminal exon 8 is shown.

CAATA-3′; 8A*R* 5′-TATCTTTGACAGCGCAGAAC-3′; and 8B*R*, 5′-TGTCCTTAACAGCTGCAAAC-3′. 5′ sequences of *dgqA* and *B* were analyzed by PCR as above, using whole head cDNA. Primers from the second 5′ untranslated exon (we refer to it as exon 2U) of the antennal *dgqB* cDNA (5′-CAAAGCAAATCGCGTGATT-3′; ref. 6) and two sequences taken from the published 5′ terminus of *dgqA* (5′-TATATCCGACGTCGTCATCG-3′ and 5′-ACAGTTCG-GTTATGCAAGCA-3′; ref. 7) were used in combination with primers 8A*R* or 8B*R*. The sequences of non-*dgq* primers are available upon request.

RESULTS AND DISCUSSION

Discovery of Novel *dgq* Exons. In the introns of the *dgq* gene, we found three previously unrecognized exons of *dgq* (5) by similarity in a computer-based search (13). Fig. 1 shows the gene structure and sequences of the original (visual isoform; ref. 5) exons 4A, 7A, and 8A, and the novel exons, 4B, 7B, and 8B. [Recently, these novel exons were described in an antennal cDNA (6).] The gene structure is consistent with three independent sequence duplication events. The crystal structures of $G\alpha$ (14, 15) and $G\alpha\beta\gamma$ (16) and an abundance of biochemical studies (reviewed in ref. 17) show that the regions encoded by exons 7 and 8 are involved in PLC-binding and receptor-binding, respectively. The role of the exon 4 peptide product is complex: (*i*) it has one third of the $G\alpha$ residues that interact with $G\beta$ and (*ii*) it is a key component of the GTPase, having all of the first switch region as well as residues that bind guanine, phosphate, ribose, and magnesium (Fig. 2). Alternative splicing of the exon 4 domain in a $G\alpha$ has not been observed before, but there is one case of exon 7 and 8 alternative splicing in a mammalian $G_o\alpha$ (17). We completed the *dgq* coding sequence by correcting a frame shift in the published exon 7B and determining the missing sequence of the exon 8B terminus (ref. 5; Fig. 1*B*).

Relationship of the Alternative DGq Sequences to the $G_q\alpha$ Family. We compared the six alternative exons independently to all $G\alpha$s in SwissProt and determined that all are of the "q" class (17). Fig. 2 shows a comparative sequence logo analysis of the variant exons of DGq in the context of the $G_q\alpha$ family. Remarkably, all three nonvisual B exons of DGq are highly similar (82% identity at the amino acid level) to the $G_q\alpha$ family consensus (topmost letter in each column of the logo), from snail to human, but all three visual A exons are divergent (61% identity; Fig. 2). In the exon 4 region, which has roles in GTPase activity and $G\beta$-binding, there are seven positions at which only one DGq isoform matches the consensus; six of these matches are to isoform B. Similarly, the B isoform has the consensus amino acid in 12 of 14 conflicts between A and B within the effector-binding region of exon 7. In the region of exons 7 and 8 implicated in receptor binding, there are 12 positions where the A and B exons differ; 11 of these are matches to the B isoform. These sequence relationships suggest that DGqB has GTPase and/or $G\beta$-binding, effector-binding and receptor-binding properties characteristic of the $G_q\alpha$ family, whereas DGqA is likely to have more idiosyncratic properties. The most parsimonious scenario is that the ancestral DGq was DGqB-like (i.e., $G_q\alpha$-family-like) and that three exon duplications in that gene led to the evolution of DGqA.

***In Vivo* Splicing Patterns of *dgq*.** RT-PCR analysis of adult flies shows that all three novel exons are used. In females, and in all tissues but the gonads of males, only two isoforms are expressed. They differ at the three alternative positions, where

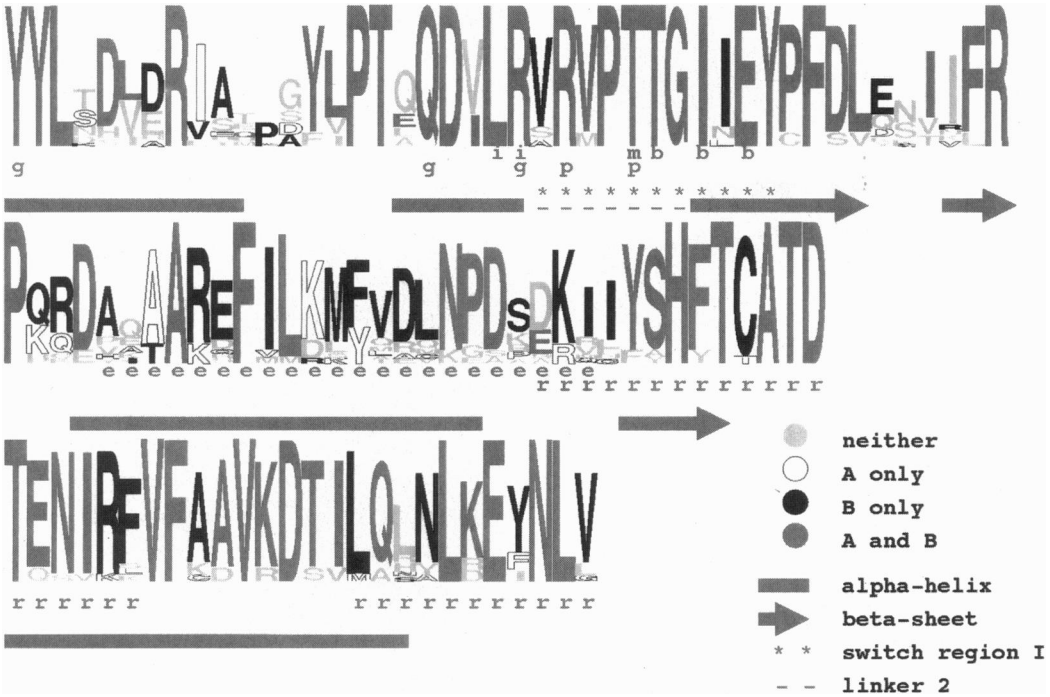

FIG. 2. Comparative sequence logo analysis of DGq isoforms A and B. The regions encoded by exons 4, 7, and 8 of all $G_q\alpha$ family members (including DGqA and DGqB) were used to build a comparative sequence logo, in which the letter height of a residue represents the frequency at which it is represented (see *Materials and Methods*). The legend gives grayscale designations, but most important is the comparison between the variant DGq positions: A in outlined letters, and B in solid black. The logo is underlayed with secondary-structure and binding-region assignments (14, 16). Boxes represent α-helices and arrows represent β-sheets. Linker 2 (dashed line) is one of two polypeptides that connect the highly helical domain to the GTPase domain. Switch region I (asterisks) is one of two regions that show dramatic conformational changes between GDP and GTPγS-bound forms (14, 15). Protein-interacting sequence is marked as "b" for G$\beta\gamma$-contacting residues, "e" for effector-binding region, and "r" for receptor-binding region. GTPase residues are noted for binding the following: g, guanine ring; m, Mg^{2+}; p, phosphate; i, ribose.

one has all A exons and the other has all B exons (Fig. 3*A*). These spliced forms correspond to cDNAs previously isolated from eyes and antennae; we call these isoforms DGqA and DGqB, respectively. [The original *dgq* work described transcripts lacking exon 7 (5). We tested for possible rare transcripts lacking exons 7A or B by probing RT-PCR Southern blots with an oligo probe spanning the putative exon 6–8A/B boundary (data not shown), but obtained no evidence for the presence of such transcripts. Scott *et al.* (2), using antipeptide antibodies specific for this putative isoform, also failed to detect such a protein.]

The antennal *dgqB* cDNA (6) showed a different 5′ untranslated region (composed of two novel exons we refer to as 1U and 2U) from that given for the the visual-specific *dgqA* transcript (deduced by primer extension, ref. 7). We made primers to exon 2U and to the published 5′ terminus of *dgqA*. PCR showed that the second novel untranslated exon is contained in both *dgqA* and *dgqB* (data not shown) and that the

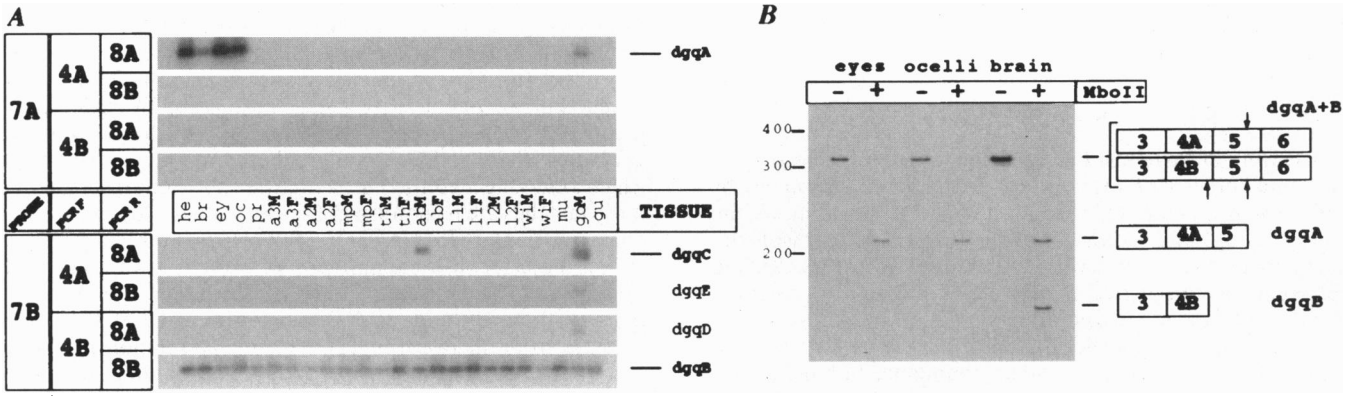

FIG. 3. (*A*) Alternative splicing of *dgq* characterized by Southern blot analysis of RT-PCR products. Tissue-specific cDNAs were tested by PCR using all combinations of alternative exons 4A/B and 8A/B primers, followed by exon 7A and 7B probing. Three main species are observed: *dgqA* in photosensitive and brain tissue (and little in male gonad), *dgqB* in all tissues, and dgqC in the male gonad/abdomen. Two minor species, *dgqD* and *dgqE*, are male gonad-specific. Tissues marked by M or F are male or female. Abbreviations follow: he, head; br, brain; ey, eye; oc, ocellus; pr, proboscis; a3, antenna segment III; a2, antenna segment II; mp, maxillary palp; th, thorax; ab, abdomen; l1, first leg; l2, second and third legs; wi, wing; mu, flight muscle; go, gonad; gu, gut. (*B*) Ratio of dgqA:dgqB in tissues that express both. PCR was primed at constant exons 3 (^{32}P-labeled oligo) and 6, cut at constant exon 5 and isoform-specific exon 4B with *Mbo*II, then separated on a denaturing polyacrylamide gel. The uncut bands in the *Mbo*II lanes are due to incomplete digestion. Eyes and ocelli express *dgqA* almost exclusively and brain expresses approximately equal amounts of both. DNA marker sizes are in base pairs.

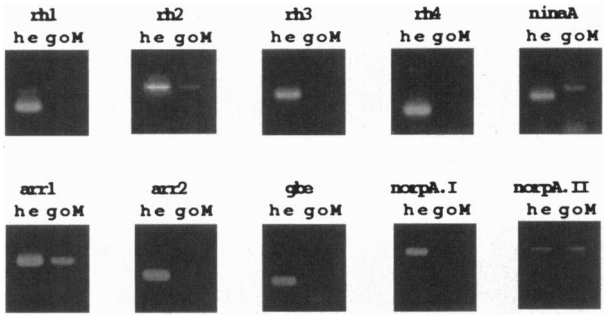

FIG. 4. Search for phototransduction machinery in male gonad. Expression of Gα-proximal photoreceptor cell-specific genes was assayed by PCR in male gonad (goM) and, as a positive control, head (he). The genes tested were Rh1, -2, -3, and -4; *ninaA*; Arr1 and -2; *gbe*; *norpA.I* (visual isoform); and *norpA.II* (widely expressed isoform). Of these, the photoreceptor-specific *rh2* and *arr1*, and the nonvisual *norpA.II* transcripts are expressed in male gonads. The larger product seen in the *ninaA*/male-gonad lane corresponds to the size of genomic PCR product.

published 5′ terminus of *dgqA* is intronic sequence. The full 5′ region and promoter structures remain to be determined.

To study the tissue distribution of the *dgq* isoforms, we microdissected adult flies for the following parts: eyes and ocelli (photosensory system), antennae and maxillary palps (olfactory system), probosci, legs, and wings (gustatory system), whole head, brain (with optic lobes), thorax, abdomen, flight muscle, male gonad, and gut. We focused on the chemosenses because there is an overlap in signal transduction machinery between fly vision and olfaction (18), including the sharing of a PLC gene, *norpA* (19). As both olfaction and taste are sexually dimorphic in flies (20), we examined those tissues (and the gonad-containing abdomens) individually in males and females. RT-PCR analysis shows that eyes, ocelli, and brain express *dgqA* and *B* exclusively, but that the other parts (except male gonads; see below) express only *dgqB* (Fig. 3*A*).

Could the chemosensory-tissue expression of *dgqB* be linked with primary sensory pathways? Since olfactory neurons are located in segment III, but not segment II, of the antennae (20), we compared these two tissues (Fig. 3*A*). No gross enrichment is seen in the third segment, suggesting that *dgqB* is not the primary olfactory Gα. Instead, its ubiquitous expression is consistent with either a general cellular function or multiple roles. As eyes, ocelli, and brain contained both isoforms, we determined their relative quantities in those tissues. Fig. 3*B* shows that eyes and ocelli only have trace amounts of *dgqB* (0.3% and 3%, respectively), while brain has ≈50% of each isoform. A study (21) of the immunohistochemistry of fly heads with pan-DGq antiserum shows that eyes and ocelli stain very intensely and that the only other area stained, weakly, is the lamina ganglionaris of the optic lobe, while DGqB-specific antiserum shows predominant head expression in the lamina (6). We detect high levels of *dgqA* message in eyes and ocelli, but there is also some brain expression. If DGqA interacts exclusively with Rh, one possible expression site in the brain is in putative photoreceptors known as Hofbauer–Buchner cells (22).

Testes-Specific *dgq* Isoforms. In the male gonads, three novel isoforms appear along with *dgqA* and *dgqB*: *dgqC* (4A, 7B, and 8A), *dgqD* (4A, 7B, and 8B), and *dgqE* (4B, 7B, and 8A). *dgqC* is highly expressed, suggesting that at least this form is functional. Further analysis is needed to determine whether *dgqA*, *dgqB*, *dgqD*, and *dgqE* are functional in the testes or products of loose splicing regulation. Notably, only *dgqA* has the visual exon 7A.

Since high levels of *dgqA* and *dgqB* in the head might prohibit detection of rarer isoforms, we performed PCR (under saturating conditions for *dgqA* and *dgqB*) on head cDNA with the following exon primer combinations: 3–8A/B, 4A/B–8A/B, and 7A/B–8A/B (data not shown). Southern transfers of these products were probed with internal alternative exon sequences. Only *dgqA* and *dgqB* were detected, showing that the mutually exclusive splicing of these two forms is exquisitely stringent. We also tested specifically for the presence of *dgqC* in head, brain, eyes, and ocelli by RT-PCR using 4A and *dgqC*-specific 7B/8A-junction primers. *dgqC* is expressed exclusively in male gonads.

Evidence for Rh-Mediated Phototransduction in Testes. The finding of a testes-specific *dgq* form with a highly divergent Rh-binding exon lead us to suspect a phototransduction function for this tissue. We searched for some testes expression of genes known to interact with DGq in the eye. To search upstream for a Rh-mediated signaling pathway, we examined photoreceptor-specific genes (reviewed in ref. 1): for rhodopsins, Rh1, -2 (ref. 7), -3, and -4; for *ninaA* (cyclophilin required for *in vivo Rh1* function); and for arrestins (Arrs), Arr1 (23, 24) and Arr2 (Rh inactivators). Fig. 4 shows two of these genes, *Rh2* and *Arr1*, express in the testes. [These PCR products were confirmed as follows: (*i*) primer sites were selected to span introns, and both genomic and cDNA PCRs result in single bands of the expected sizes; and (*ii*) the products were identified by Southern blot analysis with internal probes (data not shown).] As Arr1 is only expressed in photoreceptor cells and has been demonstrated to interact with Rh, its presence in another organ is significant. The testes are the first example of a tissue that expresses *Arr1* but not *Arr2* (25). Both Arr1 and Arr2 induce Rh innactivation, but Arr2 is functionally dominant (25). Arr1 may have a nonvisual phototransduction function, while being redundantly expressed in eyes. Rh2 is the ocellar opsin (26, 27), so it is notable that it is expressed in a second nonvisual photosensory organ. These findings suggest that a Rh-mediated phototransduction pathway exists in the male gonads of *Drosophila*.

Downstream, we examined *norpA* and *gbe* (Rh-activated Gβ; ref. 3). NorpA has two cloned isoforms that vary at one alternatively spliced exon: one (I) is expressed at high levels and appears to be eye-specific, and the other (II) is expressed at low levels throughout development and in various tissues (head, thorax, abdomen, and legs; ref. 28). Fig. 4 shows that the visual *norpA.I* is not expressed in testes, but that *norpA.II* is. (*norpA.II* is expressed in male and female abdomens, but female gonads were not tested; data not shown.) We detected no *gbe* expression outside of the visual system. Possibly the *dgq* exon 4A is not used in the testes for its Gβe-interacting specificity, but for its GTPase properties; however, there may be alternative forms of Gβe.

Putative Role of DGqC in the Testes. DGqC is an ideal candidate signal-transducer for a function that has been characterized in several moth species (see ref. 29). In the gypsy moth, a circadian rhythm of sperm release and transfer has been shown to operate via an autonomous photoreceptive pacemaker located in the testes-vas deferens complex (30). There are indications that fly testes may have such a clock function. Per, a key pacemaker component (reviewed in ref. 31), has been shown to be expressed in both male and female gonads; however, male abdomens show a circadian oscillation of transcript levels that is absent in female abdomens (32). DGqC is an ideal transducer in this putative clock-input pathway, as it has the divergent Rh-binding exon and the nonvisual PLC-binding exon.

Conclusions and a Model of *dgq* Natural History. Our analysis suggests that the visual DGqA was generated by the duplication of three DGqB exons. This allowed the photoreceptor-specific DGqA to be optimized for phototransduction at Gβ-interacting/GTPase, NorpA-binding, and Rh-binding sites. This model is supported by the existence of eye-specific forms of Gβ, NorpA, and Rh. [Vertebrate sensory systems also show this type of adaptation, having specialized Gαs (of non-q

class) for rod-vision ($G_{t1}\alpha$), cone-vision ($G_{t2}\alpha$), olfaction ($G_{olf}\alpha$), and taste ($G_{gust}\alpha$) (17).] However, squid, the only other invertebrate with a known Rh-linked $G_q\alpha$, uses one more similar to DGqB than to the the visual DGqA (33). This suggests that before the divergence of squid and fly, there was a single DGqB-like $G_q\alpha$ that mediated vision and at least one other, more ancient, signaling pathway.

One clue about current DGqB function(s) is its tissue expression: seemingly ubiquitous, but most prominently in the lamina of the optic lobe (6). The known function of the lamina ganglionaris is that it converges the axons of optically aligned photoreceptors into cartridges that sum and transmit light-activated signals (34). Is there any known $G_q\alpha$-coupled pathway in the lamina? Hardie (35) has shown evidence that a lamina-generated component (the "off" transient) of the visual response of flies is mediated by muscarinic acetylcholine receptors that are characteristically $G_q\alpha$-linked (17). Acetylcholine is a key neurotransmitter in the modulation of behavioral rhythms in both inverterbrates (37) and vertebrates (38). One possible signaling role for DGqB is in retinal photoentrainment of a lamina ganglionaris pacemaker.

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1. Ranganathan, R., Malicki, D. M. & Zuker, C. S. (1995) *Annu. Rev. Neurosci.* **18,** 283–317.
2. Scott, K., Becker, A., Sun, Y., Hardy, R. & Zuker, C. (1995) *Neuron* **15,** 919–927.
3. Dolph, P. J., Man-Son-Hing, H., Yarfitz, S., Colley, N. J., Running Deer, J., Spencer, M., Hurley, J. B. & Zuker, C. S. (1994) *Nature (London)* **370,** 59–61.
4. Bloomquist, B. T., Shortridge, R. D., Schneuwly, M., Perdew, C., Montell, C., Steller, H., Rubin, G. & Pak, W. L. (1988) *Cell* **54,** 723–733.
5. Lee, Y.-J., Dobbs, M. B., Verardi, M. L. & Hyde, D. R. (1990) *Neuron* **5,** 889–898.
6. Talluri, S., Bhatt, A. & Smith, D. P. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 11475–11479.
7. Pollock, J. A. & Benzer, S. (1988) *Nature (London)* **333,** 779–782.
8. Altschul, S. F., Gish, W., Miller, W., Meyers, E. W. & Lipman, D. J. (1990) *J. Mol. Biol.* **215,** 403–410.
9. Higgins, D. G., Bleasby, A. J. & Fuchs, R. (1992) *Comput. Appl. Biol. Sci.* **8,** 181–191.
10. Schneider, T. D. & Stephens, R. M. (1990) *Nucleic Acids Res.* **18,** 6097–6100.
11. Fujita, S. C., Inoue, H., Yoshioka, T. & Hotta, Y. (1987) *Biochem. J.* **243,** 97–104.
12. Tso, J. Y., Sun, X.-H. & Wu, R. (1985) *J. Biol. Chem.* **260,** 8220–8228.
13. Robison, K., Gilbert, W. & Church, G. M. (1994) *Nat. Genet.* **7,** 205–214.
14. Noel, J. P., Hamm, H. E. & Sigler, P. B. (1993) *Nature (London)* **366,** 654–663.
15. Lambright, D. G., Noel, J. P., Hamm, H. E. & Sigler, P. B. (1994) *Nature (London)* **369,** 621–628.
16. Lambright, D. G., Sondek, J., Bohm, A., Skiba, N. P., Hamm, H. E. & Sigler, P. B. (1996) *Nature (London)* **379,** 311–319.
17. Pennington, S. R. (1995) *Protein Profile* **2,** 167–315.
18. Carlson, J. R. (1996) *Trends Genet.* **12,** 175–180.
19. Riesgo-Escovar, J., Raha, D. & Carlson, J. R. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 2864–2868.
20. Stocker, R. F. (1994) *Cell Tissue Res.* **275,** 3–26.
21. Lee, Y.-J., Shah, S., Suzuki, E., Zars, T., O'Day, P. M. & Hyde, D. R. (1994) *Neuron* **13,** 1143–1157.
22. Hofbauer, A. & Buchner, E. (1989) *Naturwissenschaften* **76,** 335–336.
23. Smith, D. P., Shieh, D.-H. & Zuker, C. S. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 1003–1007.
24. Hyde, D. R., Mecklenburg, K. L., Pollock, J. A., Vihtelic, T. S. & Benzer, S. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 1008–1012.
25. Dolph, P. J., Ranganathan, R., Colley, N. J., Hardy, R. W., Socolich, M. & Zuker, C. S. (1993) *Science* **260,** 1910–1916.
26. Pollock, J. A. & Benzer, S. (1988) *Nature (London)* **333,** 779–782.
27. Mismer, D., Michael, W. M., Laverty, T. R. & Rubin, G. M. (1988) *Genetics* **120,** 173–180.
28. Kim, S., McKay, R. R., Miller, K. & Shortridge, R. D. (1995) *J. Biol. Chem.* **270,** 14376–14382.
29. Giebultowicz, J. M. & Zdarek, J. (1996) *J. Insect Physiol.* **42,** 167–170.
30. Giebultowicz, J. M., Riemann, J. G., Raina, A. K. & Ridgway, R. L. (1989) *Science* **245,** 1098–1100.
31. Hall, J. C. (1995) *Trends Neurosci.* **18,** 230–240.
32. Hardin, P. E. (1994) *Mol. Cell. Biol.* **14,** 7211–7218.
33. Ryba, N. J. P., Findlay, J. B. C. & Reid, J. D. (1993) *Biochem. J.* **292,** 333–341.
34. Hardie, R. C. (1986) *Trends Neurosci.* **9,** 419–423.
35. Hardie, R. C. (1989) *Nature (London)* **339,** 704–706.
36. Marder, E. & Meyrand, P. (1989) in *Neuronal and Cellular Oscillators*, ed. Jacklet, J. W. (Dekker, New York), pp. 317–337.
37. Liu, C. & Gillette, M. U. (1996) *J. Neurosci.* **16,** 744–751.