The G-Protein β-Subunit GPB-2 in *Caenorhabditis elegans* Regulates the $G_o\alpha$–$G_q\alpha$ Signaling Network Through Interactions With the Regulator of G-Protein Signaling Proteins EGL-10 and EAT-16

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ABSTRACT

The genome of *Caenorhabditis elegans* harbors two genes for G-protein β-subunits. Here, we describe the characterization of the second G-protein β-subunit gene *gpb-2*. In contrast to *gpb-1*, *gpb-2* is not an essential gene even though, like *gpb-1*, *gpb-2* is expressed during development, in the nervous system, and in muscle cells. A loss-of-function mutation in *gpb-2* produces a variety of behavioral defects, including delayed egg laying and reduced pharyngeal pumping. Genetic analysis shows that GPB-2 interacts with the GOA-1 (homologue of mammalian $G_o\alpha$) and EGL-30 (homologue of mammalian $G_q\alpha$) signaling pathways. GPB-2 is most similar to the divergent mammalian Gβ5 subunit, which has been shown to mediate a specific interaction with a Gγ-subunit-like (GGL) domain of RGS proteins. We show here that GPB-2 physically and genetically interacts with the GGL-containing RGS proteins EGL-10 and EAT-16. Taken together, our results suggest that GPB-2 works in concert with the RGS proteins EGL-10 and EAT-16 to regulate GOA-1 ($G_o\alpha$) and EGL-30 ($G_q\alpha$) signaling.

HETEROTRIMERIC G proteins, consisting of a guanine nucleotide-binding α-subunit and a βγ dimer, act as signal-transducing molecules that couple serpentine transmembrane receptors to a wide variety of intracellular effectors (Kaziro *et al.* 1991; Simon *et al.* 1991). The Gα-subunit and the Gβγ dimer can independently interact with and regulate downstream effector molecules (Clapham and Neer 1993). Despite the extensive data on the biochemical properties of these components, there are still major questions regarding the mechanisms through which Gβγ functions *in vivo*, and the relationship between Gβγ dimers and their potential Gα partners. For example, it is unclear how a given Gβγ dimer interacts with distinct Gα signaling cascades in a given cell or tissue *in vivo*. Genetic analysis in the model organism *Caenorhabditis elegans* may provide insight into the *in vivo* Gβγ function and its interaction with previously studied Gα signaling pathways.

Recent genetic experiments in *C. elegans* demonstrate that GOA-1 ($G_o\alpha$) antagonizes EGL-30 ($G_q\alpha$) signaling, with the $G_o\alpha$ pathway acting upstream of, or parallel to, the $G_q\alpha$ pathway (Hajdu-Cronin *et al.* 1999; Miller *et al.* 1999). Both the $G_o\alpha$ and $G_q\alpha$ signaling pathway regulate neurotransmitter secretion in *C. elegans* (Rand and Nonet 1997; Lackner *et al.* 1999; Nurrish *et al.* 1999). This antagonism between $G_o\alpha$ and $G_q\alpha$ involves EAT-16, a regulator of G-protein signaling proteins (RGS) and/or DGK-1 (DAG-kinase; Hajdu-Cronin *et al.* 1999; Miller *et al.* 1999), both identified as suppressors of activated $G_o\alpha$ (Hajdu-Cronin *et al.* 1999; Miller *et al.* 1999; Nurrish *et al.* 1999). RGS proteins function as GTPase-activating proteins (GAPs) that accelerate the intrinsic GTPase activity of the Gα-subunit (N. Watson *et al.* 1996; Koelle 1997; Neer 1997). Genetic analysis shows that EAT-16 (RGS) functions downstream of, or parallel to, GOA-1 ($G_o\alpha$). However, rather than acting as a GAP for GOA-1 ($G_o\alpha$), EAT-16 acts as a GAP for EGL-30 ($G_q\alpha$; Hajdu-Cronin *et al.* 1999). Thus, $G_o\alpha$ could negatively regulate $G_q\alpha$ signaling through EAT-16 by functioning as a direct effector of GOA-1 or indirectly via the Gγ-subunit-like (GGL) domain of EAT-16 that may functionally mimic a Gγ-subunit in the heterotrimeric G-protein complex (Guan and Han 1999). Another GGL-containing RGS protein in *C. elegans*, EGL-10, was previously identified as an upstream negative regulator of GOA-1 ($G_o\alpha$) signaling (Koelle and Horvitz 1996). Together, these studies suggest that the RGS proteins EGL-10 and EAT-16 inhibit $G_o\alpha$ and $G_q\alpha$ signaling in *C. elegans*, respectively, and that especially EAT-16 has an important role in mediating cross-talk between $G_o\alpha$ and $G_q\alpha$. At this point, however, it is not known where and how the Gβ-subunits function in the $G_o\alpha$–$G_q\alpha$ signaling network.

Two genes encoding G-protein β-subunits have been identified in the complete *C. elegans* genome: *gpb-1* (van der Voorn *et al.* 1990) and *gpb-2*. The first G-protein β-subunit gene, *gpb-1*, is expressed throughout development in most tissues and in the germline, and loss of

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gpb-1 results in embryonic lethality (Zwaal *et al.* 1996). Here, we show that the second G-protein β-subunit gene, *gpb-2*, is expressed in most neurons and in muscle cells and that loss of *gpb-2* is not lethal, but affects behaviors such as egg laying and pharyngeal pumping. Animals lacking both *gpb-2* and *goa-1* function have a synthetic larval lethal phenotype, which is rescued when EGL-30 activity is reduced, suggesting that $G_q\alpha$ activity causes the *gpb-2*; *goa-1* double mutant synthetic lethality. GPB-2 is most homologous to the divergent mammalian Gβ5 subunit that, unlike the other mammalian Gβ-subunits (Gβ1-4), has the ability to interact with the GGL domain of a subset of RGS proteins *in vitro* (Snow *et al.* 1998, 1999; Levay *et al.* 1999). Our results show that GPB-2 interacts with the two GGL-containing RGS proteins in *C. elegans*, EGL-10 and EAT-16, and that GPB-2 regulates the functions of these proteins in the $G_o\alpha$–$G_q\alpha$ signaling network.

MATERIALS AND METHODS

Nematode strains, culturing, and manipulation: General methods used for culturing, manipulation, and genetics of *C. elegans* were as described by Lewis and Fleming (1995). DNA transformation assays in *C. elegans* by microinjection were as described by Mello and Fire (1995). Unless indicated, all experiments were performed at 20°. The following strains were used in this study: Bristol N2, PS4034 [*eat-16(sy438)*I; Hajdu-Cronin *et al.* 1999], DA0823 [*egl-30(ad805)*I], MT1434 [*egl-30(n686)*I; Brundage *et al.* 1996], NL561 [*goa-1(pk62)*I; Mendel *et al.* 1995], MT363 [*goa-1(n363)*I; Ségalat *et al.* 1995], DR102 [*dpy-5(e61)unc-29(e403)*I], CB73 [*unc-15(e73)*I], CB1282 [*dpy-20(e1282)*IV], MT8504 [*egl-10(md176)*V; Koelle and Horvitz 1996], MT1083 [*egl-8(n488)*V; Trent *et al.* 1983; Miller *et al.* 1999], and KP1097 [*dgk-1(nu62)*X; Nurrish *et al.* 1999].

Sequence analysis of GPB-2: The GENEFINDER prediction of the *gpb-2* open reading frame, as annotated in the *C. elegans* database ACeDB (Eeckman and Durbin 1995), was confirmed by cloning and sequencing of the cDNA (see *Yeast two-hybrid analysis*). The coding sequence of GPB-2 has been submitted to the DDBJ/EMBL/GenBank databases under accession no. AF291847. The percentages of identity and similarity given above were derived using the Genetics Computer Group (GCG, version 9.1) GAP program using default parameters. Multiple alignments of GPB-2, mammalian, Dictyostelium, and Drosophila G-protein β-subunit sequences (Swiss-Prot accession nos. P04901, P54311, P54312, P11016, P29387, AF291846, AF291847, P16520, P36408, O14775, and P29829) were made using the GCG PILEUP program using default parameters.

GFP reporter constructs: A translational fusion of *gpb-2* to *gfp* (pRP2016) was constructed by inserting a ±3-kb fragment amplified by PCR on wild-type *gpb-2* genomic sequence using the primers green fluorescent protein (GFP)-BTWO1 (5′-ATA**GCATGC**TTCCTGGTGATCAGGTCATGT) and GFP-BWTO2 (5′-TA**GGATCC**AATAGCACATGTTGAATCTCC), containing a unique *Sph*I and *Bam*HI site (shown in bold), respectively, into the *Sph*I and *Bam*HI sites of pPD95.77 (A. Fire, personal communication). The construct pRP2016 was injected at 50 μg/ml with 100 μg/ml pMH86 (Han and Sternberg 1991) into CB1282 [*dpy-20(e1282)*IV] to produce NL1562 (*dpy-20(e1362)*IV; *pkEx563*[*gpb-2::gfp,dpy-20(+)*]). A second translational fusion of *gpb-2* to *gfp* (pRP2017) was constructed by inserting a ±3-kb fragment amplified by PCR on wild-type *gpb-2* genomic sequence using the primers GFP-BTWO3 (5′-ATA**GCATGC**GGCTCTAGATAGGTATGTAGA) and GFP-BWTO4 (5′-TA**GGATCC**CATGCATAATACTTTTCCCAC), containing a unique *Sph*I and *Bam*HI site (shown in bold), respectively, into the *Sph*I and *Bam*HI sites of pPD95.77 (A. Fire, personal communication). The construct pRP2017 was injected at 50 μg/ml with 150 μg/ml pMH86 (Han and Sternberg 1991) into CB1282 [*dpy-20(e1282)*IV]. This transgenic array was integrated by irradiating animals with 40 Gy of gamma radiation from a ^{137}Cs source (Way *et al.* 1991) to produce NL2784 (*dpy-20(e1282)*IV; *pkIs1303*[*gpb-2::gfp,dpy-20(+)*]). NL2784 was backcrossed once to a N2 wild-type background to produce NL2785. Cells were identified in reference to Sulston and Horvitz (1977) and White *et al.* (1986).

Generation and transgenic rescue of a *gpb-2* loss-of-function mutation: A deletion mutant of *gpb-2*, *pk751*, was isolated from a chemical deletion library using primers BTWO5 (5′-ACAAT TGGCAAATGAAGCCG) and BTWO6 (5′-TCAACGGAAATTG AGAGATG) and nested primers BTWO7 (5′-CACAAGCT TAATGACATTCC) and BTWO8 (5′-AGAAGCCGTGACG GATGACC) as described by Jansen *et al.* (1997). The PCR product detecting the deletion in *pk751* was sequenced and confirmed by Southern analysis (data not shown). The following sequence in capitals is deleted in *pk751*: (5′—tagtcttc AAAAATTGG—TGTAAAAAaagagtg—). The *pk751* allele was backcrossed three times to an N2 wild-type background, resulting in the strain NL2001 [*gpb-2(pk751)*I].

The behavioral defects of *pk751* were complemented with two wild-type *gpb-2* genomic constructs, pRP2015 (see below) and pRP2051. The rescuing construct pRP2051, containing a 4.7-kb *Spe*I-*Xba*I fragment, including the *gpb-2* genomic sequence and ~1.3 kb of upstream sequence, was injected at 20 μg/ml with 150 μg/ml pMH86 (Han and Sternberg 1991) into NL2002 [*dpy-20(e1282)*IV; *gpb-2(pk751)*I], resulting in strains NL2771, NL2772, and NL2773 [*dpy-20(e1282)*IV; *pkEx1321*, *pkEx1322*, and *pkEx1323*, respectively]. Homozygosity for the *pk751* allele was confirmed by single worm PCR.

Overexpression of GPB-2: We generated multiple transgenic lines that overexpress GPB-2 from a transgene carrying a multicopy array, either extrachromosomal or integrated, of the *gpb-2* gene under control of its endogenous promoter. The rescuing construct pRP2051 was injected at different concentrations of plasmid DNA with 150 μg/ml pMH86 (Han and Sternberg 1991) into CB1282 [*dpy-20(e1282)*IV] at 20 μg/ml to produce NL2757 (*dpy-20(e1282)*IV; *pkEx1318* [*gpb-2(+),dpy-20(+)*]), at 30 μg/ml to produce NL2758 [*dpy-20(e1282)*IV; *pkEx1319*], and at 50 μg/ml to produce NL2759 [*dpy-20(e1282)*IV; *pkEx1320*]. All strains obtained showed no defects in egg laying, locomotion, and pharyngeal pumping compared to wild type (data not shown). Another rescuing construct pRP2015, containing a 6.9-kb *Spe*I fragment including the *gpb-2* genomic sequence and ~3.5 kb of upstream sequence, was injected at 50 μg/ml with 150 μg/ml pMH86 (Han and Sternberg 1991) into CB1282 [*dpy-20(e1282)*IV] to produce NL1557 (*dpy-20(e1282)*IV; *pEx557*[*gpb-2(+),dpy-20(+)*]). This transgenic array was integrated by irradiating animals with 40 Gy of gamma radiation from a ^{137}Cs source (Way *et al.* 1991) to produce NL1559 (*dpy-20(e1282)*IV; *pkIs559*[*gpb-2(+),dpy-20(+)*]). NL1559 was backcrossed two times to an N2 wild-type background to produce NL2004. The strain NL2004 was used in behavioral assays as described below. To confirm that the integrated transgene *pkIs559*[*gpb-2(+)*] is functional, we crossed *pkIs559*[*gpb-2(+)*] into NL2002 [*dpy-20(e1282)*IV; *gpb-2(pk751)*I], resulting in strain NL2006 (*dpy-20(e1282)*IV; *gpb-2(pk751)*I; *pkIs559*[*gpb-2(+),dpy-20(+)*]).

A construct with *gpb-2* expression under the control of a heat-shock promoter (*hsp*) was generated by cloning a ±800-

bp *Spe*I-*Avr*II *hsp16.2-gpb-2* containing PCR fragment in front of an *Avr*II-*Spe*I fragment of *gpb-2* from pRP2051. In a first PCR, two fragments were amplified. The first 518-bp fragment was amplified on the *hsp16.2* promoter from pPD49.78 (Stringham *et al.* 1992) using the primers HSP1 (5′-GGTCGA C**ACTAGT**GGATCAAGAGC) and HSP3 (5′-GAGTTTTCTG GCATGGTACCGTAGACGC). In this way, the PCR fragment becomes flanked on one side by a unique *Spe*I site (shown in boldface type) and on the other side by the first 14 bp of *gpb-2*. The second 296-bp fragment was amplified on *gpb-2* using the primers HSP2 (5′-CGACGGTACCATGCCAGAA AACTCTCAG) and HSP4 (5′-CTAA**CCATGG**AATTGTAAAG). This PCR fragment is flanked on one side by the last 10 bp of *hsp16.2* and on the other side by a unique *Avr*II site (shown in bold). In a second PCR, both fragments were allowed to anneal the overlapping flanks (underlined) and used as a template to amplify a ±800-bp fragment using the outer primers (HSP1 and HSP4). The *Spe*I-*Avr*II PCR fragment was cut and cloned into the *Xba*I-*Avr*II sites of the construct pRP2051, resulting in pRP2201. The region of the PCR fragment used in cloning was sequenced completely and found to be free of amplification errors. Injection of pRP2201 was done with varying concentrations of plasmid DNA (10–50 μg/ml) with 150 μg/ml pMH86 (Han and Sternberg 1991) into CB1282 [*dpy-20(e1282)*IV] to produce NL2775, NL2776, and NL2780 [*dpy-20(e1282)*IV; *pkEx1324*, *pkEx1325*, and *pkEx1326*, respectively]. Transgenic animals of different larval stages (L1 to adult) were placed on *Escherichia coli* OP50-seeded NGM agar plates and heat shocked for 2 hr at 33° to induce promoter activity.

Construction of double mutant strains: Double mutants used in this study were generated by standard genetic methods. Homozygosity of the alleles was confirmed by either sequencing amplified genomic DNA of strains containing *eat-16(sy438)*, *egl-30(ad805)*, *egl-30(n686)*, and *dgk-1(nu62)* or by single worm PCR for strains containing *gpb-2(pk751)*, *egl-8(n488)*, and *goa-1 (pk62)*. To detect *egl-10(md176)*, we used single worm PCR using primers that fail to amplify on the rearranged *egl-10* region (Koelle and Horvitz 1996). In addition, all double mutant strains were confirmed by crossing with N2 wild-type males to re-isolate both mutations.

Phenotypic analyses of the *gpb-2* mutant, double mutant, and transgenic lines: The rate of egg laying was assayed by two methods as described (Brundage *et al.* 1996; Koelle and Horvitz 1996). In short, in the first egg-laying assay the number of unlaid eggs in the uterus was determined. L4 staged hermaphrodites were placed on *E. coli* OP50-seeded NGM agar plates and were allowed to develop for 36 hr at 20°. Adults were treated with sodium hypochlorite and eggs were counted using a dissection microscope (Leica MZ75). In the second egg-laying assay, the developmental stage of newly laid eggs was determined by placing L4 staged hermaphrodites on OP50-seeded NGM agar plates. After 29 or 53 hr at 20°, the developmental stage of newly laid eggs was determined in a 2.5-hr interval by counting the cells with a high power dissection microscope (Wild M3C). Newly laid eggs were divided into three categories: one to eight-cell, nine-cell to comma stage, and the post-comma stage. The rate of locomotion was assayed by measuring the frequency of body bends of a hermaphrodite. L4 staged hermaphrodites were placed on OP50-seeded NGM agar plates and were allowed to develop for 24 hr at 20°. At this stage, the locomotion of an adult hermaphrodite is less affected by the presence of excessive unlaid eggs. Each animal was transferred to a thin lawn of a freshly OP50-seeded NGM agar plate 15 min before measuring locomotion by counting body bends in 3-min intervals. A body bend was defined as movement of a quarter of a body length in a forward or backward direction (Brundage *et al.* 1996; Koelle and Horvitz 1996). Pharyngeal pumping on food was assayed by measuring the number of pumps per minute. L4 staged hermaphrodites were placed on separate NGM agar plates seeded with 10 μl OP50 and were allowed to develop for 29 hr at 20°. Pharyngeal pumps were counted in 2-min intervals in which one count represents three pumps. The number of the counts was multiplied by 1.5 to yield pumps per minute (Hajdu-Cronin *et al.* 1999). Assays for egg laying in 5 mg/ml serotonin (5-HT) and 0.75 mg/ml imipramine were performed and interpreted as described by Trent *et al.* (1983), with the exception that newly laid eggs were counted after 90 min. Imipramine and 5-HT were dissolved in M9 buffer. Aldicarb sensitivity was measured either by assaying the time course of the onset of paralysis following acute exposure of a population of animals to aldicarb or by quantifying the population growth rates to various concentrations of aldicarb as described by Lackner *et al.* (1999) and Miller *et al.* (1999). For statistical analysis among multiple groups, an ANOVA followed by directed Student's *t*-test was used. All results are given as mean ± standard errors.

Synthetic lethality: We constructed a *gpb-2(pk751)goa-1(pk62)/ unc-15(e73)* mutant in which all mutations are very closely linked on the right arm of chromosome I. The percentage of survival of the *gpb-2 goa-1* double mutant was scored by placing five *gpb-2 goa-1 / unc-15* L4 heterozygotes on separate OP50-seeded NGM agar plates. The heterozygous animals were given the opportunity to lay eggs for 1–2 days. All progeny were transferred to separate plates and followed for one generation to determine their genotype. This yielded 131 Unc animals, 635 heterozygotes, and 195 *gpb-2 goa-1* double mutant animals of which 95% were arrested at different larval stages. Similarly, we picked all progeny of two *gpb-2(pk751)/+*; *dgk-1(nu62)* heterozygotes. This yielded 69 hyperactive *dgk-1* animals, 121 heterozygotes that segregated arrested larvae, and 53 *gpb-2*; *dgk-1* double mutant animals.

We constructed transgenic *gpb-2 goa-1* double mutant animals containing the *gpb-2* wild-type sequence, which rescues the synthetic lethal phenotype. The rescuing construct pRP2051 was injected at 50 μg/ml with 100 μg/ml pRF4 and 50 μg/ml pRP2017 as markers into *gpb-2(pk751)goa-1(pk62)/ unc-15(e73)* heterozygous animals to produce NL2728 *gpb-2 (pk751)goa-1(pk62);pk1344Ex*[*gpb-2(+),gpb-2::gfp, rol-6 (su1006)*]. Animals that lost this transgenic array in the germline resulted in larval arrest (>96%).

To generate an *egl-30(n686)gpb-2(pk751)goa-1(pk62)* triple mutant, *egl-30 /+* males were mated with *gpb-2 goa-1/++* hermaphrodites, and F_1 cross-progeny were transferred to separate plates. From plates that segregated Egl and arrested larvae, 52 Egl F_2 progeny were transferred to separate plates. Two of the 52 Egls were *egl-30 gpb-2 goa-1 / egl-30 ++* and *egl-30 gpb-2 goa-1* triple mutants were isolated. As a control, we crossed *egl-30 gpb-2 goa-1* hermaphrodites with *dpy-5 unc-29/ ++* males and selected 19 Dpy non-Unc and 16 Unc non-Dpy F_2 recombinants. Fourteen of the 19 Dpy non-Unc were *dpy-5 gpb-2 goa-1/ dpy-5++ unc-29* recombinants and about one-fourth of their progeny arrested as larvae. Five of the 16 Unc non-Dpy were *egl-30 gpb-2 goa-1 unc-29/+ dpy-5 ++ unc-29* recombinants and did not produce lethal progeny.

An *egl-8(n488); dpy-5(e61)gpb-2(pk751)goa-1(pk62)/+++* mutant was generated. We placed 10 *egl-8*; *dpy-5 gpb-2 goa-1/+++* L4 heterozygotes on separate plates. The heterozygous animals were given the opportunity to lay eggs for 2 days. A total of 119 Dpy F_1 larvae were transferred to separate plates and followed for a generation to determine their genotype. Of the 119 Dpy larvae, 101 arrested and did not produce progeny, and 18 viable Dpy animals were *dpy-5 gpb-2 goa-1/ dpy-5++* recombinants. Arrested Dpy larvae were seen among the progeny of all recombinants. In all experiments, homozygosity for

gpb-2(pk751) and *egl-8(n488)* and the presence of *goa-1(pk62)* was confirmed by single worm PCR. Homozygosity of the *egl-30* and *dgk-1* allele was confirmed by sequencing genomic DNA of animals containing the *n686* and the *nu62* mutations, respectively.

Yeast two-hybrid analysis: The coding sequence of GPB-1 (GenBank accession no. AF291846), GPB-2 (AF291847), GPC-1 (AF291848), and GPC-2 (AF291849) and the regions encoding the GGL of EGL-10 (AF291850) and EAT-16 (AF291851) were amplified from total RNA of the *C. elegans* N2 strain by RT-PCR. First strand synthesis was initiated using a mixture of random hexamers and oligo(dT) oligonucleotides [100 ng/μl total RNA, 200 nM oligo(20)dT, 180 units/μl random hexamers, 0.5 units/μl avian myeloblastosis virus reverse transcriptase (Roche), 1 mM dNTP, 50 mM Tris-HCl pH 8.5, 8 mM $MgCl_2$, 70 mM KCl, 1 mM dithiothreitol, 10 ng/μl BSA], and cDNAs were amplified by PCR [10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.025 units/μl Taq polymerase (GIBCO, Gaithersburg, MD), 0.2 mM dNTP, 200 nM each primer] using specific oligonucleotides (GPB-1: 5′-GGGAATTCATGAGCGAACTTGACCAAC and 5′-CCCTCGAGTTAATTCCAGATCTTGAG; GPB-2: 5′-GGGAATTCATGCCAGAAAACTCTCAGC and 5′-CCCTCGAGTCAAGCCCAAATGCGAATTG; GPC-1: 5′-GGGAATTCATGGAAAACATCAAGGCATC and 5′-CCCTCGAGTTAGAGTACTGAACAGCTT; GPC-2: 5′-GGGAATTCATGGATAAATCTGACATGC and 5′-CCCTCGAGTTAGAGCATGCTGCACTTG; EGL-10-GGL: 5′-CCCCATATGCCTGGATTACGCCGGTGTAC and 5′-CCCCTCGAGACTATCCTCCCAAAGCTTGAG; EAT-16-GGL: 5′-GGCCATATGCGGCAGAATGCACAAGGTTA and 5′-GGCTCGAGTGTGTTTCGAGCACTTGCCGTC) containing recognition sites for restriction enzymes for in-frame cloning in the appropriate two-hybrid bait or prey vector (pGBK-T7 or pGAD-T7, CLONTECH, Palo Alto, CA). Final cDNA constructs were checked by sequencing for potential differences with the spliced gene products predicted by ACeDB (no differences were found) and for the absence of mutations. Combinations of bait and prey plasmids were introduced in yeast strain AH109 by polyethylene glycol/lithium acetate transformation and assayed for interactions by growth on medium lacking histidine and adenine, according to the manufacturer's protocols (CLONTECH).

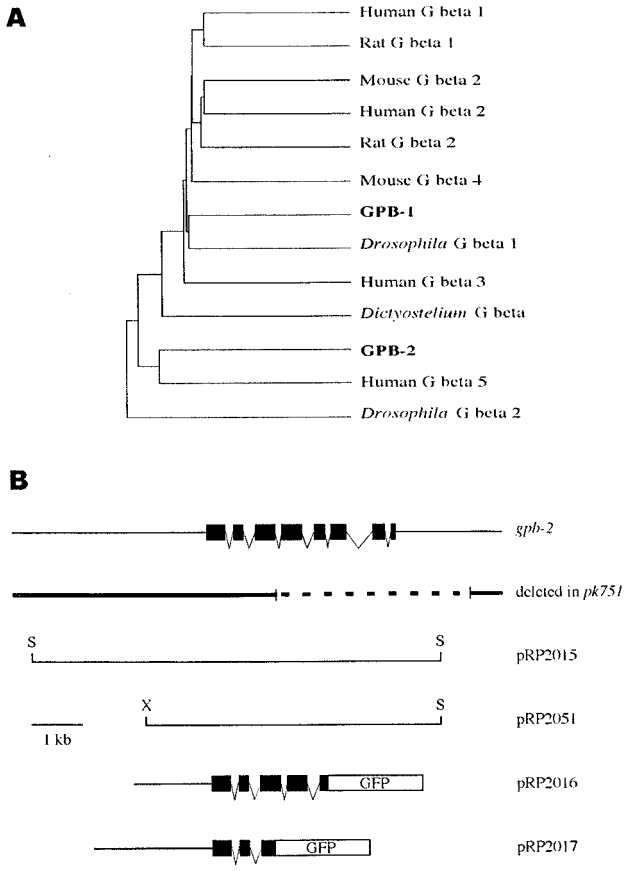

FIGURE 1.—An alignment dendogram of various G-protein β-subunits from different species and the gene structure of *gpb-2*. (A) Alignment dendogram generated with the PILEUP algorithm using default program parameters. GPB-2 is most related to human Gβ5. (B) Genomic organization of the *gpb-2* gene. The solid boxes show coding sequence. The region deleted in *gpb-2(pk751)*, two rescuing constructs, pRP2015 and pRP2051, and two *gfp* reporter constructs, pRP2016 and pRP2017, are indicated. S, *Spe*I; X, *Xba*I.

RESULTS

GPB-2 is a homologue of the divergent mammalian Gβ5 subunit: The *C. elegans* genome project identified two G-protein β-subunit genes. The first G-protein β-subunit, *gpb-1* (located on chromosome II, F13D12.7), is 86% identical to the conserved mammalian Gβ-subunits (VAN DER VOORN *et al.* 1990; ZWAAL *et al.* 1996). However, the predicted sequence of *gpb-2* (located on chromosome I, F52A8.2) indicates that the second G-protein β-subunit is much less conserved. We have isolated and sequenced full-length GPB-2 cDNA (GenBank accession no. AF291847) and found no differences with the sequence predicted by GENEFINDER. The deduced GPB-2 protein consists of 356 amino acid residues, encoding the seven WD40 repeats characteristic for the mammalian β-subunits. However, the amino acid sequence shares only ~50% identity with GPB-1 and the mammalian Gβ1-4 subunits (Figure 1A). Interestingly, GPB-2 is most similar to the divergent mammalian Gβ5 subunit—64% identity and 73% similarity at the amino acid sequence level.

***gpb-2* is widely expressed in neurons and muscle cells:** To analyze GPB-2 expression, we used two translational fusions of the *gpb-2* upstream control sequence with the gene encoding GFP (CHALFIE *et al.* 1994). One translational fusion contained *gfp* fused to the third exon and the other to the fifth exon of *gpb-2* (Figure 1B). Both *gfp* reporter constructs showed identical expression patterns throughout development in the nervous system and in muscle cells. Animals with an integrated *gpb-2::gfp* array showed no expression in embryos until the comma stage when broad expression was seen, excluding the dorsal posterior site of the comma stage embryo (Figure 2A). From the comma stage onward, when tissues differentiate and become organized, until hatching of the embryos, *gpb-2* expression was stronger and broadly expressed in the head and tail ganglia. In larvae and adult animals, *gpb-2* expression was seen in most or all neurons, including neurons located in the head ganglia

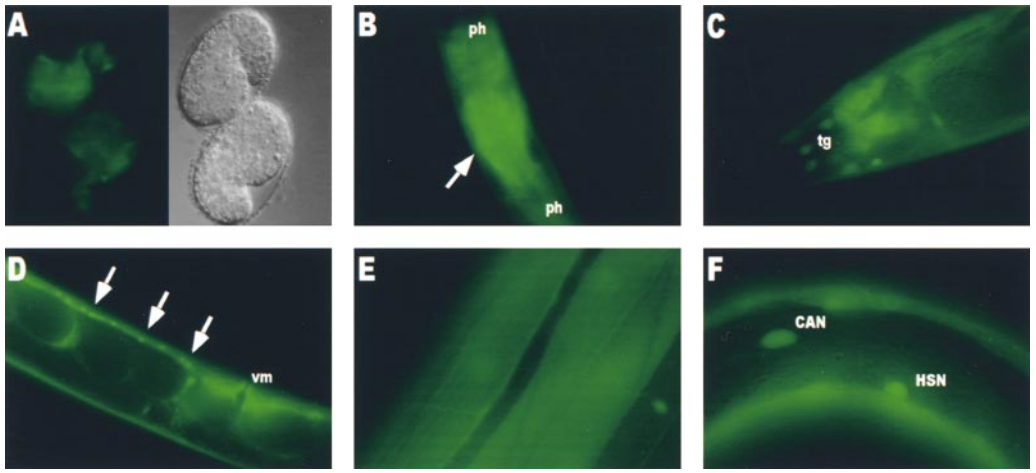

FIGURE 2.—Expression pattern of *gpb-2* as detected using translational GFP gene fusions. In the embryo, expression of *gbp-2* is first detected in the comma stage (A, lower embryo) and the 1.5-fold stage (A, upper embryo). In adult animals, expression is observed in neurons in the head (B; arrow indicates head ganglia; ph, pharyngeal muscle) and tail (C; tg, tail ganglia) regions. (D) Expression of *gpb-2* in the vulva muscle cells (vm) is shown, and in the region surrounding the vulva expression of *gpb-2* can be seen in the ventral nerve cord neurons (arrows indicate single neurons or groups of neurons). Furthermore, high expression levels of *gpb-2* are found in body-wall muscle cells (E) and in the CAN and HSN cells (F).

(Figure 2B), the ventral nerve cord (Figure 2D), and the tail ganglia (Figure 2C). Additionally, *gpb-2* expression was seen in the hermaphrodite-specific neurons (HSNs), which control egg laying (Figure 2F) and the canal cell-associated neurons (CANs), which are thought to function in osmoregulation (Figure 2F). Besides neuronal expression, high expression levels of *gpb-2* were also seen in muscle cells of the pharynx (Figure 2B), the vulva muscle cells (Figure 2D), and the body-wall muscle cells (Figure 2E). In males, similar neuronal and muscle expression was observed. Thus, *gpb-2* is widely expressed in most neurons and in muscle cells that control several distinct behaviors.

***gpb-2* is not an essential gene:** To examine GPB-2 function *in vivo*, we identified a loss-of-function *gpb-2* mutant using a reverse-genetic approach. An EMS-derived deletion library (JANSEN *et al.* 1997) was screened for deletions in the *gpb-2* genomic sequence. We isolated an allele, *gpb-2(pk751)*, in which part of the *gpb-2* coding sequence was removed. As is shown in Figure 1B, this 3380-bp deletion of genomic sequence removes the last five exons of *gpb-2* together with 1.5 kb of downstream sequence. Consequently, *pk751* leaves only one of the seven WD repeats intact. Because seven WD repeats are needed to form a functional β-subunit, it is likely that *pk751* is a null allele. Animals heterozygous for *pk751* were wild type in development and behavior. Animals homozygous for *pk751* were viable, but showed a variety of behavioral defects (discussed below). The behavioral defects of *pk751* were complemented by introducing a transgene carrying a multicopy array, either extrachromosomal or integrated, of the wild-type *gpb-2* genomic sequence, demonstrating that the defects are caused by loss of GPB-2 function (Table 1 and Figure 3).

Mutant *gpb-2* animals are viable, either because GPB-2 does not have an essential function or because GPB-1 is redundant. To address this last question genetically is difficult, since *gpb-1* embryos arrest at the four-cell stage, whereas maternally rescued animals arrest at the first stage of larval development (ZWAAL *et al.* 1996). However, the fact that *gpb-2(pk751)* animals are viable, unlike *gpb-1* mutants, allows us to study the function of a Gβ-subunit in adult behaviors using genetic tools.

GPB-2 modulates behaviors such as egg laying, locomotion, and pharyngeal pumping: To investigate the function of GPB-2 in adult animals, we quantified the behavioral defects induced by a loss-of-function *gpb-2 (pk751)* mutant and by overexpression of GPB-2 (Table 1 and Figure 3). *gpb-2* mutant animals were less active in egg laying and pharyngeal pumping and had a subtle defect in backward movement; *gpb-2* mutants tended to move backward with exaggerated flexes in which the tip of the tail touches the head during each body bend (data not shown). No defect in defecation or dauer formation was observed (data not shown). The rate of egg laying was determined by analyzing the stage of newly laid eggs and the number of unlaid eggs in the uterus. We found that *gpb-2* adult hermaphrodites laid fewer eggs at the nine-cell to comma stage of development (74%) compared to the wild type (96%) and laid more of their eggs at the post-comma stage (26%) compared to the wild type (0%, Table 1). More strikingly, 24 hr later, the same *gpb-2* adult hermaphrodites laid 97% of their eggs at the post-comma stage compared to 6% for wild-type animals. The number of unlaid eggs that accumulated in *gpb-2* mutant animals (32.1 ± 1.6) was also higher than in wild-type animals (19 ± 1.2, Figure 3). One possibility is that the serotonergic HSNs or the vulva muscle cells that control egg laying are defective in *gpb-2* mutant animals. Egg laying in wild-type animals is stimulated by adding serotonin (5-HT) and imipramine, a reuptake inhibitor of 5-HT. Animals with nonfunctional HSNs respond to exogenous 5-HT, but are resistant to imipramine (TRENT *et al.* 1983). Animals that are resistant to both agents have defects in the response of the egg-laying muscles. Adult *gpb-2*

TABLE 1

Rate of egg-laying behavior of gain- and loss-of-function *gpb-2* mutant animals and rescued transgenics

Genotype	Transgene	Time assayed post-L4 larval stage (hr)	Developmental stage of newly laid eggs (% total no. of eggs laid)			
			One- to eight-cell	Nine-cell comma	Post-comma	No. of eggs laid
N2 (wild type)	None	29	6	94	0	189
gpb-2(pk751)	None	29	0	74	26	114
gpb-2(pk751);pkIs559[*gpb-2(+)*]	pRP2015	29	4	93	3	197
pkIs559[*gpb-2(+)*]	pRP2015	29	8	92	0	204
N2 (wild type)	None	53	0	94	6	136
gpb-2(pk751)	None	53	0	3	97	104
gpb-2(pk751);pkIs559[*gpb-2(+)*]	pRP2015	53	0	95	5	132
pkIs559[*gpb-2(+)*]	PRP2015	53	0	97	3	140

At least 20 adult animals were assayed for each genotype (see MATERIALS AND METHODS). Newly laid eggs were divided into three categories: one- to eight-cell stage, nine-cell to comma stage, and post-comma stage. *pkIs559*[*gpb-2(+)*] is an integrated transgene carrying multiple copies of the *gpb-2* wild-type sequence that overexpress GPB-2.

mutant animals respond both to 5-HT and imipramine, indicating that the HSNs and the response of the egg-laying muscles are normal (Table 2). Despite the unusual backward motion described, the frequency of body bends during locomotion was not significantly different in *gpb-2* mutant animals compared to the wild type (Figure 3). In addition to these behaviors, we noticed that well-fed adult *gpb-2* mutant animals had a starved appearance after ~3 days. Although an animal can appear starved for a number of reasons, a starved appearance is often associated with a feeding defect (AVERY 1993). Because *gpb-2* was highly expressed in the pharyngeal muscle cells, we determined whether the starvation phenotype of *gpb-2* mutant animals was caused by a reduced pharyngeal pumping. Indeed, the pharynx pumped slowly in *gpb-2* mutant animals (129 ± 8 pumps/min) compared to the wild type (230 ± 10 pumps/min, Figure 3).

To analyze the effect of *gpb-2* gene dosage on behavior, we made transgenic animals that overexpress GPB-2 from a chromosomal integrated transgene carrying a multicopy array of the *gpb-2* gene, *pkIs559*[*gpb-2(+)*]. Functionality of the transgene was demonstrated by its ability to complement the *gpb-2(pk751)* behavioral defects (Table 1 and Figure 3). However, we found that overexpression of *gpb-2* under control of its endogenous (*pkIs559*[*gpb-2(+)*]) or heat-shock promoter (*pkEx1324*, *pkEx1325*, and *pkEx1326*; data not shown) did not show any obvious phenotypes with respect to egg laying, locomotion, and pharyngeal pumping (Table 1 and Figure 3). Thus, while GPB-2 is necessary for normal egg laying, locomotion, and pharyngeal pumping, overexpression of GPB-2 does not affect these behaviors.

GPB-2 genetically interacts with GOA-1 ($G_o\alpha$) and EGL-30 ($G_q\alpha$): The behavioral phenotype of *gpb-2* mutants affects similar behaviors as *goa-1* ($G_o\alpha$) and *egl-30* ($G_q\alpha$) mutants. Reduction-of-function *egl-30* alleles induce lethargy and delayed egg laying, whereas a putative null allele results in larval lethality (TRENT *et al.* 1983; BRUNDAGE *et al.* 1996). Loss-of-function mutations in *goa-1* result in a starvation phenotype, premature egg laying, and hyperactive locomotion (MENDEL *et al.* 1995; SÉGALAT *et al.* 1995). To determine whether GPB-2 plays a role in GOA-1 and EGL-30 signaling, we generated double mutants between *gpb-2* and *goa-1* and *gpb-2* and *egl-30*. When we crossed *gpb-2(pk751)* into a *goa-1(pk62)* background, we saw a synthetic phenotype—lethality at the larval stage (>95%). Of the 195 *gpb-2 goa-1* double mutant progeny of *goa-1 gpb-2/unc-15* heterozygous animals, 10 escaped to adulthood, but they also in turn produced inviable progeny (>95%). There was no phenotypic difference between the progeny of *gpb-2 goa-1* mutant animals that escaped to adulthood and *gpb-2 goa-1* progeny of heterozygous mothers. It can be argued that this synthetic lethality is a result of an additive feeding defect of both *gpb-2* and *goa-1* mutants. Therefore, we investigated the pumping rate in *gpb-2 goa-1* double mutant animals (155 ± 11 pumps/min, $n = 10$) from *gpb-2 goa-1* heterozygous mothers and found that there was no significant difference ($P = 0.18$) in the rate of pharyngeal pumping compared to *gpb-2* single mutant adult animals (140 ± 8 pumps/min, Table 3). Thus, the synthetic lethality of *gpb-2 goa-1* animals is not due to a feeding defect that is a result of a decrease in the pumping rate. To further analyze the synthetic phenotype of *gpb-2 goa-1* larvae, we generated *gpb-2 goa-1* double mutant animals rescued with the transgene containing the *gpb-2* wild-type sequence. Animals that lose the transgene in the germline give broods that lack functional *gpb-2* expression (ZWAAL *et al.* 1996). We found that the progeny of *gpb-2 goa-1* double mutant animals that lost the *gpb-2* rescuing transgene pumped slowly (163 ± 9 pumps/min, $n = 10$) and showed pharyngeal defects (data not shown); the pharynx was com-

pressed or shrunken in 80% of the animals. This defect may severely impair feeding, causing the lethality that we observed. However, we cannot exclude other explanations for the lethality. Interestingly, there is evidence that behaviors other than feeding are affected by the *gpb-2 goa-1* double mutants and not by each single mutant. Before *gpb-2 goa-1* double mutant larvae arrested, these animals moved in an uncoordinated fashion and occasionally we observed that *gpb-2 goa-1* larvae rotated half or full turns around their longitudinal axes when elongated.

If combining mutations in *gpb-2* and *goa-1* causes a synthetic lethal phenotype, then double mutants of *gpb-2* and *dgk-1*, which encode a putative downstream effector of GOA-1 signaling, could also result in a similar synthetic phenotype. Like *goa-1*, *dgk-1* mutants are hyperactive in locomotion and egg laying and also have a starvation phenotype, albeit less severe than *goa-1* mutants. We constructed double mutants between *gpb-2 (pk751)* and *dgk-1(nu62)* and found that animals defective in both *gpb-2* and *dgk-1* also arrest (>98%). One of the 53 *gpb-2*; *dgk-1* double mutant progeny of *gpb-2/+*; *dgk-1* animals survived to adulthood and produced progeny that arrested as larvae (>98%). As for *gpb-2 goa-1* double mutants, the phenotype of the progeny of homozygous *gpb-2*; *dgk-1* animals does not differ from the phenotype of the first generation of *gpb-2*; *dgk-1* mutants, excluding any maternal effects. In addition, the phenotype of *gpb-2*; *dgk-1* double mutants resembles the phenotype of *gpb-2 goa-1* mutants, *e.g.*, pharyngeal defects (95% of animals) and locomotory behavior. These genetic data suggest that *gpb-2* acts in a parallel pathway with *goa-1* and *dgk-1*.

Surprisingly, when EGL-30 activity was reduced in *gpb-2 (pk751)goa-1(pk62)* double mutant animals by constructing an *egl-30(n686)gpb-2(pk751)goa-1(pk62)* triple mutant, the lethality of *gpb-2 goa-1* double mutants was suppressed. Only 1 of the 169 *egl-30 gpb-2 goa-1* triple mutants of *egl-30 gpb-2 goa-1/egl-30 ++* heterozygous animals arrested at the larval stage and did not produce offspring. We measured the egg-laying activity, the pharyngeal pumping rate, and the frequency of body bends during locomotion in *egl-30(n686)gpb-2(pk751)goa-1 (pk62)* triple mutants compared to *egl-30(n686)* single mutants. Like *egl-30(n686)* animals, the triple mutant

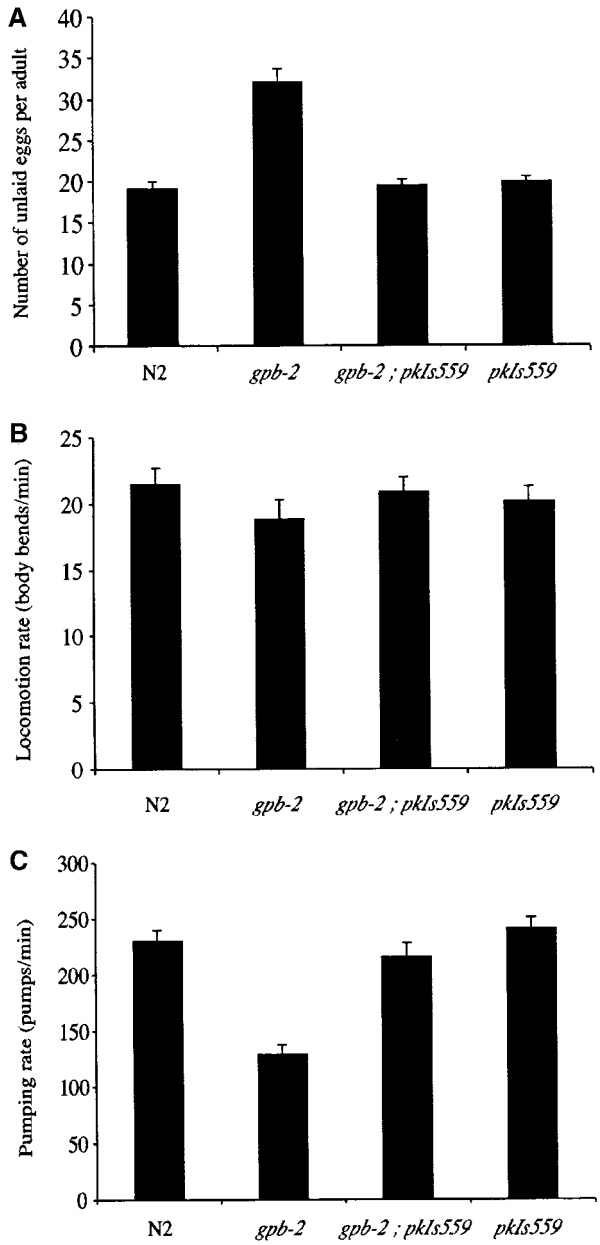

FIGURE 3.—A loss-of-function mutation in *gpb-2* affects egg laying and pharyngeal pumping; overexpression of GPB-2 does not have a significant effect on the frequency of body bends during locomotion, egg laying, and pharyngeal pumping. Egg laying assayed as the number of eggs contained within the uterus (A), the frequency of body bends during locomotion (B), and the pumping rate (C) of N2 (wild-type) animals, *gpb-2* loss-of-function animals (*pk751*), and animals transgenic for a multicopy array containing the *gpb-2* gene (*pkIs559*[*gpb-2 (+)*]) in a *gpb-2* loss-of-function (*pk751*) background and a wild-type background. ANOVA comparing groups for different variables was conducted. A significant effect of group was noted for the amount of unlaid eggs per adult ($F = 43.7$, $P < 0.001$) and for pharyngeal pumping ($F = 26.7$, $P < 0.001$). No significant effect of group was noted for frequency of body bends during locomotion ($F = 0.85$, $P = 0.47$). Differences between genotypes for the unlaid eggs per adult and pharyngeal pumping were analyzed using Student's *t*-test. *gpb-2 (pk751)* mutant animals are significantly different with respect to the amount of unlaid eggs per adult and the rate of pharyngeal pumping from N2 (wild-type) animals ($P < 0.001$), *gpb-2*; (*pkIs559*[*gpb-2(+)*]) animals ($P < 0.001$), and (*pkIs559*[*gpb-2(+)*]) animals ($P < 0.001$). There was no significant difference between (*pkIs559*[*gpb-2(+)*]) and N2 (wild type) for the unlaid eggs per adult ($P = 0.051$) and the pharyngeal pumping rate ($P = 0.22$) or between *gpb-2(pk751)*; (*pkIs559*[*gpb-2(+)*]) and N2 (wild type) for the unlaid eggs per adult ($P = 0.059$) and the pharyngeal pumping rate ($P = 0.15$). About 20 adult animals were assayed for each genotype (see MATERIALS AND METHODS). Values reported are the means ± standard errors.

TABLE 2

Egg-laying assay of loss-of-function *gpb-2* animals in the presence of 5-HT and imipramine

	No. of animals laying the indicated number of eggs after treatment with:											
	M9 buffer				5-HT				Imipramine			
Genotype	0	1–3	4–7	>7	0	1–3	4–7	>7	0	1–3	4–7	>7
N2 (wild type)	19	7	0	0	0	0	7	18	1	7	8	10
gpb-2(pk751)	21	5	0	0	0	0	2	19	1	6	10	7

The response of egg laying of adult hermaphrodites was tested by adding exogenous serotonin (5-HT) and imipramine (see MATERIALS AND METHODS). M9 buffer was used for the control. The number of eggs laid by the hermaphrodites are indicated as follows: 0 eggs, 1–3 eggs, 4–7 eggs, and >7 eggs. Both N2 (wild type) and *gpb-2(pk751)* are sensitive to 5-HT and imipramine.

laid most eggs at the post-comma stage and became as bloated with retained eggs as the *egl-30(n686)* single mutant (Table 3). Moreover, the frequency of body bends during locomotion of the triple mutant is not significantly different from *egl-30(n686)* single mutants (Table 3), although we did observe that *egl-30 gpb-2 goa-1* triple mutants have brief periods of rapid forward and backward movement. Because *egl-8*, which encodes a phospholipase β (PLCβ), is a putative downstream effector of EGL-30, we also generated an *egl-8(n488)*; *gpb-2 (pk751)goa-1(pk62)* triple mutant. Unlike *egl-30*, *egl-8* did not suppress the synthetic lethality of *gpb-2(pk751) goa-1(pk62)* double mutants, suggesting that EGL-30 activity causes the lethality of *gpb-2(pk751)goa-2(pk62)* double mutants via an as yet unknown downstream effector. The results of this epistasis analysis are consistent with *gpb-2* and *goa-1* acting in parallel pathways and upstream of, or parallel to, *egl-30*.

If *egl-30* acts downstream of *gpb-2*, we would expect *egl-30 gpb-2* double mutants to exhibit the phenotypes of *egl-30* single mutants. Therefore, we constructed double mutants between *gpb-2(pk751)* and hypomorphs of *egl-30*. We found that animals having both mutations in *egl-30(ad805)*, the strongest reduction-of-function allele of *egl-30*, and *gpb-2(pk751)* were highly similar to *egl-30 (ad805)* single mutants. *egl-30(ad805)gpb-2(pk751)* double mutant animals were severely lethargic and laid 100% of their eggs at the post-comma stage (Table 3). However, egg laying was very infrequent, suggesting that *gpb-2* may enhance the *egl-30* phenotype (Table 3). We also made a double mutant between a loss-of-function *gpb-2* mutation and a reduction-of-function *egl-30* mutation, *n686*, and again found that *gpb-2(pk751)* did not suppress the behavioral defects of *egl-30(n686)* single mutants; if anything, there was a slight increase in the *egl-30* egg-laying defect (Table 3). Again these results suggest that *gpb-2* acts upstream of, or parallel to, *egl-30* to control egg-laying activity, the locomotion rate, and the pumping rate. Taken together, the genetic data indicate that *gpb-2* may regulate both *goa-1* ($G_o\alpha$) and *egl-30* ($G_q\alpha$) signaling.

GPB-2 interacts with, and is necessary for, both EGL-10 and EAT-16 function: The mammalian Gβ5 subunit interacts with Gγ-subunits (A. J. WATSON *et al.* 1996; ZHANG *et al.* 1996), but Gβ5 also has the unique ability to interact with the GGL domain of a subset of RGS proteins (SNOW *et al.* 1998, 1999; LEVAY *et al.* 1999). The complete genome of *C. elegans* contains two GGL-containing RGS proteins, EGL-10 and EAT-16, and two canonical G-protein γ-subunits, GPC-1 and GPC-2. We examined the possibility that GPB-2, the closest *C. elegans* homologue of Gβ5, could physically interact with the GGL domain of EGL-10 and EAT-16 and the Gγ-subunits, GPC-1 and GPC-2. Yeast two-hybrid analysis showed that GPB-2 bound to the GGL domain of EGL-10 and EAT-16 and the G-protein γ-subunits, GPC-1 and GPC-2 (Figure 4). We found similar results with GPB-1 (Figure 4). These findings suggest that there is no specificity for GPB-2 and GPB-1 in their ability to bind to EGL-10, EAT-16, GPC-1, or GPC-2 *in vitro*. However, these results may not reflect the *in vivo* situation.

Mutations in *eat-16* (RGS) result in similar behavioral phenotypes as described for *goa-1* ($G_o\alpha$)—starvation, premature egg laying, and hyperactive locomotion (HAJDU-CRONIN *et al.* 1999)—but mutations in *egl-10* (RGS) result in opposite phenotypes (KOELLE and HORVITZ 1996). Genetic analysis showed that EGL-10 is an upstream inhibitor of GOA-1 ($G_o\alpha$) (KOELLE and HORVITZ 1996), whereas EAT-16 acts downstream of, or parallel to, GOA-1 ($G_o\alpha$), which in turn inhibits EGL-30 ($G_q\alpha$) activity (HAJDU-CRONIN *et al.* 1999). Given the physical interaction of GPB-2 with EGL-10 and EAT-16 *in vitro* and the behavioral phenotypes of *gpb-2* mutant animals, GPB-2 may be required for the function of either EGL-10 or EAT-16 or both. To test this hypothesis, we analyzed the phenotype of double mutants between *gpb-2* and *eat-16* or *egl-10*. We found that the phenotype of double mutants of *gpb-2(pk751)eat-16(sy438)* and *gpb-2(pk751)*; *egl-10(md176)* was indistinguishable from the phenotype of *gpb-2* mutant animals with respect to the rate of egg laying, pharyngeal pumping, and the frequency of body bends during locomotion (Table 4). Also the phenotype of *gpb-2(pk751)eat-16(sy438)*; *egl-10 (md176)* triple mutant animals was not different from

TABLE 3

Genetic interactions between *gpb-2*, *egl-30*, and *goa-1*

Genotype	Developmental stage of newly laid eggs (% total no. of eggs laid)				Locomation rate (body bends per min)	Unlaid eggs per adult	Pumping rate (pumps per min)
	One- to eight-cell	Nine-cell comma	Post-comma	No. of eggs laid			
N2 (wild type)	5	95	0	197	18.4 ± 2.0	17.6 ± 0.6	258 ± 12
gpb-2(pk751)	0	80	20	156	17.5 ± 2.1	30.3 ± 1.6	140 ± 8
egl-30(n686)	0	25	75	131	10.8 ± 1.5	39.3 ± 1.3	279 ± 12
egl-30(n686)gpb-2(pk751)	0	13	87	128	10.0 ± 1.2	37.1 ± 2.1	256 ± 15
egl-30(ad805)	0	0	100	67	ND	46.1 ± 1.9	210 ± 14
egl-30(ad805)gpb-2(pk751)	0	0	100	14	ND	39.1 ± 1.7	190 ± 9
egl-30(n686)gpb-2(pk751)goa-1(pk62)	0	46	54	170	11.4 ± 1.0	40.5 ± 0.8	267 ± 19

Twenty adult animals were assayed for each genotype to determine the developmental stage of newly laid eggs. To determine the frequency of body bends, the unlaid eggs per adult, and the pharyngeal pumping rate, 10 adult animals were assayed for each genotype (see MATERIALS AND METHODS). Values reported are the means ± standard errors. The *goa-1(pk62)* allele is a putative loss-of-function mutation (MENDEL *et al.* 1995). *egl-30(ad805)* is a stronger reduction-of-function mutation compared to *egl-30(n686)* (TRENT *et al.* 1983; BRUNDAGE *et al.* 1996). Both *egl-30(ad805)* mutants and *egl-30(ad805)gpb-2(pk751)* double mutants made few tracks and were almost paralyzed when undisturbed (ND, not determined). ANOVA comparing groups for different variables was conducted. We found a significant effect of group for locomotion ($F = 6.12$, $P < 0.001$), for unlaid eggs per adult ($F = 37.0$, $P < 0.001$), and for the rate of pharyngeal pumping ($F = 16.94$, $P < 0.001$). We used a Student's *t*-test to analyze the differences between genotypes. No significant differences were obtained for the frequency of body bends during locomotion between *egl-30(n686)* and *egl-30(n686)gpb-2(pk751)* ($P = 0.35$) and *egl-30(n686)gpb-2(pk751)goa-1(pk62)* ($P = 0.34$). The following differences were obtained for the unlaid eggs per adult: *egl-30(ad805)* from *egl-30(ad805)gpb-2(pk751)* ($P = 0.018$). No significant differences were obtained for the unlaid eggs per adult between *egl-30(n686)* and *egl-30(n686)gpb-2(pk751)* ($P = 0.18$), and *egl-30(n686)gpb-2(pk751)goa-1(pk62)* ($P = 0.27$). No significant differences were found for the pharyngeal pumping rate between *egl-30(n686)* and *egl-30(n686)gpb-2(pk751)* ($P = 0.11$) and *egl-30(n686)gpb-2(pk751)goa-1(pk62)* ($P = 0.33$), and between *egl-30(ad805)* and *egl-30(ad805)gpb-2(pk751)* ($P = 0.056$).

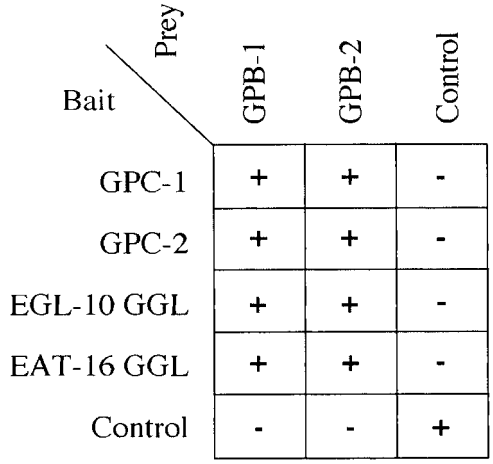

FIGURE 4.—G-protein β-subunits interact directly with G-protein γ-subunits. The cDNA sequences encoding GPB-1 and GPB-2 and GPC-1 and GPC-2, as well as the GGL domains of EGL-10 and EAT-16, were cloned into the two-hybrid prey and bait vector, respectively. Specific two-hybrid interactions were observed for both β-subunits with all γ-subunits and γ-like domains, whereas no interaction was detected with control bait (P53) or prey (SV40 large T) proteins. +, growth on medium lacking histidine and adenine; −, no growth detectable within 10 days on medium lacking histidine and adenine.

the phenotype of *gpb-2* mutants (data not shown). These results suggest that *gpb-2* acts downstream of, or parallel to, *egl-10* and *eat-16* and are also consistent with GPB-2 being needed for both EGL-10 and EAT-16 function.

If it is the case that GPB-2 is required for both EGL-10 and EAT-16 function, then animals defective in both *egl-10* and *eat-16* should result in a phenotype resembling the *gpb-2* mutant phenotype. Indeed, we found that the phenotype of double mutant animals that contain a putative loss-of-function mutation in *eat-16* [*eat-16(sy438)*] and a loss-of-function mutation in *egl-10* [*egl-10(md176)*] resembled the *gpb-2* mutant phenotypes with respect to the rate of egg laying, pharyngeal pumping, and the frequency of body bends during locomotion, rather than an *eat-16(sy438)* or *egl-10(md176)* phenotype. As shown in Table 4, *eat-16(sy438)*; *egl-10(md176)* double mutants were wild type with respect to the frequency of body bends during locomotion; however, the egg-laying rate was intermediate to those of *gpb-2(pk751)* and *egl-10(md176)* single mutants. Moreover, well-fed adult *eat-16(sy438)*; *egl-10(md176)* double mutant animals had a similar starved phenotype as *gpb-2(pk751)* mutants; pharyngeal pumping in *eat-16(sy438)*; *egl-10(md176)* double mutant animals was reduced (Table 4). Taken together, the genetic and physical interaction of GPB-2 with EGL-10 and EAT-16 is consistent with GPB-2 function being required for both EGL-10 and EAT-16 function and vice versa.

***gpb-2* mutant animals are sensitive to the acetylcholinesterase inhibitor aldicarb:** Recent studies showed that the $G_o\alpha$–$G_q\alpha$ signaling network regulates acetylcholine release at the *C. elegans* neuromuscular junction (LACKNER *et al.* 1999; MILLER *et al.* 1999; NURRISH *et al.* 1999). These studies have been facilitated by assaying the response of animals to the drug aldicarb, which is used as a measure of acetylcholine levels at synapses. Aldicarb treatment in wild-type animals causes hypercontraction of body wall muscles and paralysis, as well as inhibition of growth and reproduction. Mutations that decrease acetylcholine release show reduced sensitivity or resistance to aldicarb, whereas mutations that cause elevated levels of acetylcholine at synapses are hypersensitive to aldicarb. For instance, *goa-1* ($G_o\alpha$) null mutant animals exhibit hyperactive locomotion and hypersensitivity to aldicarb, while *egl-30* ($G_q\alpha$) reduction-of-function mutant animals are lethargic and resistant to aldicarb (MILLER *et al.* 1999).

Since *gpb-2* interacts with the *goa-1* ($G_o\alpha$) and *egl-30* ($G_q\alpha$) signaling pathways, we measured the sensitivity of *gpb-2* animals to the acetylcholinesterase inhibitor aldicarb. We observed that *gpb-2* mutant animals exhibit an altered response to aldicarb-induced paralysis compared to wild-type animals; *gpb-2(pk751)* animals are more sensitive to aldicarb (Figure 5A). Similar aldicarb sensitivities of *gpb-2(pk751)* mutant animals were obtained by measuring the population growth rates (Figure 5B). These results suggest a presynaptic locus of action of *gpb-2*. Although *gpb-2* mutant animals did not exhibit hyperactive locomotion, in the absence of aldicarb they did tend to move backward with exaggerated body flexion. To further investigate whether GPB-2 is required for both EGL-10 and EAT-16 function, we analyzed the response of *gpb-2 eat-16* and *gpb-2*; *egl-10* double mutant animals to aldicarb. A putative loss-of-function mutation in *eat-16* [*eat-16(sy438)*] and a loss-of-function mutation in *egl-10* [*egl-10(md176)*] alone showed opposite effects to aldicarb—hypersensitivity and resistance, respectively (Figure 5B). When we crossed *gpb-2* into an *eat-16* or *egl-10* background, we found that the aldicarb sensitivity of *gpb-2(pk751)eat-16(sy438)* and *gpb-2(pk751)*; *egl-10(md176)* double mutants was indistinguishable from the *gpb-2(pk751)* single mutant (data not shown), confirming again that GPB-2 is needed for both EGL-10 and EAT-16 function.

Despite the fact that *eat-16*; *egl-10* double mutant animals resemble the phenotype of *gpb-2* mutant animals with respect to the rate of egg laying, pharyngeal pumping, and the frequency of body bends during locomotion, we found that *eat-16*; *egl-10* double mutant animals had aldicarb sensitivities that were not significantly different from those of wild type, whereas *gpb-2* mutant animals were sensitive (Figure 5B). Interestingly, *eat-16*; *egl-10* double mutants did not exhibit the exaggerated backward flexion seen in *gpb-2* mutant animals, suggesting that the hyperflexion and aldicarb sensitivity phenotypes are related. While it is not completely clear whether *eat-16* and *egl-10* are complete loss-of-function

TABLE 4

Genetic interaction between *gbp-2*, *eat-16*, and *egl-10*

Genotype	Developmental stage of newly laid eggs (% total no. of eggs laid)				Locomotion rate (body bends per min)	Unlaid eggs per adult	Pumping rate (pumps per min)
	One-to eight-cell	Nine-cell comma	Post-comma	No. of eggs laid			
N2 (wild type)	6	94	0	189	21.5 ± 1.2	19.0 ± 1.2	230 ± 10
gpb-2(pk751)	0	74	26	114	18.8 ± 1.5	32.1 ± 1.6	129 ± 8
egl-10(md176)	0	0	100	134	2.9 ± 0.3	43.1 ± 1.1	238 ± 8
gpb-2(pk751);egl-10(md176)	0	78	22	120	18.5 ± 1.7	31.3 ± 1.3	120 ± 7
eat-16(sy438)	95	5	0	176	39.2 ± 1.1	3.8 ± 0.3	146 ± 6
gpb-2(pk751)eat-16(sy438)	0	68	32	109	17.5 ± 1.6	31.0 ± 1.5	135 ± 6
eat-16(sy438);egl-10(md176)	0	38	62	170	20.5 ± 1.0	30.0 ± 1.1	141 ± 7

At least 20 adult animals were assayed for each genotype (see MATERIALS AND METHODS). Values reported are the means ± standard errors. The *eat-16(sy438)* and *egl-10(md176)* alleles are putative loss-of-function mutations (KOELLE and HORVITZ 1996; HAIDUCRONIN *et al.* 1999). ANOVA comparing groups for different variables was conducted. A significant effect of group was noted for locomotion ($F = 75.9$, $P < 0.001$), for unlaid eggs per adult ($F = 142.5$, $P < 0.001$), and for pharyngeal pumping ($F = 44.5$, $P < 0.001$). Differences between genotypes for the frequency of body bends during locomotion, unlaid eggs per adult, and pharyngeal pumping were analyzed using Student's *t*-test. Differences from *gpb-2(pk751)* for the frequency of body bends during locomotion include: *eat-16(sy438)* ($P < 0.001$) and *egl-10(md176)* ($P < 0.001$). No significant difference was obtained for the frequency of body bends during locomotion between *gpb-2(pk751)* and N2 (wild type) ($P < 0.064$), *gpb-2(pk751);egl-10(md176)* ($P = 0.44$), *gpb-2(pk751)eat-16(sy438)* ($P = 0.23$), and *eat-16(sy438);egl-10(md176)* ($P = 0.17$). Differences from *gpb-2(pk751)* for the unlaid eggs per adult include: N2 (wild type) ($P < 0.001$), *eat-16(sy438)* ($P < 0.001$), and *egl-10(md176)* ($P < 0.001$). No significant difference was obtained for the amount of unlaid eggs per adult between *gpb-2 (pk751)* and *gpb-2(pk751);egl-10(md176)* ($P = 0.31$), *gpb-2(pk751)eat-16(sy438)* ($P = 0.30$) and *eat-16(sy438);egl-10(md176)* ($P = 0.11$). The following differences were obtained for pharyngeal pumping: *gpb-2(*$_{\beta}$*k751)* from N2 (wild type) ($P < 0.001$) and *egl-10(md176)* ($P < 0.001$). No significant differences were found for pharyngeal pumping between *gpb-2(pk751)* and *gpb-2(pk751);egl-10(md176)* ($P = 0.18$), *gpb-2(pk751)eat-16(sy438)* ($P = 0.26$), and *eat-16(sy438);egl-10(md176)* ($P = 0.15$).

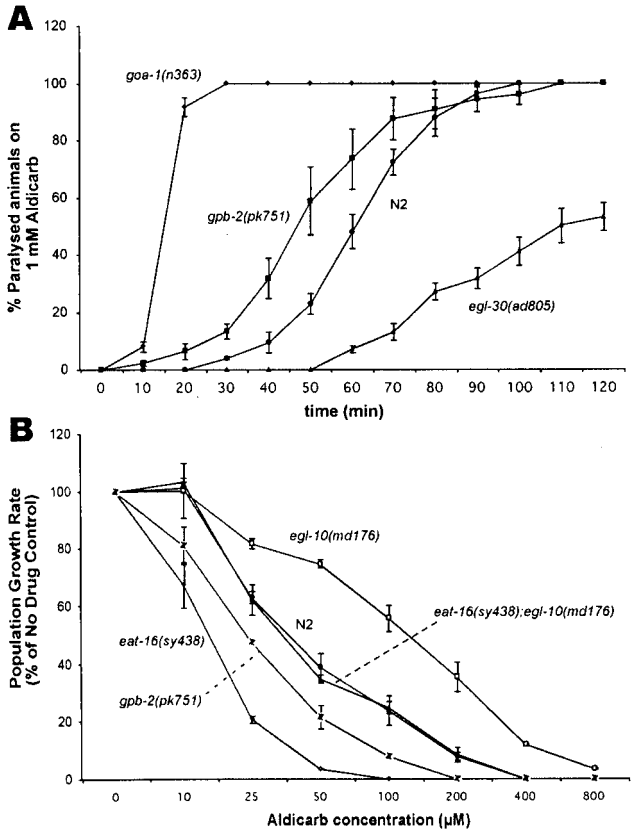

FIGURE 5.—Loss-of-function *gpb-2* mutant animals cause aldicarb sensitivity. (A) Aldicarb sensitivity in *gpb-2(pk751)*, *goa-1(n363)*, and *egl-30(ad805)* single mutants is quantified by assaying the time course of the onset of paralysis following acute exposure to aldicarb. In each experiment, ~25 animals were placed on aldicarb plates and prodded for 10 min over a 2-hr period to determine if they were able to move. The *goa-1(n363)* allele is a deletion allele that removes a region containing the *goa-1* gene (SÉGALAT *et al.* 1995). *egl-30(ad805)* is the strongest reduction-of-function allele (BRUNDAGE *et al.* 1996). Reducing the function of *gpb-2* leads to a significantly increased rate of paralysis compared to N2 (wild type), whereas reduction of *goa-1* and *egl-30* function results in animals that show an increased and decreased rate of paralysis compared to N2 (wild type), respectively. A two-factor repeated-measure ANOVA comparing groups over time was conducted. A significant effect between *gpb-2(pk751)* and N2 (wild type) was noted ($F = 19$, $P < 0.001$), over a time course of 10 min to 90 min ($F = 71.6$, $P < 0.001$) with no significant interaction. (B) Aldicarb sensitivity is quantified by measuring the population growth rates of N2 (wild-type) animals, *gpb-2 (pk751)*, *eat-16(sy438)*, or *egl-10(md176)* single mutants, and *eat-16(sy438); egl-10(md176)* double mutants on various concentrations of aldicarb. One hundred percent represents the number of progeny produced from a starting population of L1 larvae over a 96-hr period in the absence of aldicarb. Animals that lost both *eat-16* and *egl-10* function result in aldicarb sensitivities not significantly different from wild type (N2), whereas *gpb-2* animals are significantly sensitive to aldicarb. Significant effects between groups over increasing aldicarb concentration were analyzed using a two-factor repeated-measure ANOVA. The following significant effects were obtained: N2 (wild type) from *gpb-2(pk751)* ($F = 12.6$, $P = 0.005$), over an aldicarb concentration range of 10 to 200 μM ($F = 50.8$, $P < 0.001$) with no significant interaction. No significant effect between N2 (wild type) and *eat-16(sy438); egl-10(md176)* was observed ($F = 0.006$, $P = 0.94$), with a significant effect over an aldicarb concentration range of 10 to 200 μM ($F = 66.8$, $P < 0.001$). Error bars represent the standard error of the mean of triplicate experiments.

mutations, our data suggest that GPB-2 has functions that are independent of EAT-16 and EGL-10 function.

DISCUSSION

Two G-protein β-subunit genes, *gpb-1* and *gpb-2*, are present in the complete *C. elegans* genome. We show here that, like GPB-1, GPB-2 is widely expressed, even though both have different and specific functions. GPB-2 is most related to mammalian Gβ5, which specifically interacts with the GGL domain of a subset of RGS proteins (SNOW *et al.* 1998; LEVAY *et al.* 1999). Our data illustrate a genetic and physical interaction of GPB-2 with the *C. elegans* GGL-containing RGS proteins, EGL-10 and EAT-16, which functionally regulate the $G_o\alpha$–$G_q\alpha$ signaling network.

What is the *in vivo* function of G-protein β-subunits? The first G-protein β-subunit in *C. elegans*, GPB-1, is highly similar to the mammalian Gβ1-4 subunits (VAN DER VOORN *et al.* 1990; ZWAAL *et al.* 1996), whereas GPB-2 is most related to the mammalian Gβ5 subunit. Both GPB-2 and Gβ5 differ in amino acid sequence from the other four mammalian Gβ-subunits in conserved regions, but particularly in their amino-terminal regions. The Gβ1-4 subunits contain glutamine residues in the conserved amino-terminal region that are absent in GPB-2 and Gβ5 (WATSON *et al.* 1994). The amino-terminal region of the Gβ-subunit is involved in the coiled-coil interaction with the amino-terminal region of the Gγ-subunit (LUPAS *et al.* 1991; SONDEK *et al.* 1996).

Like Gβ5, GPB-2 is expressed in neuronal tissues. In addition, GPB-2 is widely expressed in muscle cells. Both GPB-1 and GPB-2 have similar expression patterns and therefore may have redundant function in certain cells. However, on the basis of the phenotypes of *gpb-1* and *gpb-2* mutants, they seem to have distinct functions. *gpb-1* null mutants are embryonic lethal and progeny of maternally provided *gpb-1* animals arrest at the first stage of larval development (ZWAAL *et al.* 1996); *gpb-2* null mutant animals develop normally to adulthood even though GPB-2 is expressed from the embryo to the adult. These results suggest that GPB-2 cannot compensate for GPB-1, but does not exclude the possibility that GPB-1 can compensate for GPB-2. Mosaic *gpb-1* animals exhibit hyperactive locomotion and premature egg laying (ZWAAL *et al.* 1996), whereas loss of *gpb-2* results in animals that have delayed egg laying and reduced pharyngeal pumping. Thus, loss of *gpb-1* results in different adult behavioral phenotypes compared to loss of *gpb-2*, suggesting that GPB-1 and GPB-2 have distinct and

specific functions. In agreement with this hypothesis, overexpression of GPB-1 results in animals that are lethargic and bloated with eggs (Zwaal *et al.* 1996), whereas overexpression of GPB-2 has no effect on egg laying, pharyngeal pumping, or the frequency of body bends during locomotion.

A possible explanation for these distinct phenotypic patterns is that overexpression of GPB-1, but not GPB-2, may sequester one or more specific Gα-subunits. For example, reduction-of-function mutation in *egl-30* ($G_q\alpha$) has a phenotype similar to GPB-1 overexpression—lethargic and delayed egg laying (Brundage *et al.* 1996). The GPB-1 overexpression phenotype may therefore be due to sequestration of EGL-30. Consistent with this hypothesis, we identified a putative gain-of-function allele of *egl-30* as a suppressor of the lethargy and egg-laying defective phenotype that results from overexpression of GPB-1 (A. M. van der Linden, H. C. Korswagen and R. H. A. Plasterk, unpublished results). Furthermore, *gpb-2*—or components that interact with GPB-2—may already be expressed at high levels in a wild-type background, in such a way that extra GPB-2 will not have an effect. Finally, it is possible that differential interactions with Gγ-subunits or Gγ-like subunits account for the activation of different downstream signaling pathways, resulting in different effects on behavior.

Interaction of GPB-2 with EGL-10 and EAT-16: The genome of *C. elegans* harbors two canonical G-protein γ-subunits, GPC-1 and GPC-2 (Jansen *et al.* 1999), and two GGL-containing RGS proteins, EGL-10 and EAT-16 (Koelle and Horvitz 1996; Hajdu-Cronin *et al.* 1999). Like the specific interaction of Gβ5 with the γ-like (GGL) domains of RGS6, 7, 9, and 11 (Snow *et al.* 1998, 1999; Levay *et al.* 1999), we show that GPB-2, the closest *C. elegans* homologue of Gβ5, interacts with EGL-10 and EAT-16. Using the yeast two-hybrid interaction assay *in vitro*, we demonstrate specific interactions between Gβ-subunits and Gγ-like subunits, but we do not see any differences between GPB-1 and GPB-2 in this regard. *In vivo*, we found genetic interactions of GPB-2 with EGL-10 and EAT-16, consistent with GPB-2 function being required for both EGL-10 and EAT-16 function. In *gpb-2 eat-16* and *gpb-2*; *egl-10* double mutants, functional GPB-1 is present in the cells that control the observed phenotypes, *e.g.*, egg laying and muscle activity, suggesting that GPB-1 is not functionally redundant with GPB-2 for the phenotypes described. After completion of this study we became aware of two recent results that support these conclusions: first, interactions between GPB-2 and EGL-10 and EAT-16 have been independently observed (Chase *et al.* 2001), and second, consistent with the observed feeding defects, *eat-11* is allelic to *gpb-2* and appears to interact with *eat-16* and *egl-10* (Robatzek *et al.* 2001).

Role of GPB-2 in the GOA-1 ($G_o\alpha$)–EGL-30 ($G_q\alpha$) signaling network: In mammals, it is clear that Gβ5-RGS complexes can act as GAPs for Gα-subunits. GAPs inhibit Gα activity by catalyzing the exchange of GTP bound to Gα to GDP. Genetic and biochemical data argue that EAT-16 (RGS) functions as a GAP for EGL-30 ($G_q\alpha$; Hajdu-Cronin *et al.* 1999), whereas genetic data suggest that EGL-10 (RGS) inhibits GOA-1 ($G_o\alpha$) activity (Koelle and Horvitz 1996). The results show that GPB-2 interacts with both EGL-10 and EAT-16 to regulate GOA-1 and EGL-30 activity, respectively. This conclusion is emphasized by the finding that loss of *gpb-2* does not result in phenotypes similar to either *eat-16* or *egl-10*. Only *eat-16*; *egl-10* double mutant animals resemble a *gpb-2* mutant phenotype, *e.g.*, the rate of egg laying, pharyngeal pumping, or the frequency of body bends during locomotion. In line with data from studies on Gβ5, a possible role of GPB-2 may thus be to modulate the specificity and/or activity of EGL-10 and EAT-16 for GOA-1 and EGL-30, respectively (Witherow *et al.* 2000). It is, however, also likely that GPB-2 function is not completely dependent on EGL-10 and EAT-16. For example, animals defective in *gpb-2* exhibit exaggerated

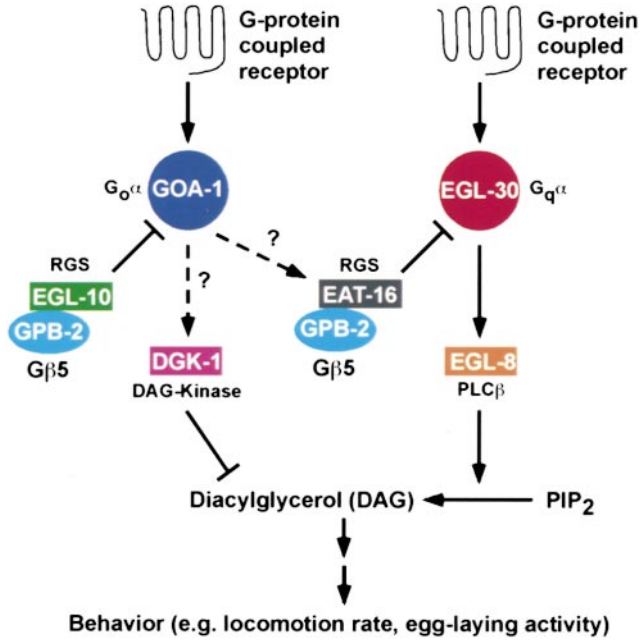

Figure 6.—A model for GPB-2 to regulate behavior (*e.g.*, locomotion rate, egg-laying activity) by interacting with both EGL-10 and EAT-16, which in turn inhibit GOA-1 ($G_o\alpha$) and EGL-30 ($G_q\alpha$) activity, respectively. This model is based on data presented here and on data from Koelle and Horvitz (1996), Hajdu-Cronin *et al.* (1999), Nurrish *et al.* (1999), Lackner *et al.* (1999), and Miller *et al.* (1999). GPB-2—most closely related to mammalian Gβ5—is required for the function of the GGL-containing RGS proteins EGL-10 and EAT-16. EGL-30 activates EGL-8—encoding a phospholipase Cβ (PLCβ)—which cleaves phosphatidylinositol 4,5-biphosphate (PIP_2) into diacylglycerol (DAG) and inositol-1,4,5-triphosphate (Berridge 1984). DGK-1—encoding a diacylglycerol kinase (DAG-kinase)—may be activated by GOA-1 to reduce DAG levels. The locomotion rate and egg-laying activity is indirectly regulated by levels of DAG. GOA-1 may inhibit EGL-30 ($G_q\alpha$) activity directly through downstream effectors such as EAT-16 and/or DGK-1, indirectly via the GGL domain of EAT-16 as proposed by Guan and Hall (1999), or act in parallel to EGL-30.

backward motion and aldicarb sensitivity phenotypes, whereas *eat-16*; *egl-10* double mutant animals do not.

Previous genetic data indicated that GOA-1 ($G_o\alpha$) antagonize EGL-30 ($G_q\alpha$) signaling directly or indirectly through EAT-16 and/or DGK-1 (DAG-kinase; Hajdu-Cronin *et al.* 1999; Miller *et al.* 1999). In this model both EAT-16 and DGK-1 function downstream of, or parallel to, GOA-1 ($G_o\alpha$) and act synergistically to negatively regulate EGL-30 ($G_q\alpha$) signaling (Figure 6). This synergy was proposed because animals defective in both EAT-16 and DGK-1 function are synthetic lethal, and this lethality could be suppressed by reducing EGL-30 activity (Hajdu-Cronin *et al.* 1999). Thus, downstream signaling components of EGL-30 may cause the lethality of the *eat-16*; *dgk-1* double mutant. In support of this model, we found similar genetic interactions with GPB-2, GOA-1, and DGK-1. *gpb-2*; *dgk-1* and *gpb-2 goa-1* double mutants resulted in a synthetic lethal phenotype. Moreover, the synthetic lethal phenotype of *gpb-2 goa-1* double mutants was suppressed when EGL-30 activity was reduced. No suppression was observed when the activity of EGL-8—a potential downstream effector of EGL-30—was reduced. This result is in agreement with the model that EGL-8 does not mediate all the effects of EGL-30 (Miller *et al.* 1999), but that there should be other, so far unknown, downstream effectors. An interesting finding is that, unlike *gpb-2 goa-1* double mutants, *goa-1 eat-16* double mutants are not lethal (Hajdu-Cronin *et al.* 1999), suggesting that *gpb-2* does not interact with only *eat-16* but also with another unknown pathway. A potential pathway is the *dgk-1* pathway, since *dgk-1*; *gpb-2* double mutants also result in a synthetic lethal phenotype.

Recent literature has designed a number of interactions in the GOA-1 ($G_o\alpha$)–EGL-30 ($G_q\alpha$) network that functions in locomotion and egg laying in *C. elegans* (Hajdu-Cronin *et al.* 1999; Lackner *et al.* 1999; Miller *et al.* 1999; Nurrish *et al.* 1999). In this signaling network, GOA-1 ($G_o\alpha$) and EGL-30 ($G_q\alpha$) are assumed to couple to G-protein-coupled receptors and are shown to be negatively regulated by the GGL-containing RGS proteins EGL-10 and EAT-16, respectively (Figure 6). The rate of locomotion and the activity of egg laying are stimulated by activation of EGL-30 via EGL-8 (phospholipase Cβ), whereas activation of GOA-1 inhibits both behaviors, probably via DGK-1 (DAG-kinase). The data presented here support a model in which GPB-2 plays an important role in the GOA-1–EGL-30 signaling network by interacting with both GGL-containing RGS proteins EAT-16 and EGL-10 (Figure 6). In view of this model, two questions can be asked. First, does GPB-2 inhibit both EGL-30 and GOA-1 and, second, do our data distinguish between GOA-1 acting in parallel to EGL-30 or GOA-1 acting upstream of EGL-30? With respect to the first question, our epistatic analysis of *gpb-2* with *goa-1* and *egl-30* appears consistent with two hypotheses: either GPB-2 inhibits both GOA-1 and EGL-30 or GPB-2 inhibits GOA-1 and activates EGL-30. However, since we found that GPB-2 interacts with both EGL-10 and EAT-16, we favor the hypothesis that GPB-2 inhibits both GOA-1 and EGL-30. It is plausible that the apparent activation of events downstream of EGL-30 by GPB-2 as suggested by the slight enhancement of the *egl-30* phenotype in the *egl-30 gpb-2* double mutants can be easily caused by inhibition of GOA-1, because GOA-1 antagonizes EGL-30 signaling. With regard to the second question, our data do not explicitly distinguish between the two models described. In either case, we propose that GPB-2 plays an important role in the regulation of GOA-1 ($G_o\alpha$) and EGL-30 ($G_q\alpha$) signaling through EGL-10 and EAT-16.

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