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MAU-8 is a Phosducin-like Protein required for G protein signaling in *C. elegans*

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Abstract

The *mau-8(qm57)* mutation inhibits the function of GPB-2, a heterotrimeric G protein β subunit, and profoundly affects behavior through the Gαq/Gαo signaling network in *C. elegans*. *mau-8* encodes a nematode Phosducin-like Protein (PhLP), and the *qm57* mutation leads to the loss of a predicted phosphorylation site in the C-terminal domain of PhLP that binds the Gβγ surface implicated in membrane interactions. In developing embryos, MAU-8/PhLP localizes to the cortical region, concentrates at the centrosomes of mitotic cells and remains associated with the germline blastomere. In adult animals, MAU-8/PhLP is ubiquitously expressed in somatic tissues and germline cells. MAU-8/PhLP interacts with the PAR-5/14.3.3 protein and with the Gβ subunit GPB-1. In *mau-8* mutants, the disruption of MAU-8/PhLP stabilizes the association of GPB-1 with the microtubules of centrosomes. Our results indicate that MAU-8/PhLP modulates G protein signaling, stability and subcellular location to regulate various physiological functions, and they suggest that MAU-8 might not be limited to the Gαq/Gαo network.

Keywords: C. elegans; G protein signaling; Neurotransmission; Cell division; PAR-5/14.3.3 proteins

Introduction

Heterotrimeric Gαβγ proteins mediate signal transduction through seven-transmembrane domain receptors. In response to neurotransmitters or hormones, activated receptors bind GTP via the Gα subunit leading to the dissociation of Gα from the Gβγ dimer. G proteins regulate the levels of second messengers (e.g. cyclic nucleotides and phospholipids) which in turn modulate the activities of downstream effectors (such as ion channels and enzymes) in the nervous system. G protein signaling has been extensively analyzed in *C. elegans* in recent years to try to understand how G protein complexes function in vivo. In the nervous system of *C. elegans*, different heterotrimeric G proteins signaling pathways have characteristic effects on development, neural degeneration and behavior, including locomotion, egg laying and defecation (Bargmann and Kaplan, 1998).

Locomotion and egg laying activity in *C. elegans* are controlled by the antagonistic effects of the Gαq/EGL-30 and Gαo/GOA-1 signaling pathways (Brundage et al., 1996; Hajdu-Cronin et al., 1999). This Gαq/Gαo network can regulate synaptic transmission via several downstream effectors including UNC-13, a DAG binding protein that modulates synaptic vesicle release (Lackner et al., 1999; Miller et al., 1999; Nurrish et al., 1999). The antagonistic activities of the Gα subunits EGL-30 and GOA-1 are in turn regulated by Gβγ complexes. Genetic analyses have shown that GPB-2, the second Gβ subunit of the nematode, interacts with both EGL-10 and EAT-16 RGS (Regulator of G protein Signaling) proteins to modulate locomotion and egg laying. Upon binding of the ligand to its receptor, GPB-2/RGS is released by its cognate Gα and the complex can then bind and inhibit the opposing Gα by enhancing its GTPase activity (Robatzek et al., 2001; Van den Linden et al., 2001). The RIC-8 protein is also a component of the G protein network. It acts upstream of Gαq, and it is linked to the Gαs pathway (Miller et al., 2000; Schade et al., 2005; Reynolds et al., 2005).

In early *C. elegans* embryos, the Gβ subunit GPB-1 is required for correct centrosome migration around the nucleus

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and thus plays a role in orienting the mitotic spindle (Zwaal et al., 1996). In parallel, two Gα subunits, GOA-1 and GPA-16, control spindle asymmetry (Ahringer, 2003). Nuclear migration is crucial for axis determination, and there is evidence that centrosomes and various motor proteins associated with the microtubule cytoskeleton are required to position nuclei at specific locations in the cytoplasm. *ric-8* is also involved in spindle morphology and position, but its role in this process still remains to be determined. Moreover, the *par* (partitioning defective) genes are required for the establishment and maintenance of anterio-posterior (A/P) body asymmetry after fertilization (Rose and Kemphues, 1998). Although recent data also indicate that PAR polarity proteins and heterotrimeric G protein signaling regulate the stability of microtubules at the cortex of the embryo (Labbé et al., 2003), targets of heterotrimeric G proteins have not been identified yet. Beside G proteins, other molecules including *let-99* and *spn-1* may also be involved in regulating centrosome position, but their role in this pathway is not clear (Rose and Kemphues, 1998).

G protein function is regulated by different classes of proteins, and signaling through the βγ heterodimer is modulated by binding to members of the Phosducin (Pd) and Phosducin-like Protein (PhLP) family (Gaudet et al., 1996; Thibault et al., 1997). Pd and PhLP are highly homologous acidic phosphoproteins. Pd has a well-characterized role in retinal signal transduction, while PhLP is considered to be a potentially ubiquitous regulator of Gβγ signaling. Several studies have established that the physiological control of G protein function by PhLP involves phosphorylation by casein kinase II and calcium/calmodulin kinase II (Thulin et al., 2001; Humrich et al., 2003). The dephosphorylated form of PhLP binds Gβγ which then prevents receptor-mediated Gα reactivation and blocks interactions between Gβγ and its effectors (Yoshida et al., 1994). PhLP has multiple partners: it can bind p45/SUG-1, the regulatory subunit of the 26S proteasome, and it regulates protein folding through interaction with the cytosolic chaperonin CCT (Barhite et al., 1998; Martin Benito et al., 2004). PhLP also interacts with 14.3.3 proteins in brain extracts (Garzon et al., 2002). Multiple 14.3.3 isoforms play roles in numerous cellular processes including signal transduction. Previous studies have shown that 14.3.3 can protect its target protein from proteolysis and dephosphorylation. It has been suggested that, in retinal photoreceptors, 14.3.3 proteins inhibit binding of Pd to Gβγ by sequestering phosphorylated Pd molecules (Nakano et al., 2001). In *C. elegans*, two 14.3.3 genes, *par-5* and *ftt-2*, have been reported, but only PAR-5 acts in gonads and early embryos (Morton et al., 2002).

Although the interactions of PhLP with Gβγ and its other partners have been convincingly documented through in vitro studies, it is not clear how PhLP controls G protein signaling in vivo. *C. elegans* provides a powerful genetic model to analyze Phosducin function and study its interacting partners in signaling pathways. In this report, we describe the phenotypic and molecular characterization of *mau-8*, a *ma*ternal-effect *u*ncoordinated mutant of *C. elegans*. Our results indicate that the *mau-8* gene encodes a ubiquitous Phosducin-like Protein that regulates various physiological functions in the nematode.

Materials and methods

Strains and genetic analysis

Worms were cultivated on NGM plates using standard methods. The Bristol strain N2 was used as a standard wild type strain. The *mau-8(qm57)* allele is an ethyl methane sulfonate-induced allele which was isolated in a screen for maternal-effect mutations (Hekimi et al., 1995). Prior to our work, *mau-8(qm57)* was linked to chromosome IV using *dpy-20(e1282ts)*, and it was suspected to lie close to *unc-24* at 3.45 cM (Hekimi et al., 1995). To check *mau-8* position, we subsequently performed additional mapping experiments. The initial 3-factor mapping experiment, using *unc-24(e138)dpy-20(e1282)* double mutant, gave us the following result: *unc-24(4)mau-8(0)dpy-20*. This mapping was further confirmed using a 2-point mapping: *unc-24mau-8/++* animals were allow to lay eggs and 25/76 pools of 3 UNC progeny segregated UNCnonMAU progeny. Altogether, these results showed that *mau-8* was initially mispositioned and that it was lying around 9 cM. Additional mapping experiments were performed by mating *unc-26(e205)dpy-4(e1166)/+* or *unc-31(e169)dpy-4(e1166)/+* males with *mau-8(qm57)* hermaphrodites. In fact, *mau-8* was placed between *unc-26* and *dpy-4* by the following 3-factor mapping results: *unc-31(6)mau-8(4)dpy-4* and *unc-26(6)mau-8(29)dpy-4*.

The *qm57* mutation was precisely mapped with respect to SNP using the CB4856 SNP mapping strain (Wicks et al., 2001). SNP mapping was performed as described in http://niob.knaw.nl/researchpages/plasterk/wickssnip.html and the *C. elegans* SNP website http://genome.wustl.edu/gsa/C_elegans/SNP. Recombinants were analyzed as previously reported using SNP pkP4089, pkP4058 and pkP4059 (Wicks et al., 2001; and Supplementary data).

Synthetic lethality

The survival of *mau-8;goa-1* and *mau-8;gpb-2* double mutants was scored as described (Van den Linden et al., 2001). Briefly, *goa-1/+* or *gpb-2/+* males were mated with *mau-8(qm57)dpy-4(e1282)* and 10 F1 heterozygotes were given the opportunity to lay eggs for 2–3 days. For the *mau-8;goa-1* crosses, a total of 87 hyperactive animals of the progeny were transferred to separate plates and followed for one generation to determine their genotype. Of 87 hyperactive animals, 18 were also DPY and did not produce progeny. In all experiments, homozygosity of the mutants was confirmed by single worm PCR.

DNA cloning and sequencing

YAC or genomic DNAs were extracted by standard procedures and amplified by PCR using upstream (forward) and downstream (reverse) primers to cover each ORF or group of ORFs within the 8.79 cM region. To amplify OP4540, DNA was amplified by the Expand Long Template PCR system (Roche) using CACAACTTTCTGACTTCCAAC (forward) and AATACATTTAATAGCTTTGAGTTT (reverse). To clone Y62E10A.8, primers TTGCCGTGTAAATATGGTTG and AAAGGAGAATGGGAGGCTCTT were used. PCR products were sequenced by Genome Express.

To identify lesions in *mau-8(qm56)*, genomic DNA, including all exons, introns and 1.5 kb upstream regulatory region of Y62E10A.8, was amplified and sequenced. The lesion was confirmed on both strands and from DNA prepared in independent PCRs. In addition, 11 putative genes located upstream of Y62E10A.8 on Y62E10A and Y67H2 and 7 putative genes located downstream of Y62E10A.8 were amplified and sequenced. None of these putative genes showed any mutations in their exons.

Germline transformations were performed using 10 ng/μl of DNA and 100 ng/μl pRF4 *rol-6(su1006)*. Transformations were carried out using Biolistic methods.

RNA interference

Double-stranded RNA (dsRNA) was delivered by feeding *rrf-3* animals with bacteria producing either L4440, the empty cloning vector (Simmer et al., 2002),

or RCE1182, a *C. elegans* RNAi feeding clone (Open Biosystems). The RCE1182 clone is derived from the *C. elegans* ORFeome Library v1.1. and contains the Y62E10A.8 coding sequences located exactly between the initiation and termination codons, excluding the 5′ and 3′ mRNA untranslated regions. Phenotypes were monitored in the F1 progeny of the animals placed on the plate.

Immunostaining

The dissection procedure used in the immunostaining of gonads and intestines was performed as described in http://www.wormatlas.org/anatmeth/gonadintes.htm. Slides were observed with a Leica confocal microscope.

Protein extraction, immunoprecipitation and Western blots

Proteins were extracted as previously described (Burbea et al., 2002). Immunoprecipitation was performed with antibodies against 14.3.3β(H8) antibodies or PhLP and magnetic Protein-A beads (Dynal). Immunoprecipitated proteins were analyzed by Western blotting with anti-PhLP (gift from P. Sanchez-Blasquez, Instituto Cajal, Madrid and F. Cuzin, University of Nice) or GPB-1 antibodies (gift from R. Plasterk, Hubrecht Laboratory, The Netherlands).

Egg laying assays

The number of unlaid eggs in the uterus was determined as described (Van den Linden et al., 2001). Briefly, L4 animals were placed on OP50-seeded NGM plates and were allowed to develop for 36 h at 20°C. Fifteen to twenty adults were individually dissolved a few minutes in 5% sodium hypochlorite, and their eggs, which survive because of their protective eggshells, were counted using a Nikon T200 microscope.

The developmental stage of newly laid eggs was quantified as reported (Robatzek and Thomas, 2000). Animals were picked as L4 hermaphrodites and assayed after 24 h of growth at 20°C. Animals were then placed on the assay NGM plates and allowed to recover from the transfer for 30 min. Eggs laid during the recovery period were removed, and, at 20 min intervals, eggs laid were examined under the microscope. Wild type and mutant animals were tested for 2 h and categorized as having one to four cells, from 8 cells to gastrulation and after gastrulation.

Results

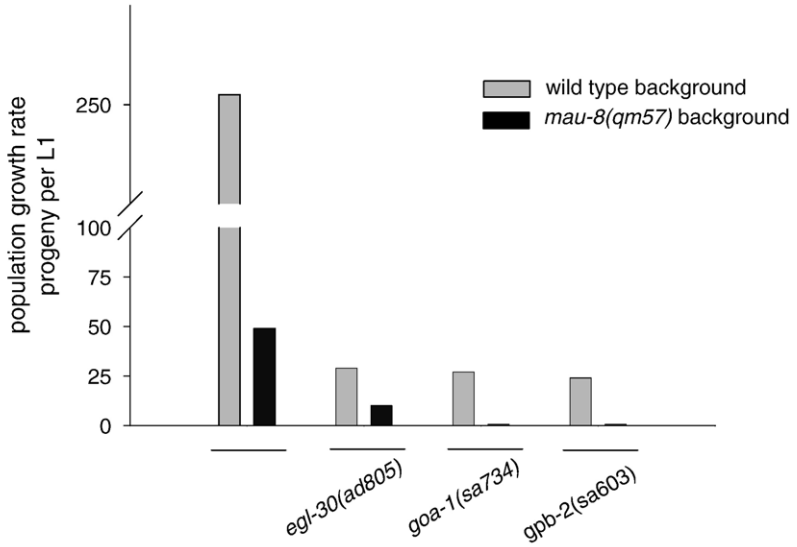
MAU-8 is required for normal development and modulates behavior through the Gαo/Gαq network

mau-8 homozygous mutants display a pronounced maternal rescue of their developmental and behavioral defects, which was the basis for selection in the mutant screen (Hekimi et al., 1995). *mau-8* is defined as a single allele, *qm57*, and could correspond to a rare hypomorphic allele of a gene of which total loss of function leads to lethality. In fact, *mau-8(qm57)* mutant animals display a high incidence of lethality (18%) during embryonic development. Embryonic arrests include disorganized gastrulating embryos, deformed 3-fold stage embryos and embryos of abnormal sizes (data not shown). In addition, significant postembryonic lethality was also observed (16% larval lethality). Later, at the postembryonic stage, all adult survivors show an uncoordinated phenotype. *mau-8(qm57)* mutant animals tended to move with reduced flexes, the head and the anterior half of the body propagate only very shallow waves. Intermittently, they display frequent very rapid forward or backward movements, and, when induced to move backward, these mutants display a discontinuous ratchet-like movement. The flattened sinusoidal locomotion of *mau-8(qm57)* mutants is very similar to that of *egl-30(lf)* and activated GOA-1, whereas their hyperactivity resembles that described in *goa-1(lf)* mutants and in animals overexpressing EGL-30 (Brundage et al., 1996). The Gαq/EGL-30 and Gαo/GOA-1 signaling pathways regulate locomotion in *C. elegans* (Brundage et al., 1996; Hajdu-Cronin et al., 1999). To determine whether G protein signaling was affected in *mau-8*, we generated double mutants between *mau-8(qm57)* and *egl-30(ad805)* and between *mau-8(qm57)* and *goa-1(sa734)*. Decreasing MAU-8 in animals expressing low levels of EGL-30 reduced the growth rates of *egl-30(ad803)* and strongly increased paralysis (Fig. 1A and data not shown). In parallel, *mau-8(qm57)* lacking *goa-1* function have a synthetic lethal phenotype. These data thus indicate that MAU-8 strongly inhibits the Gαq pathway and profoundly affects the Gαo signaling pathway. Interestingly, these results are very similar to those reported for *gpb-2(lf)* mutants indicating that GPB-2, the mammalian Gβ5 ortholog, interacts with Gαq and Gαo to regulate behavior (Robatzek et al., 2001; Van den Linden et al., 2001). In fact, the intermittent locomotion of *mau-8(qm57)* mutants, including periods of inactivity and periods of hyperactive movement, resembles that of putative *gpb-2(sa603)* null mutants (Robatzek et al., 2001). To investigate the possible interaction of MAU-8 with GPB-2, we examined *mau-8(qm57)*; *gpb-2(sa603)* double mutants. We found that reducing MAU-8 in a *gpb-2* null background gave a synthetic lethal phenotype (Fig. 1A). These results thus indicate that MAU-8 inhibits GPB-2 function and suggest that its role might not be limited to the Gαq/Gαo network.

mau-8 mutants also display slight defects in egg laying activity. The rate of egg laying was determined by analyzing the stage of laid eggs and the number of unlaid eggs in the uterus. As shown in Fig. 1B, *mau-8* adult hermaphrodites laid fewer eggs at the 8 cells to gastrulation stage of development (83%) as compared to wild type (99%) and laid more of their eggs at the postgastrulation stage (12%) compared to wild type (0%). As in *mau-8* mutants, egg laying activity in *gpb-2* mutants is also moderately affected and *gpb-2(sa603)* hermaphrodites laid eggs of significantly later stages compared to wild type (Robatzek et al., 2001; Van den Linden et al., 2001 and Fig. 1B). Moreover, *mau-8* hermaphrodites accumulated wild type numbers of eggs in their uterus (Fig. 1B), but 27% of the mutant animals retained less than 10 eggs in their uterus. Furthermore, microscopic analysis of *mau-8(qm57)* hermaphrodites revealed abnormal thin gonads containing only a few germ nuclei suggesting that the reduced brood size and death of embryos could result from a failure of the gonad or germline to develop properly.

In summary, the significant embryonic lethality of *mau-8 (qm57)* suggests that maternal MAU-8 activity is required for early development. Later, *mau-8 (qm57)* was involved in regulating behavior and acts like a *gpb-2* null allele on the Gαq/Gαo network. Genetic data suggested that the *qm57* mutation might also disturb other pathways.

A. Population growth rate of mutants affecting G protein signaling in *mau-8(qm57)* background.



B. Rate of egg laying behavior of *mau-8(qm57)* and *gpb-2(sa603)* mutants.

Genotype	Stages of eggs laid[a]			No of Eggs laid	No of unlaid eggs[b]
	1 to 4 cells	*8 cells to gastrulation*	*after gastrulation*		
Wild type	1	99		114	18,1 ± 1,5
mau-8 (qm57)	5	83	12	40	17,8 ± 1,8
gpb-2 (sa603)	1	89	10	32	17,3 ± 1,4

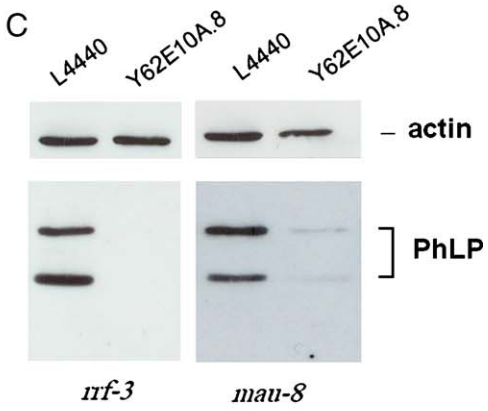
[a]. % total number of eggs laid
[b]. number of unlaid eggs in the uterus

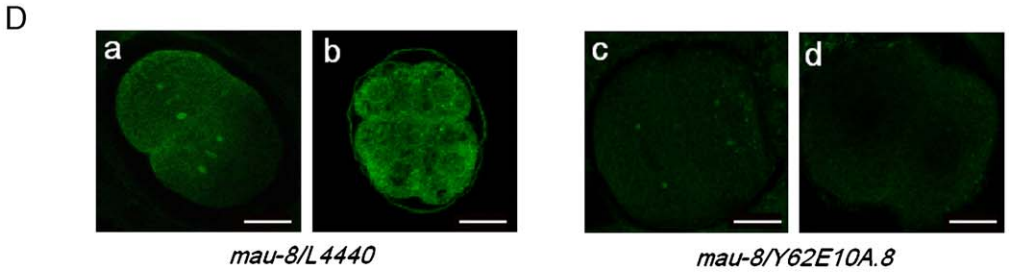
Fig. 1. Population growth rate and egg laying behavior in *mau-8(qm57)* and in mutants affecting G protein signaling. (A) Population growth rate of mutants affecting G protein signaling in a *mau-8(qm57)* background. Combining mutation in *mau-8(qm57)* and *egl-30(ad805)* causes a decrease in the population growth rate whereas reducing *mau-8* in *goa-1* and *gpb-2* putative null mutants induces a lethal phenotype. Population growth rate is defined as the average number of L1 produced by a hermaphrodite after 3 days at 20°C. (B) Newly laid eggs were divided into three groups: one to four cells, from eight cells to gastrulation and after gastrulation. Fifteen to twenty animals were assayed for each genotype.

MAU-8 is a Phosducin-like Protein

Using three-point and SNP mapping (Wicks et al., 2001), we positioned *mau-8* close to 8.79 cM on chromosome IV. This region is covered by Y67H2 and Y62E10. We amplified DNA covering each ORF or group of ORFs within this region (Wormbase111), and a long PCR fragment corresponding to operon 4540 linked to 1.5 kb upstream sequences successfully rescued the *mau-8* uncoordinated phenotype (Fig. 2A). Sequencing and rescue experiments subsequently identified

Fig. 2. Genomic organization of *mau-8* and sequence conservation. (A) Positional cloning of *mau-8*. The mutation was genetically mapped in a region covered by Y62E10. The mau-8 gene was then molecularly positioned close to 8.47 cM and 8.79 cM. The *mau-8* phenotype was rescued by operon 4540, and subsequent rescue experiments and sequencing data finally positioned *mau-8(qm57)* mutation in Y62E10A.8. (B) Predicted gene structure of *mau-8* and representation of the protein product. The *mau-8 (qm57)* mutation was mapped onto the 5th exon, changing C to T at position 2284 from the ATG of Y62E10A.8. The schema of the domain structure of the protein shows the position of three Gβγ-binding sites deduced from data obtained from Pd/PhLP/Gβγ complexes. In addition, a potential PDZ-1 binding site is predicted in the C-terminal portion of MAU-8/PhLP. This binding site overlaps with one potential casein kinase II consensus site (underlined). Two additional casein kinase II consensus binding sites are also predicted by the Scansite website together with a putative calcium/calmodulin kinase II binding site (dotted lines). (C) Detection of the MAU-8/PhLP protein by Western blot analysis in total protein lysates. Protein extracts from mixed developmental stages were prepared from *rrf-3* and *mau-8* mutants. Two protein bands corresponding to the size of the predicted PhLP proteins were detected in blots from *rrf-3* and *mau-8* animals probed with anti-PhLP antibodies. After feeding with Y62E10A.8 RNAi, these bands completely disappeared in extracts of *rrf-3* animals while silencing of PhLP was almost complete in *mau-8* mutants. As loading control, the blot was probed with anti-actin antibodies. (D) PhLP immunostaining in *mau-8* mutants: 2-cell embryo (a) and 4-cell embryo (b). In embryos laid by *mau-8* mutants fed with Y62E10A.8 RNAi, there was no detectable PhLP staining [(two-cell embryo (d)] or a strongly reduced level of the protein [(two-cell embryo (c)]. (E) Sequence conservation of *mau-8*. Alignment of MAU-8/PhLP with human and *Drosophila* PhLP. The boxshade program was used to shade the amino acids black that are identical in at least two species and to shade the similar amino acids gray. The red star labels the position of the mutated serine (ser 254) in *mau-8* (*qm57*). Accession numbers are Q9U1X7 for *C. elegans* PhLP, Q13371 for human PhLP and Q9VUR7 for *Drosophila* PhLP.





E

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human       MTTLDDKLLGEKLQYYSSSEDEDSDHEDKD------RGRCAPASSSVPAEAELAG---E
drosophila  NATLEDKLLGEKLEYYCSSSEGEDNGDEGGDNKGASGKSRCSGLTIDTNPDATEAGGFRQ
Y62E10A.8   -MNLESRILDGRPAGYCSSSEGEEDD---------------FKVVNDEEEHQANVMKRM

                ------------
human       GISVNTGPKGVINDWPRFKQLETECREEQCREMERLIKKISMTCRSHLDEEEEQQKCKDL
drosophila  QSSTNTGPKGVVKDWCRFKQLEAERRDETERQRLALAKKLTITATTSAEEEE-RKPQEEL
Y62E10A.8   GPSSNTGAKGVLNEFKARREQTRLAVESKNQKLIECAKKGMIGSKEERPKAQEEDDEDE

human       QEKISGKMTLKEFAIMNPDQDDEEPLQQYRKQRNEEMECCLHKGPQFKQVPPISSGEGFL
drosophila  LAELD--------ELMSED-----FLQQYCKQRNAEMLPCTGHHCQFGQVQQLTSHEPFL
Y62E10A.8   IFRMT--------------------LFGTLKARRLRFMRKTAAN-----RVTRMLDKKQYS

human       LMIDPEQKSIVLMVHIYEDGIPGTEANGQMICLAAEYEAVKTCKVKSSVIGASSCFTRN
drosophila  ACVEQPNRHTTIIIHIYERQLAACATLNKCLDSLASDYESIKRAKICSSVAGMSRDFRTK
Y62E10A.8   LAVD-GSSSYLLCVLIYEPESDECEYGTRIVKILAADCFKTKFVRATSTLLDMSRAFRTN

human       ALPALLIYKCGELIGNFVRVTDCLGDDFFAVDLEAFLCGPCLLPEKEVLVLTSVRNSATC
drosophila  GLPALLVYKAQAVIGNFVRLTDDLSDDFFASDVESFLIPHGLIVDR------ALYN-----
Y62E10A.8   GVECLQFYSNGNLIGNFVKISAILGCDYDCKKLTKFLRGGHIDLMAG-----GYASDSDN

human       PSEDSDLEID
drosophila  ----------
Y62E10A.8   BSEDDL----
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Y62E10A.8 as *mau-8*. Database searches for proteins similar to the predicted MAU-8/Y62E10A.8 sequence revealed similarity to the vertebrate Phosducin-like Protein (PhLP). The high degree of homology (45%) between MAU-8/PhLP and human PhLP suggests that these two proteins are probably derived from a common ancestor and are orthologous genes (Fig. 2E). PhLP is a member of the Phosducin (Pd) family known to regulate G protein signaling pathways by binding the Gβγ subunit complex (Gaudet et al., 1996; Thibault et al., 1997). Y62E10A.8 appears to be the only member of the Phosducin family in the nematode. A motif search program (Scansite website) identified three consensus sequences for Gβγ binding, one consensus sequence for phosphorylation by calcium/calmodulin-dependent protein kinase I, three putative casein kinase II phosphorylation sites and a consensus sequence for PDZ-1 binding (Fig. 2B).

Sequencing of Y62E10A.8 from the mutant genome revealed a point mutation that transforms a serine to phenylalanine at amino acid position 254. This substitution is located in the putative PDZ-1 binding site and leads to the loss of a predicted casein kinase II phosphorylation site in the mutant protein (Fig. 2B).

To further confirm that Y62E10A.8 corresponds to the *mau-8* locus, we used RNA-mediated interference (RNAi). RNAi was delivered by feeding *rrf-3*, an RNAi-hypersensitive strain (Simmer et al., 2002). Although *rrf-3* animals fed with Y62E10A.8 RNAi did not display any locomotory defects, *mau-8(qm57)* mutants treated by RNAi laid a high frequency of dead embryos (90%) and the eggs that successfully hatched developed as young lethargic larvae that did not reach the adult stage. In agreement with our genetic analyses, *egl-30(ad803)* mutants treated with Y62E10A.8 RNAi were more severely paralyzed than untreated animals while *goa-1(sa734)* and *gbp-2 (sa603)* mutants fed with RNAi did not give any progeny (data not shown). These results further demonstrate that Y62E10A.8 corresponds to the *mau-8* gene and that *qm57* is a hypomorphic mutation of an essential gene.

The *mau-8* gene encodes a putative acidic protein containing 258 amino acids. To detect MAU-8/PhLP, we used polyclonal antibodies directed against a conserved PhLP sequence (Garzon et al., 2002; Lopez et al., 2003). In mammalian tissues, PhLP exists in two splice variants: the long (PhLPL) and short (PhLPS) isoforms (Humrich et al., 2003). In extracts of adult worms, the polyclonal antisera immunoreacted with two PhLP proteins, migrating at 34 and 26 kDa (Fig. 2C). The bands corresponding to PhLP disappeared in animals fed with Y62E10A.8.RNAi confirming antibody specificity (Fig. 2C). In *mau-8* mutants, silencing of PhLP was not complete and two faint PhLP bands are visible in the Western blot (Fig. 2C). These results probably represent the small percentage of eggs (10%) that successfully hatched because they inherited a small amount of maternal *mau-8* activity. PhLP is expressed in early embryos (see Fig. 3), and the specificity of the antibody was also confirmed after immunostaining of embryos laid by *mau-8* mutants fed with Y62E10A.8 RNAi. As shown in Fig. 2D, most embryos did not exhibit any PhLP staining, although a few embryos still expressed low level of the protein.

mau-8 is ubiquitously expressed in somatic and germline tissues

To determine how MAU-8/PhLP might function, we examined its expression pattern and subcellular localization by immunofluorescence microscopy. During embryonic development, from the first cell stage to the end of morphogenesis, MAU-8/PhLP staining was faint and diffuse in the cytoplasm and strongly concentrated at the cell membranes (Figs. 3C and 5A). In addition, MAU-8/PhLP exhibits a dynamic cell-cycle-dependent distribution with staining concentrated at the centrosomes and at the central spindle region of mitotic cells (Figs. 3A, B). From the 2-cell stage, MAU-8/PhLP is also specifically expressed in the germline blastomere where it co-immunolocalized with P granules (Figs. 3B, D–F). In adult hermaphrodites, MAU-8/PhLP expression was detected in most tissues, including body wall muscles, hypodermal cells, pharynx and intestine (Fig. 3I, data not shown). MAU-8/PhLP also remained associated with the germline lineage throughout the life of the worm. Staining appears throughout the gonad as small punctate structures concentrated in the cytoplasm and the cortex of oocytes and germline cells (Figs. 3G, H). This expression pattern is consistent with a ubiquitous role of MAU-8 protein in the nematode and suggests multiple functions of this molecule including a role in germline development.

In *mau-8* embryos, MAU-8/PhLP also localizes to the cell membranes and to the centrosomes of dividing cells (Fig. 3J). However, the distribution MAU-8/PhLP is heterogeneous with a higher concentration of MAU-8/PhLP protein at the nuclear periphery (Fig. 3J), suggesting that the mutant MAU-8/PhLP protein is preferentially associated with the nuclear envelope. Staining of the germline blastomere was also detected in *mau-8* embryos (Fig. 3K), although later, in adult hermaphrodites, the gonad is thin and filled with only a few germline cells (Fig. 3L). These gonads show a heterogeneous distribution of MAU-8/PhLP, suggesting that the germline does not develop properly.

MAU-8/PhLP interacts with the Gβ subunit GPB-1

Biochemical studies have abundantly documented the binding of Pd/PhLP to Gβγ subunits (Gaudet et al., 1996; Thibault et al., 1997; Thulin et al., 2001). In *C. elegans*, two Gβ (*gpb-1* and *gpb-2*) and two Gγ (*gpc-1* and *gpc-2*) subunits have been identified (Jansen et al., 1999). Both GPB-1 and GPC-2 are expressed in embryos where they appear to play essential roles in the proper orientation of the planes of cell division (Zwaal et al., 1996, Jansen et al., 1999; Gotta and Ahringer, 2001). During early embryogenesis, GPB-1 was detected in all cells with staining concentrated at cell membranes and co-localized with asters (Figs. 4A, B; and Zwaal et al., 1996). Later, in adult animals, GPB-1 was found in various tissues, including the germline where staining appeared at the membrane. This distribution pattern was identical to the immunostaining observed with MAU-8/PhLP antibodies (Fig. 3). To determine whether MAU-8/PhLP interacts with Gβγ, we evaluated MAU-8 and GPB-1 interactions in total worm lysates. As shown in Fig. 4E, a complex between MAU-8/PhLP and GPB-1 was

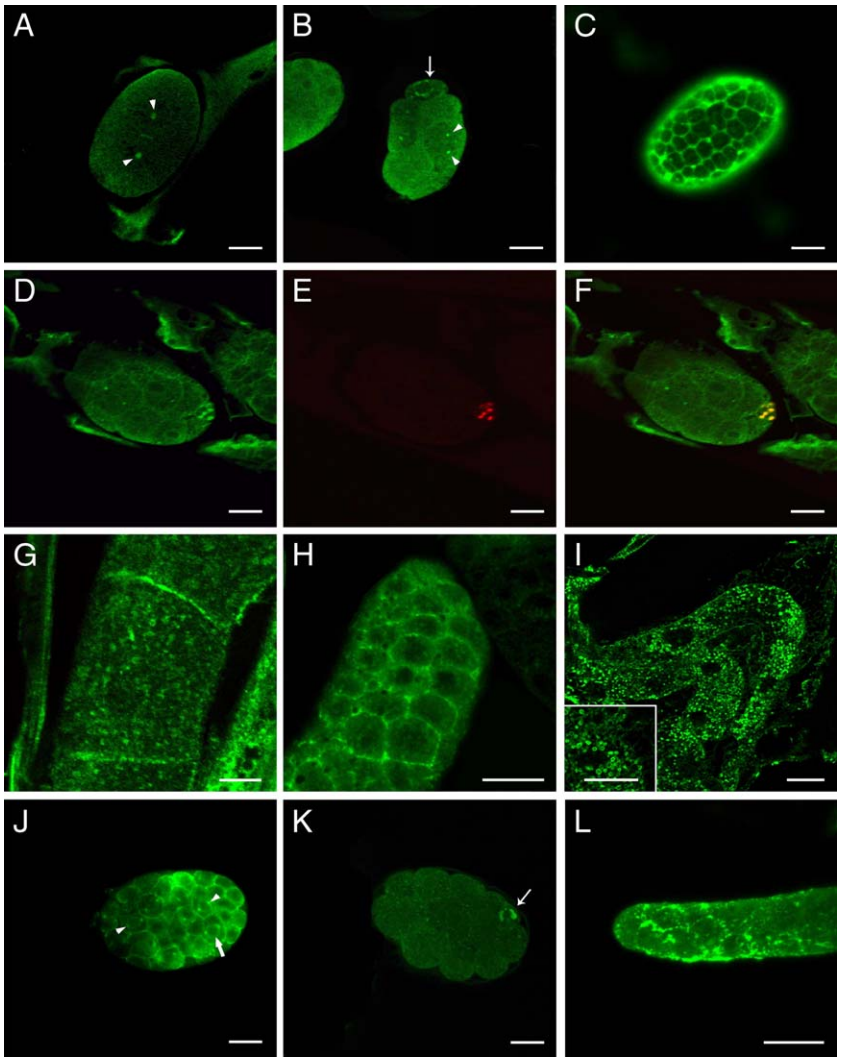

Fig. 3. Expression pattern and subcellular localization of MAU-8/PhLP. (A–I) Immunolocalization of MAU-8/PhLP in wild type embryos and adult hermaphrodites. Embryos show PhLP staining at the centrosomes (arrowheads) and the central spindle region of mitotic cells (star, 1-cell embryo (A), 4-cell embryo (B)). Staining also localizes to the cell membranes (comma-stage embryo) (C). From 2-cell stage onward, PhLP staining concentrates in the germline blastomere (B and D–F). Co-immunolocalization of MAU-8/PhLP (green, D) and P granules (red, E) is shown in an 8-cell embryo (F). In dissected gonads of young hermaphrodites, immunostaining is detected in the cytoplasm and plasma membrane of oocytes (G) and gametes (H). Immunostaining of the intestine appears as small rings resembling endosomes (I). (J–L) Confocal micrographs showing anti-PhLP immunostaining in embryos (J and K) and adult gonad (L) of *mau-8* origin. As in wild type, staining localizes to the cell membrane and to the centrosomes (arrowheads) of dividing cells but the fluorescence of the nuclear membrane appears stronger than in wild type (arrow, late gastrulation (J)). PhLP staining also concentrates in the germline blastomere (arrow, 16-cell embryo (K)). In dissected gonads, heterogeneous staining is observed at the membranes of germline cells (L). Scale bar, 10 μm.

revealed in Western blots after immunoprecipitation of the lysates with anti-PhLP antibodies and blotting with anti-GPB-1 antibodies.

Although the C-terminal domain of PhLP binds Gβγ with low affinity, this interaction is probably important because it occurs at the membrane associating surface of Gβγ (Savage et al., 2000). We thus tried to determine whether the association of Gβγ with the membrane was perturbed in *mau-8* mutant animals. Although the staining of GPB-1 at the cortex of early embryos seemed normal, higher concentrations of GPB-1 were detected in microtubules emanating from the centrosomes of *mau-8* early embryos (Figs. 4C, D). These results are in agreement with the coimmunoprecipitation experiments showing sequestering of GPB-1 by MAU-8/PhLP in *mau-8* mutants (Fig. 4E). These results thus suggest that the mutation in the C-terminal domain of MAU-8/PhLP stabilizes the association of GPB-1 with the microtubules of centrosomes.

MAU-8/PhLP interacts with PAR-5/14.3.3

Pd and PhLP have been recently reported to associate with proteins of the 14.3.3 family (Nakano et al., 2001; Garzon et al., 2002). The 14.3.3 family includes a series of closely related proteins that bind phosphorylated components of signal transduction pathways. One ortholog of 14.3.3 in *C. elegans* is PAR-5, a maternally expressed protein required in the early embryo to establish cellular asymmetry (Morton et al., 2002).

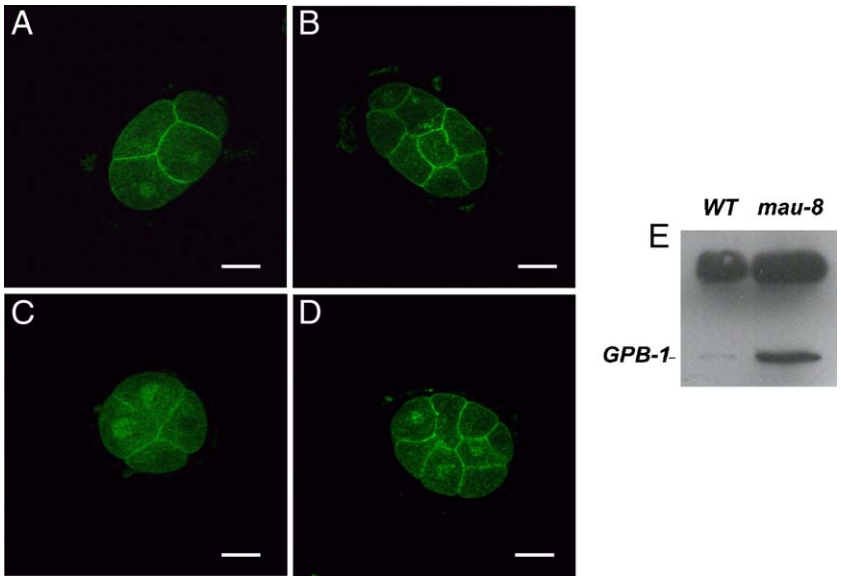

Fig. 4. MAU-8/PhLP interacts with Gβ. (A–D) Immunolocalization of GPB-1 in wild type (A and B) and *mau-8* (C and D) embryos. Staining is observed at the cell membranes and concentrates in microtubules emanating from the centrosomes. Stronger staining accumulates in microtubules of *mau-8* mutants (C and D). Micrographs of a focal plane in 4-cell (A and C) and 8-cell (B and D) embryos. Scale bars, 10 μm. (E) Western blot analysis of mixed developmental stages. Proteins were immunoprecipitated with anti-PhLP antibodies, and the blot was hybridized with anti-GPB-1 antiserum. The MAU-8/PhLP/Gβ complex is more apparent in *mau-8* extracts.

To determine whether MAU-8/PhLP interacts with PAR-5, we searched for co-localization of the two proteins using immunofluorescence analysis in embryos and gonads of wild type and mutant animals. Immunostaining of embryos with PAR-5 antibodies was uniformly localized in the cytoplasm of early embryos (Fig. 5, Morton et al., 2002). Diffuse expression of MAU-8/PhLP was also seen in the cytoplasm with a stronger immunostaining in the cortex of the 1-cell stage (Fig. 5A). Co-localization of MAU-8/PhLP and PAR-5/14.3.3 was observed in the cytoplasm of the zygote (Fig. 5C). In later embryos, both proteins co-localized to the cortex of embryonic cells (Figs. 5D–F). In adult hermaphrodites, PAR-5 staining appeared to be concentrated in the cortex of germ cells (Fig. 5H; Morton et al., 2002). This staining was identical to MAU-8 staining in the gonad (Fig. 5G), and almost complete co-immunostaining was observed, suggesting that MAU-8 co-localized with PAR-5 in embryos and gonads of wild type animals (Fig. 5I).

In *mau-8* mutant worms, immunostaining appeared identical to that observed in wild type animals, suggesting that, in these tissues, the mutation does not disturb the distribution of PAR-5 proteins (data not shown).

In agreement with immunostaining analysis, a complex of the MAU-8/PhLP protein with PAR-5/14.3.3 protein was also shown by immunoprecipitation of embryos and total adult worm lysates with PhLP antiserum followed by Western blot analysis of the precipitated complexes with PAR-5/14.3.3 antibodies (Fig. 5J). This interaction is not disturbed in embryos and gonads of *mau-8* animal mutants, suggesting that the carboxy-terminal domain of MAU-8/PhLP is not involved in PAR-5/14.3.3 binding and/or that MAU-8/PhLP acts after PAR-5.

14.3.3 proteins have been reported to interact with consensus motifs containing a phosphorylated serine. However, since no potential 14.3.3-binding sites of this kind were predicted in MAU-8 (see Fig. 2E), we conclude that MAU-8 interacts with PAR-5 through a non-conventional 14.3.3 interacting protein sequence.

Discussion

mau-8(qm57) encodes a Phosducin-like Protein causing altered G protein signaling

Several genes have been isolated through a maternal-effect viable screen in the nematode. These genes are essential for embryonic development and are required for various aspects of morphogenesis and differentiation or for normal timing of development and life span (Lakowski and Hekimi, 1996). *mau-8* is a maternal-effect mutant isolated on the basis of its abnormal locomotion in an F3 population of mutagenized animals, and a previous study has shown that this phenotype can be maternally rescued (Hekimi et al., 1995). We have shown in this study that *mau-8* is a Phosducin-*like* Protein required for G protein signaling and that the *qm57* mutation leads to the loss of a predicted phosphorylation site in the C-terminal domain of MAU-8/PhLP. PhLP regulate G protein signaling pathways by binding the Gβγ subunit complex. The crystal structure of the Pd/Gβγ complex has been solved, and several biochemical studies support a model in which the N-terminal domain of PhLP binds to Gβγ in a region that also binds to Gα, thus preventing reassociation of G protein subunits to form the heterotrimer Gαβγ. The C-terminal domain of PhLP appears to contact the Gβγ propeller at a site facing the lipid bilayer. This interaction promotes the release of Gβγ from the membrane and blocks interactions between Gβγ and other effectors. In the nematode, the *mau-8(qm57)* mutation may disturb Gβγ

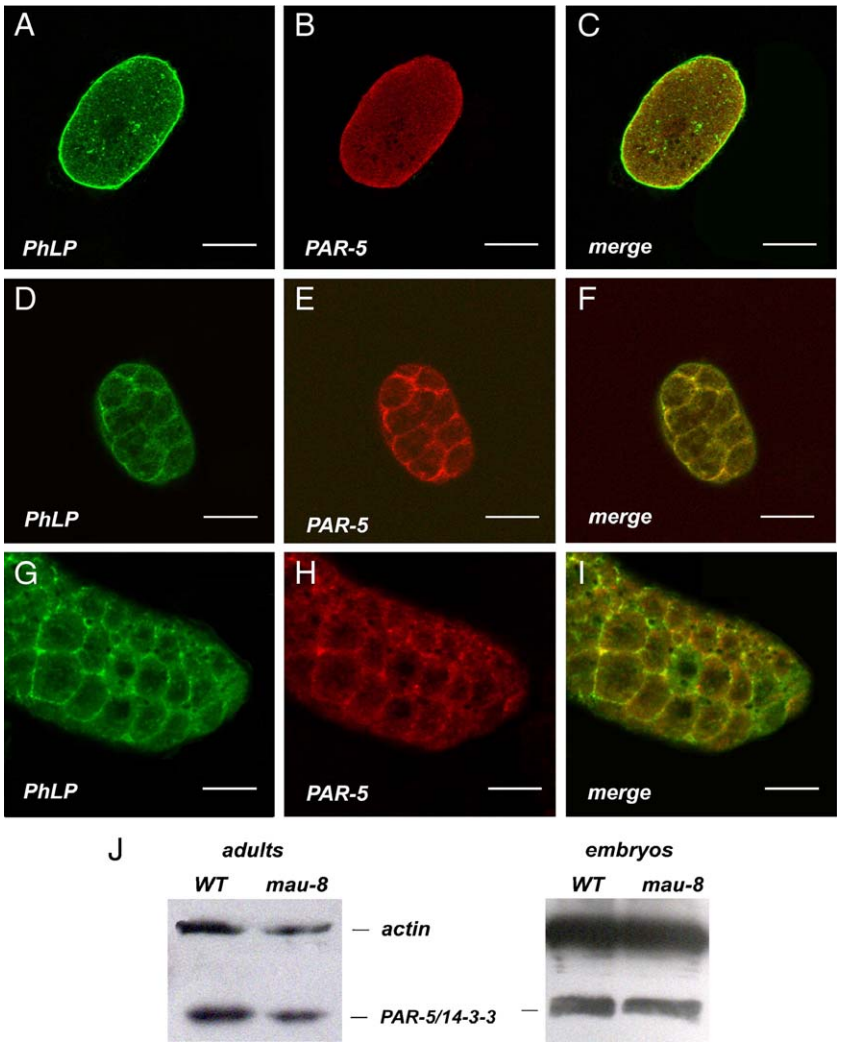

Fig. 5. MAU-8/PhLP interacts with PAR-5/14.3.3. (A–F) Colocalization of MAU-8/PhLP and PAR-5/14.3.3 in one cell embryo (A–C) and in 8–16-cell embryos (D–F). Embryos were stained with anti PhLP antibodies (green) and PAR-5 antibodies (red). The merged images show colocalization (yellow). Scale bar, 10 μm. (G–I) MAU-8/PhLP and PAR-5 immunofluorescence in gonads. Gonads were stained with anti-PhLP antibodies (green) and PAR-5 antibodies (red). The merge image shows co-localization of both proteins in the cortex of germ cells (yellow). Scale bar, 10 μm. (J) Detection of the PAR-5/14.3.3 protein by Western blot analysis in extracts of proteins immunoprecipitated with anti-PhLP antibodies. The PAR-5/14.3.3 protein migrates as a band of approximately 30 kDa. The blot was probed with anti-actin antibodies as control.

anchoring to the membrane. The abnormal accumulation of the Gβ subunit GPB-1 that we observed in asters could result from an abnormal level of folding of Gβ due to altered co-chaperonin activity induced by the *qm57* mutation. Two recent reports describe a novel role for PhLP as an essential chaperone in Gβγ dimer formation (Humrich et al., 2005; Lukov et al., 2005). In addition, the cooperation of molecular chaperones with the proteosome machinery allows the cell to maintain highly efficient quality control of proteins and the *mau-8(qm57)* mutation might protect Gβ from proteosomal degradation. In agreement with this proteosomal model, the binding of Phosducin to Gβγ has been reported to block both ubiquitination of Gβγ and its subsequent degradation in photoreceptor cells (Obin et al., 2002). These results suggest that the primary defect in G-protein signaling in *mau-8* mutants may be due to a non-functional Gβ or Gβγ complex and favor the hypothesis that MAU-8/PhLP acts as a positive Regulator of G protein Signaling. Since Gβ has been shown to act as a limiting component (Zwaal et al., 1996; Tsou et al., 2003), Gβ sequestering by mutant MAU-8/PhLP could prevent reassociation of G protein subunits to form a transduction-competent heterotrimeric Gαβγ complex. These results suggest that the disruption of MAU-8/PhLP produces a phenocopy of the Gβ null mutant.

mau-8 functions in early embryogenesis

mau-8(qm57) is a hypomorphic mutation of the essential PhLP gene. This mutation alters but does not completely eliminate the activity of essential cellular functions because more than 80% of the embryos develop into adults. An important role for PhLP was previously suspected from work in yeast and Dictyostelium showing that the Phlp2 homologue was essential to maintain cell viability (Flanary et al., 2000; Blaauw et al., 2003). We have shown that MAU-8/PhLP is

expressed in early embryos and that the protein localizes to the centrosomes, the central spindle region of mitotic cells and to the perinuclear area. Centrosomes are required to precisely position nuclei in the cytoplasm, and they are closely associated with the nucleus in interphase cells possibly to maintain the proximity of centrosomes to chromosomes upon the onset of mitosis. Several observations in worms and flies have suggested that the centrosome attaches to the nucleus by an interaction between astral microtubules of the centrosome and perinuclear dynein. In early *C. elegans* embryos, both Gα and Gβ subunits are required for the correct positioning of the centrosome (Gotta et al., 2003). We have shown that reducing MAU-8/PhLP alters essential cellular functions and initially induces the sequestering of GPB-1 in asters in mutant embryos. These data suggest that MAU-8/PhLP may regulate centrosome movements during embryogenesis.

MAU-8/PhLP also interacts with PAR-5/14.3.3 in early *C. elegans* embryos. PAR-5/14.3.3 protein plays an essential role in the early events leading to polarization of the zygote (Morton et al., 2002). MAU-8/PhLP protein concentrates at the centrosome of mitotic cells, but it is also found in the cell cortex where it localizes with PAR-5/14.3.3. In view of the possible roles of 14.3.3, these results allow us to speculate that PAR-5/14.3.3 is sequestering MAU-8/PhLP in the cortex preventing interactions with Gβγ and/or its other partners. In mitotic cells, MAU-8/PhLP could cycle from its phosphorylated 14.3.3-binding form to its dephosphorylated Gβγ-binding form. This mechanism would not be disturbed in *mau-8* mutants since PAR-5 localization is not affected in these animals, suggesting that MAU-8/PhLP is acting downstream of PAR-5. Altogether, these observations suggest that, by regulating the function of G proteins in spindle positioning, MAU-8/PhLP provides a link between factors that determine polarity and G protein function in early embryos.

MAU-8 regulates behavior

MAU-8/PhLP is expressed in a variety of tissues, suggesting that it plays multiple functions. Although its binding partners in adult tissues may diverge from those found in embryos, MAU-8/PhLP can interact with Gβ subunits and PAR-5/14.3.3 in a variety of different tissues including the germline cells and the nervous system. In hermaphrodites, reducing *mau-8* activity has broad pleotropic effects: in addition to their sluggish locomotion and altered egg laying activity, *mau-8(qm57)* mutants have defects in the defecation cycle (with frequent absence of the expulsion step), and they are slightly hypersensitive to aldicarb and levamisole (data not shown). In addition, both axon guidance and migration are disturbed in various neuronal cell types (Fig. S6 and Table S1). Altogether, the phenotypic features of *mau-8(qm57)* mutants are very similar to those in mutants showing altered Gα signaling pathways. In *C. elegans*, the Gαq/Gαo signaling network regulates behavior and GPB-2 plays a central role in mediating the opposing activities of Gαo (GOA-1) and Gαq (EGL-30) (Robatzek et al., 2001; Van den Linden et al., 2001). Our in vivo analyses of *mau-8* function have shown that MAU-8/PhLP profoundly affects the Gαq/Gαo signaling network and inhibits Gβ function.

Our results further suggested that MAU-8 might not be limited to the Gαq/Gαo network. Recent data have shown that the Gαs pathway is also interconnected with the Gαq/Gα network (Reynolds et al., 2005; Schade et al., 2005). Since neurodegeneration can be initiated by a constitutively active mutation in the Gαs/GSA-1 protein (Korswagen et al., 1997) and since *mau-8(qm57)* mutants showed signs of neurodegeneration (Fig. S7), some defects of *mau-8* mutants could reflect activated Gαs signaling.

MAU-8/PhLP interacts with PAR-5/14.3.3. In vertebrates, 14.3.3 proteins have been found at the membrane of chromaffin granules where they stimulate calcium-dependent exocytosis (Chamberlain et al., 1995). More recently, 14.3.3 proteins have been shown to associate with KIF1C, a kinesin-like motor protein of the KIF1/UNC-104 subfamily (Dorner et al., 1999). In *C. elegans*, UNC-104 is a kinesin motor that underlies the anterograde axonal transport of synaptic vesicles. Since the dimeric configuration of 14.3.3 allows simultaneous binding to two proteins, 14.3.3 proteins may provide a scaffold on which other proteins interact. These features suggest that 14.3.3 might control the localization of PhLP within the cell by bridging it to a kinesin motor protein. In the nervous system of *C. elegans*, PAR-5/14.3.3 could then regulate the release of MAU-8/PhLP to ultimately modulate neurotransmission.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ydbio.2006.02.039.

References

Ahringer, J., 2003. Control of cell polarity and mitotic spindle positioning in animal cells. Curr. Opin. Cell Biol. 15, 73–81.

Bargmann, C.I., Kaplan, J.M., 1998. Signal transduction in the *Caenorhabditis elegans* nervous system. Annu. Rev. Neurosci. 21, 279–308.

Barhite, S., Thibault, C., Miles, M.F., 1998. Phosducin-like protein (PhLP), a regulator of G beta gamma function, interacts with the proteasomal protein SUG1. Biochim. Biophys. Acta 1402, 95–101.

Blaauw, M., Knol, J.C., Kortholt, A., Roelofs, J., Engel, R., Postma, M., Visser, A.J., van Haastert, P.J., 2003. Phosducin-like proteins in *Dictyostelium discoideum*: implications for the phosducin family of proteins. EMBO J. 22, 5047–5057.

Brundage, L.A., Avery, L., Katz, A., Kim, U.J., Mendel, J.E., Sternberg, P.W., Simon, M.I., 1996. Mutations in a *C. elegans* Gqalpha gene disrupt movement, egg laying, and viability. Neuron 16, 999–1009.

Burbea, M., Dreier, L., Dittman, J.S., Grunwald, M.E., Kaplan, J.M., 2002. Ubiquitin and AP180 regulate the abundance of GLR-1 glutamate receptors at postsynaptic elements in *C. elegans*. Neuron 35, 107–120.

Chamberlain, L.H., Roth, D., Morgan, A., Burgoyne, R.D., 1995. Distinct effects of alpha-SNAP, 14-3-3 proteins, and calmodulin on priming and triggering of regulated exocytosis. J. Cell Biol. 130, 1063–1070.

Dorner, C., Ullrich, A., Haring, H.U., Lammers, R., 1999. The kinesin-like motor protein KIF1C occurs in intact cells as a dimer and associates with proteins of the 14-3-3 family. J. Biol. Chem. 274, 33654–33660.

Flanary, P.L., DiBello, P.R., Estrada, P., Dohlman, H.G., 2000. Functional analysis of Plp1 and Plp2, two homologues of phosducin in yeast. J. Biol. Chem. 275, 18462.

Garzon, J., Rodriguez-Diaz, M., Lopez-Fando, A., Garcia-Espana, A., Sanchez-Blasquez, P., 2002. Glycosylated phosducin-like protein long regulates opioid receptor function in mouse brain. Neuropharmacology 42, 813–828.

Gaudet, R., Bohm, A., Sigler, P.B., 1996. Crystal structure at 2.4 a resolution of the complex of transducin βγ and its regulator, phosducin. Cell 87, 577–588.

Gotta, M., Ahringer, J., 2001. Distinct roles for G alpha and G beta gamma in regulating spindle position and orientation in *Caenorhabditis elegans* embryos. Nat. Cell Biol. 3, 297–300.

Gotta, M., Dong, Y., Peterson, Y.K., Lanier, S., Ahringer, J., 2003. Asymmetric distributed *C. elegans* homologs of AGS3/PINS control spindle position in the early embryo. Curr. Biol. 13, 1029–1037.

Hajdu-Cronin, Y.M., Chen, W.J., Patikoglou, G., Koelle, M.R., Sternberg, P.W., 1999. Antagonism between G(o)alpha and G(q)alpha in *Caenorhabditis elegans*: the RGS protein EAT-16 is necessary for G(o)alpha signaling and regulates G(q)alpha activity. Genes Dev. 13, 1780–1793.

Hekimi, S., Boutis, P., Lakowski, B., 1995. Viable maternal-effect mutations that affect the development of the nematode. Genetics 141, 1351–1364.

Humrich, J., Bermel, C., Grubel, T., Quitterer, U., Lohse, M.J., 2003. Regulation of phosducin-like protein by casein kinase 2 and N-terminal splicing. J. Biol. Chem. 278, 4474–4481.

Humrich, J., Bermel, C., Bunemann, M., Harmark, L., Frost, R., Quitterer, U., Lohse, M.J., 2005. Phosducin-like protein regulates G-protein betagamma folding by interaction with tailless complex polypeptide-1alpha: dephosphorylation or splicing of PhLP turns the switch toward regulation of Gbetagamma folding. J. Biol. Chem. 280, 20042–20050.

Jansen, G., Thijssen, K.L., Werner, P., van der Horst, M., Hazendonk, E., Plasterk, R.H.A., 1999. The complete family of genes encoding G proteins of *Caenorhabditis elegans*. Nat. Genet. 21, 414–419.

Korswagen, H., Park, J.-H., Ohshima, Y., Plasterk, R., 1997. An activating mutation in a *C. elegans* Gs protein induces neural degeneration. Genes Dev. 11, 1493–1503.

Labbé, J.-C., Maddox, P.S., Salmon, E.D., Goldstein, B., 2003. PAR proteins regulate microtubule dynamics at the cell cortex in *C. elegans*. Curr. Biol. 13, 707–714.

Lackner, M.R., Nurrish, S.J., Kaplan, J.M., 1999. Facilitation of synaptic transmission by EGL-30 G(q)alpha and EGL-8 PLC beta: DAG binding to UNC-13 is required to stimulate acetylcholine release. Neuron 24, 335–346.

Lakowski, B., Hekimi, S., 1996. Determination of life span in *Caenorhabditis elegans* by four clock genes. Science 272, 1010–1013.

Lopez, P., Yaman, R., Lopez-Fernandez, L.A., Vidal, F., Puel, D., Clertant, P., Cuzin, F., Rassoulzadegan, M., 2003. A novel germ line-specific gene of the phosducin-like protein (PhLP) family. J. Biol. Chem. 278, 1751–1757.

Lukov, G.L., Hu, T., McLaughlin, J.N., Hamm, H.E., Willardson, B.M., 2005. Phosducin-like protein acts as a molecular chaperone for G protein betagamma dimer assembly. EMBO J. 24, 1965–1975.

Martin Benito, J., Bertrand, S., Hu, T., Ludtke, P.J., McLaughlin, J.N., Willardson, B.M., Carrascosa, J.L., Valpuesta, J.M., 2004. Structure of the complex between the cytosolic chaperonin CCT and phosducin-like protein. Proc. Natl. Acad. Sci U. S. A. 101, 17410–17415.

Miller, K.G., Emerson, M.D., Rand, J.B., 1999. Goα and diacylglycerol kinase negatively regulate the Gqα pathway in *C. elegans*. Neuron 24, 323–333.

Miller, K.G., Emerson, M.D., McManus, J.R., Rand, J.B., 2000. RIC-8 (synembrin): a novel conserved protein that is required for Gqα signaling in the *C. elegans* nervous system. Neuron 27, 289–299.

Morton, D.G., Shakes, D.C., Nugent, S., Dichoso, D., Wang, W., Golden, A., Kemphues, K.J., 2002. The *Caenorhabditis elegans* par-5 gene encodes a 14.3.3 protein required for cellular asymmetry in the early embryo. Dev. Biol. 241, 47–58.

Nakano, K., Chen, J., Tarr, G.E., Yoshida, T., Flynn, J.M., Bitenski, M.W., 2001. Rethinking the role of phosducin: light-regulated binding of phosducin to 14.3.3 in rod inner segments. Proc. Natl. Acad. Sci. U. S. A. 98, 4893–4898.

Nurrish, S., Segalat, L., Kaplan, J.M., 1999. Serotonin inhibition of synaptic transmission; Goα decreases the abundance of UNC-13 at release sites. Neuron 24, 231–242.

Obin, M., Lee, B.Y., Meinke, G., Bohm, A., Lee, R.H., Gaudet, R., Hopp, J.A., Arshavsky, V.Y., Willardson, B.M., Taylor, A., 2002. Ubiquitylation of transducin βγ subunit complex. J. Biol. Chem. 277, 44566–44575.

Reynolds, N.K., Shade, M.A., Miller, K.G., 2005. Convergent, RIC-8-dependent G alpha signaling pathways in the *C. elegans* synaptic signaling network. Genetics 169, 651–670.

Robatzek, M., Thomas, J.H., 2000. Calcium/calmodulin-dependent protein kinase II regulates *Caenorhabditis elegans* locomotion in concert with a Go/Gq signaling network. Genetics 156, 1069–1082.

Robatzek, M., Niacaris, T., Steger, K., Avery, L., Thomas, J.H., 2001. *eat-11* encodes GPB-2, a G beta(5) ortholog that interacts with G(o)alpha and G(q) alpha to regulate *C. elegans* behavior. Curr. Biol. 11, 288–293.

Rose, L.S, Kemphues, K., 1998. The *let-99* gene is required for proper spindle orientation during cleavage of the *C. elegans* embryo. Development 125, 1337–1346.

Savage, J.R., McLaughlin, J.N., Skiba, N.P., Hamm, H.E., Willardson, B.M., 2000. Functional roles of the two domains of phosducin and phosducin-like protein. J. Biol. Chem. 275, 30399–30407.

Schade, M.A., Reynolds, N.K., Dollins, C.M., Miller, K.G., 2005. Mutations that rescue the paralysis of *C. elegans* ric-8 (synembryn) mutants activate the Gs pathway and define a major branch of the synaptic signaling network. Genetics 169, 631–649.

Simmer, F., Tijsterman, M., Parrish, S., Koushika, S.P., Nonet, M.L., Fire, A., Ahringer, J., Plasterk, R.H.A., 2002. Genome-wide RNAi of *C. elegans* using the hypersensitive *rrf-3* strain reveals novel gene functions. Curr. Biol. 12, 1317–1319.

Thibault, C., Sgranga, M.W., Miles, M.F., 1997. Interaction of phosducin-like protein with G protein betagamma subunits. J. Biol. Chem. 272, 12253–12256.

Thulin, C.D., Savage, J.R., McLaughlin, J.N., Truscott, S.M., Old, W.M., Ahn, N.G., Resing, K.A., Hamm, H.E., Bitenski, M.W., Willardson, B.M., 2001. Modulation of the G protein regulator phosducin by Ca^{2+}/calmodulin-dependent protein kinase II phosphorylation and 14.3.3 protein binding. J. Biol. Chem. 276, 23805–23815.

Tsou, M.-F.B., Hayashi, A., Rose, L.S., 2003. LET-99 opposes Gα/GPR signaling to generate asymmetry for spindle positioning in response to PAR and MES-1/SRC-1 signaling. Development 130, 5717–5730.

Van den Linden, A.M., Simmer, F., Cuppen, E., Plasterk, R.H.A., 2001. The G-protein B-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. Genetics 158, 221–235.

Wicks, S.R., Yeh, R.T., Gish, W.R., Waterston, R.H., Plasterk, R.H., 2001. Rapid gene mapping in *Caenorhabditis elegans* using a high density polymorphism map. Nat. Genet. 2, 160–164.

Yoshida, T., Willardson, B.M., Wilkins, J.F., Jensen, G.J., Thornton, B.D., Bitensky, M.W., 1994. The phosphorylation state of phosducin determines its ability to block transducin subunit interactions and inhibit transducin binding to activated rhodopsin. J. Biol. Chem. 269, 24050–24057.

Zwaal, R.R., Ahringer, J., van Luenen, H.G.A.M., Rushforth, A., Anderson, P., Plasterk, R.H.A., 1996. G proteins are required for spatial orientation of early cell cleavages in *C. elegans* embryos. Cell 86, 619–629.