Participation of the Protein G_o in Multiple Aspects of Behavior in *C. elegans*

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The *goa-1* gene encoding the alpha subunit of the heterotrimeric guanosine triphosphate–binding protein (G protein) G_o from *Caenorhabditis elegans* is expressed in most neurons, and in the muscles involved in egg laying and male mating. Reduction-of-function mutations in *goa-1* caused a variety of behavioral defects including hyperactive movement, premature egg laying, and male impotence. Expression of the activated G_o alpha subunit ($G\alpha_o$) in transgenic nematodes resulted in lethargic movement, delayed egg laying, and reduced mating efficiency. Induced expression of activated $G\alpha_o$ in adults was sufficient to cause these phenotypes, indicating that $G\alpha_o$ mediates behavior through its role in neuronal function and the functioning of specialized muscles.

The heterotrimeric G protein G_o is abundantly expressed in mammalian and insect neurons and growth cones, suggesting that it is important for both neuronal development and function (*1*). Although G_o has been shown to associate with a variety of receptors and to modulate several cellular effectors (*2*), the full spectrum of roles that G_o plays in vivo has not been elucidated.

We previously described complementary DNA (cDNA) and genomic clones encoding the G_o alpha subunit ($G\alpha_o$) from the nematode *Caenorhabditis elegans* (*3*). To determine the expression pattern of $G\alpha_o$, we used 5 kb of DNA containing the upstream presumptive control region of *goa-1* (the gene encoding $G\alpha_o$) to direct the expression of both β-galactosidase (*lacZ*) and green fluorescent protein (GFP) (*4*). Transgenic *C. elegans* bearing *goa-1–lacZ* fusions showed β-galactosidase activity throughout the nervous system at all larval stages and in adults (Fig. 1, A and C) (*5–7*). Virtually every neuron showed *goa-1* expression, including the hermaphrodite-specific and male-specific neurons (Fig. 1, B and C). Some non-neuronal cell types also expressed $G\alpha_o$, including the vulva and uterine muscles in the hermaphrodite (Fig. 1B) and the diagonal muscles in the male (Fig. 1D). Most cells of the pharynx, the distal-tip cells in the adult hermaphrodite, and the intestinal muscle also stained positive for β-galactosidase activity. The GFP construct confirmed this pattern of expression (*8*). In *C. elegans*, as in insects and mammals, $G\alpha_o$ is expressed predominantly in neurons and a few other tissue types.

We used two reverse genetic approaches to determine the roles of $G\alpha_o$. The allele *goa-1(pk62)* was isolated from a Tc1 transposon insertion mutant bank (*9*) and contains an insertion in codon 102 that likely reduces $G\alpha_o$ function. We isolated an additional reduction-of-function allele of *goa-1*, *sy192*, in a screen for mutants defective in male copulatory behavior. The *sy192* allele maps to the genetic interval containing *goa-1* and does not complement *goa-1(pk62)*. Both *goa-1(pk62)* and *goa-1(sy192)* caused similar phenotypes: Homozygotes were hyperactive for movement, hermaphrodites laid eggs that were at an abnormally early stage of development, and males were partially impotent (Table 1 and Fig. 2, C and D) (*10*). Both *pk62* and *sy192* also reduced fertility (Table 1).

We constructed a constitutively active mutation in *goa-1* analogous to the Q227L and Q205L mutants of mammalian $G\alpha_s$ and $G\alpha_i$, respectively [where Q (Gln) is replaced with L (Leu) at the indicated position]. These substitutions occur in the conserved sequence Asp-Val-Gly-Gly-Gln-Arg, part of the guanine nucleotide–binding pocket; activation results from decreased guanosine triphosphatase activity which locks the protein in the GTP-bound conformation (*11*). We substituted a leucine codon for that of glutamine at position 205 in a *C. elegans* $G\alpha_o$ genomic clone (pJMGo) (*12*) and established a *C. elegans* strain bearing multiple copies of this mutant clone, pJMGoQL, as an integrated transgene, *syIs9* (*5*). The *syIs9* homozygotes were lethargic in movement, hermaphrodites retained eggs, and males had reduced mating efficiency (Table 1). These phenotypes were all progressive: young adults, within 24 hours of the last larval (L4) molt, moved more slowly than did control ani-

Table 1. Mutations in *goa-1* cause multiple phenotypes. Adult hermaphrodites were scored for movement (*10*), developmental stage of freshly laid eggs (*10*), the rate of egg laying, and total brood size. Males were scored for their ability to sire progeny (*10*) and to perform the several steps required for successful mating (*18*). Mean values ± standard deviations are reported. ND, not determined.

Phenotype	Genotype			
	Wild type	*goa-1(pk62)*	*goa-1(sy192)*	*syIs9*
Movement				
Uncoordinated*	0%	100%	100%	100%
(*n* = no. of animals)	(*n* = 25)	(*n* = 50)	(*n* = 50)	(*n* = 50)
Wavelength (mm)†	0.51 ± 0.03	0.43 ± 0.04	0.43 ± 0.04	0.55 ± 0.05
Amplitude (mm)†	0.13 ± 0.02	0.17 ± 0.03	0.14 ± 0.03	0.08 ± 0.02
(*n* = no. of waves)	(*n* = 100)	(*n* = 100)	(*n* = 100)	(*n* = 100)
Egg laying				
Stage (cells/egg)	15 ± 7	6 ± 5	4 ± 3	Increases to
(*n* = no. of eggs)	(*n* = 29)	(*n* = 41)	(*n* = 81)	550 with age
Rate (eggs/hour)‡	8 ± 2	3 ± 1	3 ± 1	Decreases to
(*n* = no. of animals)	(*n* = 20)	(*n* = 23)	(*n* = 22)	0 with age
Brood size	282 ± 31	82 ± 34	206 ± 36	20 ± 14
(*n* = no. of animals)	(*n* = 25)	(*n* = 36)	(*n* = 34)	(*n* = 32)
Male mating				
Potent males	100%	14%	67%	14%
(*n* = no. of males)	(*n* = 30)	(*n* = 30)	(*n* = 30)	(*n* = 30)
Normal turns§	100%	0%	0%	ND
(*n* = no. of attempts)	(*n* = 100)	(*n* = 130)	(*n* = 100)	
Spicule insertion‖	81%	0%	3%	ND
(*n* = no. of attempts)	(*n* = 100)	(*n* = 80)	(*n* = 76)	

*The *goa-1(pk62)* and *goa-1(sy192)* animals are hyperactive, and the *syIs9* animals are lethargic. †Ten individuals of each genotype were placed on bacterial lawns, and the dimensions of their tracks were measured with an ocular micrometer. Measurements were made from the central groove of each track in sections with relatively straight vectors. The measurements underestimate the differences in wave parameters: *syIs9* wavelengths increased with age, but the tracks became too irregular to measure (animals within 30 hours of the L4 molt were used). ‡Eggs laid by individual hermaphrodites over 3 hours were counted. §Ten N2, 13 *pk62*, and 10 *sy192* males were observed. ‖Ten N2, 18 *pk62*, and 14 *sy192* males were observed.

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mals (Fig. 2, A and B); older animals moved little except when prodded. Within 48 hours of the L4 molt, animals became bloated with late-stage eggs, and by 72 hours the retained embryos hatched internally, indicative of a severe egg-laying defect (Table 1) (*13–15*). The *syIs9* hermaphrodites were also semisterile (Table 1).

The movement phenotype seen in the reduction-of-function alleles appears to be opposite to that caused by the gain-of-function transgene. *Caenorhabditis elegans* moves by propagating waves of alternating dorsal and ventral flexions along its body length, which produces regular sinusoidal tracks on a bacterial lawn. Both *pk62* and *sy192* homozygotes flexed more rapidly and made deeper flexions than did the wild type, whereas young *syIs9* adults flexed slower and made shallower flexions than the wild type (Fig. 2, A to D). The alteration in flexion resulted in abnormal sinusoidal motion: The wavelength of tracks left by *pk62* and *sy192* mutants was reduced, whereas *syIs9* left tracks with reduced amplitude relative to those left by the wild type (Table 1). Therefore, $G\alpha_o$ is involved in the volume and pitch as well as the tempo of C. *elegans* movement. $G\alpha_o$ might control wave propagation by acting either in the oscillating circuit among the motor neurons or in the interneurons that control movement (*16*).

The effects of reduced and enhanced $G\alpha_o$ activity on egg laying are not necessarily opposite. Although *pk62* and *sy192* hermaphrodites laid early eggs and *syIs9* hermaphrodites laid late eggs, hermaphrodites of all three genotypes laid eggs more slowly than did the wild type (Table 1). The reduced rate of egg laying in *pk62* and *sy192* might result from slow egg production: Wild-type hermaphrodites had 11.6 ± 2.2 eggs in the uterus (n = 20 hermaphrodites), whereas *pk62* had 4.1 ± 1.6 (n = 36) and *sy192* had 3.2 ± 1.0 (n = 63) (scored at 24, 36, and 48 hours after the L4 molt). The role of $G\alpha_o$ in egg laying is likely complex because *goa-1* is expressed in both the egg-laying muscles and the serotonergic HSN neurons that innervate them (Fig. 1B) (*17*). Analysis of *syIs9* demonstrates that *goa-1* affects egg laying both pre- and postsynaptically. Egg laying by wild-type C. *elegans* is stimulated by serotonin (5-HT) or imipramine (Table 2). Animals with nonfunctional HSNs lay eggs in response to exogenous 5-HT but are resistant to imipramine, a potentiator of endogenous 5-HT. Animals with defects in the egg-laying muscles are unable to respond to either agent (*13*). Young *syIs9* adults responded partially to 5-HT, but they were completely resistant to imipramine (Table 2). Of the *syIs9* hermaphrodites, 64% were resistant to both agents, demonstrating that $G\alpha_o$ is involved postsynaptically in egg laying. Because 36% of *syIs9* homozygotes had at least partially functional egg-laying muscles (they responded to 5-HT), the complete resistance to imipramine indicates an additional, fully penetrant defect in the function of the HSNs.

Both classes of *goa-1* mutations caused male impotence (Table 1). A C. *elegans* male responds to contact with a hermaphrodite by placing the ventral surface of his tail against her, then moving backward along her body. When he approaches either her head or her tail, he executes a turn keeping his tail in contact with his mate. When he locates the vulva, he inserts his spicules and transfers sperm. The *pk62*, *sy192*, and *syIs9* males responded to contact with hermaphrodites and moved backward. Instead of turning at 10 or 90% of the hermaphrodite's body length as do wild-type males (*18*), *pk62* and *sy192* males either failed to turn or turned at the end (0 or 100% body length) of the hermaphrodite (Table 1). The *syIs9* males moved so slowly that we have not yet analyzed specific defects in mating behavior. The *goa-1* gene is expressed in the diagonal muscles and the motor neurons that innervate them (Fig. 1, B and D), ablation of which results in turns similar to those made by *pk62* and *sy192* males (*19*). The *pk62* and *sy192* males were also defective in spicule insertion (Table 1),

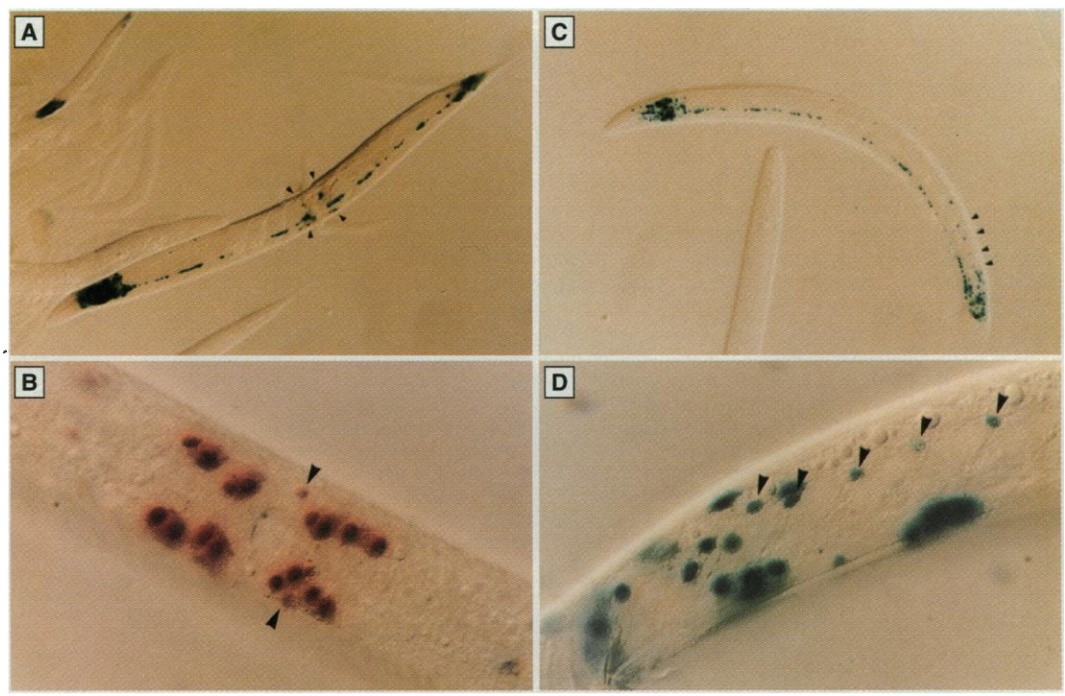

Fig. 1. Expression of G_o in *C. elegans*. Transgenic animals were stained for β-galactosidase activity to visualize expression of *goa-1–lacZ* reporter constructs (*4–7*). (**A**) Oblique left lateral view of an adult hermaphrodite showing staining in pharynx, circumpharyngeal nerve ring, ventral nerve cord, and posterior nervous system. Arrowheads indicate the position of egg-laying muscles. An L2 larva with similar expression is at the upper left. The transgene was derived from reporter construct pJMOSS.11 (*4*). Anterior of both animals is to the left, ventral is down. X-Gal was used for staining. (**B**) Ventral view of an L4 hermaphrodite showing expression in the HSN neurons (arrowheads) and vulva and uterine muscles, two nuclei each per quadrant. The vulval cavity can be seen in the center of all staining nuclei, and anterior is to the left. The transgene was derived from reporter construct pJMONN.28 (*4*), and Salmon-Gal was used for staining. (**C**) Left oblique lateral view of an adult male. Nuclei of some of the diagonal muscles are indicated by arrowheads. Note additional staining nuclei in the tail compared with the hermaphrodite. The transgene was derived from reporter construct pJMOSS.11 (*4*), and X-Gal was used for staining. (**D**) Right lateral view of an adult male. Diagonal muscle nuclei are indicated by arrowheads. The transgene was derived from reporter construct pJMONN.28 (*4*), and X-Gal was used for staining. Panels (A) and (C), magnification ~×40. Panels (B) and (D), magnification ~×190. All panels were photographed on Ektar 25 film (Kodak) with Nomarski optics.

which requires spicule muscles and sensory and motor neurons (*18*). Thus, $G\alpha_o$ is necessary for several steps in mating behavior, consistent with its expression in many male specific cells.

To determine whether alteration of $G\alpha_o$ activity affects the adult, we placed the $G\alpha_o$ gene with the Q205L substitution under the control of the C. *elegans* heat-shock promoter hsp16-2, producing the plasmid pJMGoQLH (*20*). The hsp16-2 promoter directs expression in many tissues including neurons. Animals bearing pJMGoQLH as an integrated transgenic array, *syIs17*, are wild type for fertility, movement, and egg laying in the absence of heat treatment (Fig. 2E). When heat-treated as adults, *syIs17* animals rapidly became sterile, lethargic, and egg-laying defective (*20*). One hour after a 30-min heat pulse, 100% of *syIs17* hermaphrodites (n = 28) were sluggish and had reduced flexions (wavelength = 0.48 ± 0.04 mm, amplitude = 0.07 ± 0.02 mm, n = 50 waves from nine hermaphrodites; see Table 1). Egg laying stopped within 1 hour of heat treatment, and developing embryos hatched internally. The brood size of heat-treated *syIs17* hermaphrodites was small (6.6 ± 4.5, n = 28). The uncoordinated phenotype induced in *syIs17* animals was progressive as it was in aging *syIs9* homozygotes, and within 3 hours of heat treatment, *syIs17* hermaphrodites resembled *syIs9* hermaphrodites (Fig. 2, B and F). Thus, induction of activated $G\alpha_o$ even in adults is sufficient to generate the observed phenotypes.

Alteration of $G\alpha_o$ activity resulted in a number of aberrant behaviors in C. *elegans*, presumably through its action in neurons and, for egg laying and perhaps male mating, in specific muscles. The phenotypes caused by activated $G\alpha_o$ could all be induced after adulthood, indicating that G_o is involved in the continued functioning of neurons and the other cells in which $G\alpha_o$ is expressed. Both reduction-of-function and gain-of-function mutations affected the same behaviors, and in the case of movement the mutations had opposite effects, suggesting that wild-type G_o participates in these behaviors. The multiple phenotypes caused by mutations of *goa-1* can now be used in extragenic suppressor screens to identify additional components of G_o-mediated signal transduction.

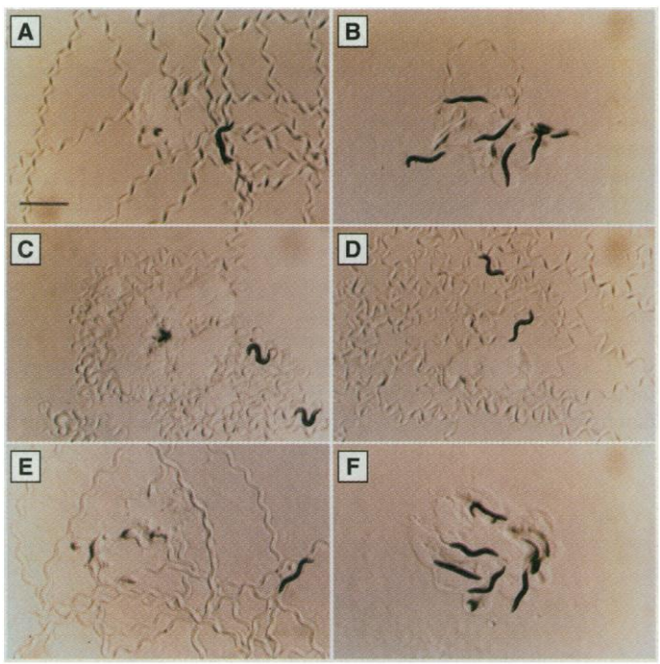

Fig. 2. Abnormal movement resulting from mutations in *goa-1*. Five adult hermaphrodites were placed on a bacterial lawn, and after 5 min the point of origin was photographed. Nematode tracks in the slurry of *Escherichia coli* can be seen. (**A**) Wild-type N2 hermaphrodites (*22*); bar = 1 mm, for all panels. (**B**) Hermaphrodites homozygous for *syIs9*, an integrated transgene derived from activated $G\alpha_o$ plasmid, pJMGoQL (*12*). All five animals are close to the point of origin, and their tracks have decreased amplitude. (**C**) Hermaphrodites homozygous for *goa-1(pk62)*. The number of tracks in the field is increased, and the tracks have decreased wavelength and increased amplitude. (**D**) Hermaphrodites homozygous for *goa-1(sy192)*. Tracks are increased in number and have decreased wavelength. (**E**) Hermaphrodites homozygous for *syIs17*, an integrated transgene derived from pJMGoQLH (*20*) before heat treatment. Sinusoidal tracks are identical to those of the wild type shown in (A). (**F**) Behavior of the same five pJMGoQLH hermaphrodites shown in (E) 3 hours after heat treatment (*20*). All five animals are close to the point of origin as in (B). All panels were photographed on Ilford XP2 ASA 400 film with a Wild M5A stereomicroscope.

Table 2. Activated $G\alpha_o$ results in pre- and postsynaptic defects in egg laying. Young adult hermaphrodites were tested for egg laying in response to 5-HT and imipramine. M9 buffer was used for the control. The number of eggs laid by the worms are indicated by the following symbols: +, >7 eggs; (+), 4 to 7 eggs; (−), 1 to 3 eggs; and −, 0 eggs, n = 25. N2 is the Bristol strain of wild-type *C. elegans* (*10*). The *syIs10* strain, used as a control for genetic background, is a *C. elegans* strain bearing an integrated transgene derived from pJMONN.28 (*4, 5*); *syIs9* is a *C. elegans* strain bearing pJMGoQL (*12*) as an integrated transgene (*5*). Assays were performed and interpreted as described (*13*), except eggs were counted after 90 min in all cases. Imipramine and 5-HT were purchased from Sigma and dissolved in M9 buffer (*22*).

Strain	Number of worms laying the indicated number of eggs after treatment with											
	M9 buffer				5-HT				Imipramine			
	+	(+)	(−)	−	+	(+)	(−)	−	+	(+)	(−)	−
N2	0	0	5	21	21	3	1	0	25	0	0	0
syIs10	0	0	2	23	14	6	5	0	23	1	0	1
syIs9	0	0	1	24	1	4	4	16	0	0	0	25

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3. M. A. Lochrie, J. E. Mendel, P. W. Sternberg, M. I. Simon, *Cell Regul.* **2**, 135 (1991).
4. DNA fragments from a *goa-1*–containing cosmid PS#21D12 (*3*), extending 5′ from and including the *goa-1* translational start, were cloned into the *lacZ* expression vectors pPD21.28 and pPD22.11 (*21*). Three clones having 3.8 kb (Nco-Nco, pJMONN.28), 5.0 kb (Bam-Sph, pJMOBS.11), and 5.5 kb (Sph-Sph, pJMOSS.11) of upstream DNA directed identical patterns of β-galactosidase expression in transgenic *C. elegans* (*5*), with longer clones showing stronger expression in ventral cord neurons. To generate a *goa-1*– GFP fusion, a 6.8-kb Bam fragment of PS#21D12 in pBS+ (Stratagene) was used as a substrate for mutagenesis in vitro (Muta-Gene phagemid kit, Bio-Rad). A Bam site at the translational start site in *goa-1* (*3*) was created with the primer: 5′-CATG-GTACAACGGATCCCACCTAGAGTT-3′. The resulting Bam fragment, containing 5 kb of 5′ *goa-1* DNA, was inserted into the GFP expression vector Tu61 [M. Chalfie, Y. Tu, G. Euskirchen, W. W. Ward, D. C. Prasher, *Science* **263**, 802 (1994)], producing the plasmid pJMOB61. Reporter gene constructs were injected at 60 μg/ml.
5. Transgenic *C. elegans* were generated by injection of DNA into the gonad arms of adult hermaphrodites [C. C. Mello, J. M. Kramer, D. Stinchcomb, V. Ambros, *EMBO J.* **10**, 3959 (1991)]. Test DNA was injected into *dpy-20(e1362)* along with pMH86, a plasmid containing wild-type *dpy-20* DNA. In all cases, similar phenotypes were observed for multiple stable transgenic lines. Transgenic arrays were chromosomally integrated as described [J. C.

Way, L. Wang, J.-Q. Run, A. Wang, *Genes Dev.* **5**, 2199 (1991)], except that animals were treated with 38 Gy (1 Gy = 100 rads) from an x-ray source. Transgenic strains were outcrossed to *dpy-20(e1282); him-5(e1490),* and the transgene was reisolated in a *dpy-20(e1362)* background with or without *him-5* [J. Hodgkin, H. R. Horvitz, S. Brenner, *Genetics* **91**, 67 (1979)]. Chromosomal integration of transgenic arrays stabilized but did not alter the observed phenotypes.

6. Nematodes were fixed and stained with X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) or Salmon-Gal (6-chloro-3-indolyl-β-D-galactopyranoside) (Biosynth) (*21*). Animals were examined with Nomarski optics.
7. Cell identifications were made by the size, shape, and position of cell bodies or their nuclei [J. E. Sulston and H. R. Horvitz, *Dev. Biol.* **56**, 110 (1977); J. E. Sulston, D. G. Albertson, J. N. Thomson, *ibid.* **78**, 542 (1980); J. G. White, E. Southgate, J. N. Thomson, S. Brenner, *Philos. Trans. R. Soc. London B. Biol. Sci.* **314**, 1 (1986)].
8. Transgenic *C. elegans* bearing a *goa-1*–GFP fusion, pJMOB61 (*4*), were examined, and cells were scored for green fluorescence.
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10. Movement, the stage of egg laying, and male mating efficiency were tested in +/+, *pk62/+, sy192/+, pk62, sy192,* and *pk62/sy192* animals. Movement was scored by direct observation of hermaphrodites and their tracks in a bacterial lawn. Eggs were harvested at 10-min intervals and examined with Nomarski optics. The percentage of potent males, relative to wild type, was determined by placing individual L4 males on culture dishes with six *unc-52(e444)* (*22*) L4 hermaphrodites. A male was scored as potent if cross progeny were observed after 5 days. All males were *him-5(e1490)*. *pk62* is semidominant for impotence (87% of *pk62/+* were potent, $n = 30$, Table 1), whereas *sy192* is semidominant for movement (26% of *sy192/+* were hyperactive, $n = 23$) and dominant for premature egg laying (4.0 ± 3.6 cells/egg, $n = 33$ eggs, Table 1). Hyperactivity and premature egg laying were enhanced in trans: 100% of *pk62/sy192* were hyperactive ($n = 30$) and freshly laid eggs had 1.8 ± 0.9 cells ($n = 24$). Of the *pk62/sy192* males 64% were potent, similar to *sy192* (Table 1). The plasmid pJMGo (*12*) rescues the phenotypes of *pk62* and *sy192*.
11. S. B. Masters *et al.*, *J. Biol. Chem.* **264**, 15467 (1989); M. P. Graziano and A. G. Gilman, *ibid.*, p. 15475; Y. H. Wong *et al.*, *Nature* **351**, 63 (1991). Mammalian $G\alpha_o$ containing a substitution at the equivalent glutamine (Q205L) is protected by GTP from tryptic digestion as are constitutively activated $G\alpha_s$ and $G\alpha_i$, suggesting that this substitution also results in constitutively activated $G\alpha_o$ [V. Z. Slepak, T. M. Wilkie, M. I. Simon, *J. Biol. Chem.* **268**, 1414 (1993); S. M. Strittmatter, M. C. Fishman, X.-P. Zhu, *J. Neurosci.* **14**, 2327 (1994)].
12. The plasmid pJMGo has a 9.0-kb insert in pBS+ (Stratagene) containing the *goa-1* coding sequence flanked by 5 kb of DNA at the 5′ end and 1 kb of DNA at the 3′ end (*3*). The extent of the 5′ sequence in pJMGo matches that of the *goa-1*-reporter plasmids pJMOBS.11 and pJMOB61 (*4*). For mutagenesis in vitro, we used the primer 5′-CCTTTCTGATCTAAGACCTCCCACATC-3′, corresponding to nucleotides 693 to 719 of the *goa-1* cDNA (*3*). The mutation was confirmed by limited sequencing of the resulting plasmid, pJMGoQL. To establish *syls9*, we injected pJMGoQL into *C. elegans* (*5*) at 5 μg/ml along with pMH86 (*dpy-20*) at 10 μg/ml and pBS+ at 100 μg/ml.
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14. Transgenic animals bearing multiple copies of wild-type $G\alpha_o$ (pJMGo) (*12*) were also defective in egg laying, although not as severely as *syls9* animals. We therefore introduced a frame-shift mutation into pJMGoQL upstream of the Q-L substitution: pJMGoQL was cleaved at a unique Sph site corresponding to nucleotide 176 in the *goa-1* cDNA sequence (*3*), treated with T4 DNA polymerase, and religated. Limited sequence analysis of the resulting plasmid, pJMStopnGo, showed a deletion of five base pairs at the expected position. The conceptual translation of pJMStopnGo matches the wild-type sequence through residue 27 with translation ceasing at codon 49. We injected pJMStopnGo into *C. elegans* (*5*) at 10 μg/ml along with pMH86 (*dpy-20*) at 10 μg/ml and pBS+ at 100 μg/ml. Transgenic animals bearing this control plasmid were indistinguishable from wild-type *C. elegans*, indicating that the abnormal phenotypes seen in *syls9* animals are due to expression of the activated $G\alpha_o$ protein.
15. Activated $G\alpha_o$ may also affect chemosensation: The *syls9* adults abandoned the bacterial lawn, as do mutants lacking functional chemosensory apparatus [J. Hodgkin, *Genetics* **103**, 43 (1983)]. *syls9* also resulted in abnormal pharyngeal pumping and defecation and small body size.
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20. A 4-kb Nco-Apa fragment of pJMGoQL, extending from the start site of translation to 1 kb 3′ to the *goa-1* coding region, was inserted between the Nco and Apa sites in pPD49.78 [C. C. Mello and A. Fire, in *Methods in Cell Biology:* C. elegans, D. Shakes and H. Epstein, Eds. (Academic Press, San Diego, CA, 1995, in press)] downstream of the hsp16-2 promoter [E. G. Stringham, D. K. Dixon, D. Jones, P. M. Candido, *Mol. Biol. Cell* **3**, 221 (1992)], producing pJMGoQLH. We injected pJMGoQLH at 5 μg/ml along with pMH86 (*dpy-20*) at 10 μg/ml and pBS+ at 100 μg/ml. A transgenic line bearing pJMGoQLH as an integrated array, *syls17,* was generated (*5*) and examined for its response to heat treatment, 33°C for 30 min in S basal buffer plus cholesterol (*22*).
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Development of a Mouse Model of *Helicobacter pylori* Infection That Mimics Human Disease

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The human pathogen *Helicobacter pylori* is associated with gastritis, peptic ulcer disease, and gastric cancer. The pathogenesis of *H. pylori* infection in vivo was studied by adapting fresh clinical isolates of bacteria to colonize the stomachs of mice. A gastric pathology resembling human disease was observed in infections with cytotoxin-producing strains but not with noncytotoxic strains. Oral immunization with purified *H. pylori* antigens protected mice from bacterial infection. This mouse model will allow the development of therapeutic agents and vaccines against *H. pylori* infection in humans.

Infection of the human stomach by *Helicobacter pylori*, a Gram-negative spiral bacterium first isolated in 1982 from a patient with chronic active gastritis (*1*), causes nearly all duodenal ulcers and most gastric ulcers and is associated with an increased risk of gastric adenocarcinoma (*2, 3*). In developing countries, *H. pylori* infection occurs early in life (often within the first 6 to 8 months) and can persist chronically; 80% of the population is infected by age 20. In developed countries, the rate of infection slowly increases with age, and 50% of people 60 years old are infected by the bacterium (*4*). However, only a minority of infected people develop signs and symptoms of gastric pathology. Host factors have been proposed to be important for the development of symptomatic gastric disease (*5*). Treatment of gastric disease is usually achieved with H2 antagonists and represents a major expense for health care systems. Recently, antibiotic therapy has been introduced to eradicate *H. pylori* infection; nevertheless, antimicrobial treatment may be limited in the future by the emergence of antibiotic-resistant strains. Vaccination thus offers the best long-term strategy for prevention and possible treatment of disease.

Clinical isolates of *H. pylori* can be divided into at least two major types (*6*). Type I bacteria express a vacuolating cytotoxin (VacA), which induces vacuole formation in epithelial cells, and an immunodominant cytotoxin-associated antigen (CagA). Type II bacteria do not express VacA or CagA. Patients with duodenal ulcers are always infected by cytotoxin-producing type I bacteria (*7, 8*); this finding suggests that only infection with type I strains is associated with the development of gastric lesions that may evolve into severe disease. These clinical observations recently were supported by the observation that lysates of type I bacteria, but not those of type II bacteria, cause gastric damage in mice and that purified VacA cy-

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