STR-33, a Novel G Protein-coupled Receptor That Regulates Locomotion and Egg Laying in Caenorhabditis elegans*

Received for publication, March 17, 2011, and in revised form, August 27, 2011 Published, JBC Papers in Press, September 21, 2011, DOI 10.1074/jbc.M111.241000

Jeong-Eui Lee†, Pan-Young Jeong‡†, Hyoe-Jin Joo‡, Heeke Yong Kim†, Taehoon Lee†‡, Hyeon-Sook Koo†, and Young-Ki Paik†‡§

From the †Department of Biochemistry and Yonsei Proteome Research Center and the ‡Department of Integrated Omics for Biomedical Sciences, World Class University Program, Graduate School, Yonsei University, Seoul 120-749, Korea

Despite their predicted functional importance, most G protein-coupled receptors (GPCRs) in Caenorhabditis elegans have remained largely uncharacterized. Here, we focused on one GPCR, STR-33, encoded by the str-33 gene, which was discovered through a ligand-based screening procedure. To characterize STR-33 function, we performed UV-trimethylpsolaren mutagenesis and isolated an str-33-null mutant. The resulting mutant showed hypersinusoidal movement and a hyperactive egg-laying phenotype. Two types of egg-laying-related mutations have been characterized: egg laying-deficient (Egl-d) and hyperactive egg laying (Egl-c). The defect responsible for the egg laying-deficient (Egl-d) phenotype is related to Goq signaling, whereas that responsible for the opposite, hyperactive egg-laying (Egl-c) phenotype is related to Goα/o signaling. We found that the hyperactive egg-laying defect of the str-33(ykp001)/H9251 mutant is dependent on the G protein GOA-1/Goα/o. Endogenous acetylcholine suppressed egg laying in C. elegans via a Goα/o-signaling pathway by inhibiting serotonin biosynthesis or release from the hermaphrodite-specific neuron. Consistent with this, in vivo expression of the serotonin biosynthetic enzyme, TPH-1, was up-regulated in the str-33(ykp001) mutant. Taken together, these results suggest that the GPCR, STR-33, may be one of the neurotransmitter receptors that regulates locomotion and egg laying in C. elegans.

Signal transduction through G protein-coupled receptors (GPCRs) is conserved from yeast to mammals and mediates cellular processes as diverse as odorant detection, hormonal signaling, vision, and drug responses (1). GPCRs, which share a conserved seven-transmembrane domain structure, transduce environmental stimuli into intracellular signals by coupling to heterotrimeric G proteins. The subfamily of G proteins to which the receptor couples defines the specific linkage to intracellular effectors and determines the nature of the intracellular signal. In Caenorhabditis elegans, GPCRs are involved in a wide range of physiological functions, including perception of odors and regulation of behaviors, such as locomotion (2), pharyngeal pumping (3), and male mating (4).

C. elegans locomotion involves sinusoidal movements that reflect neurotransmission at neuromuscular junctions regulated by dorsal and ventral motor neurons. The GPCR ligand acetylcholine (ACh) is a major neurotransmitter in C. elegans excitatory signaling pathways involved in regulating biological behaviors, including locomotion (5, 6), whereas GABA (γ-aminobutyric acid) mediates inhibitory influences on locomotion and other behaviors (7). Appropriate movement behavior requires a balance between ACh and GABA (8). For example, mutation of the cholinergic biosynthetic enzyme CHA-1 produces a severe, uncoordinated locomotor phenotype characterized by deep body-bending movement (5), whereas mutation of the UNC-25 protein, which encodes a GABA biosynthetic enzyme, produces the opposite phenotype (7).

Egg-laying behavior is primarily regulated by two classes of motor neurons: hermaphrodite-specific neurons (HSNs) and ventral cord (VC) motor neurons. Of these, HSNs play the more critical role (9). HSNs make neuromuscular junctions with vulval muscles and VC motor neurons, which are cholinergic neurons that inhibit egg laying by inhibiting HSNs. ACh signaling through a putative GPCR coupled to the G protein GOA-1/Goα/o in HSNs suppresses the egg-laying behavior of C. elegans by inhibiting biosynthesis or release of newly synthesized 5-hydroxytryptamine (5-HT) (6, 10). Expression of the tph-1 gene, which encodes tryptophan hydroxylase-1, is strongly suppressed by GOA-1/Goα/o; conversely, tph-1 expression is activated by EGL-30/Goα/o. Thus, signaling through Goα/o suppresses egg laying by inhibiting 5-HT biosynthesis in HSNs, whereas Goq-mediated signaling stimulates 5-HT biosynthesis and egg laying (9, 10).

Recent studies suggest that some mechanosensory neurons also regulate egg laying by inhibiting HSNs as well as VC motor neurons (11), although the specific signaling pathways and proteins involved have not yet been identified. Although diverse biological processes in C. elegans are mediated by GPCRs, many of these GPCRs are orphan receptors, and the specific function of only a few has been clarified. In this study, we sought to...
identify the function of nematode-specific GPCRs for which reverse genetics has failed to identify orthologs in other species. Here, we show that the newly isolated str-33, a member of the str family and one of the uncharacterized nematode-specific GPCRs, mediates locomotion and egg-laying behavior of *C. elegans* in mechanosensory neurons.

**EXPERIMENTAL PROCEDURES**

*C. elegans* Strains and Maintenance—*C. elegans* strains were maintained on nematode growth medium (NGM) agar plates at 20 °C, as described previously (12). The N2 Bristol strain was used for all experiments unless otherwise noted. Worms were fed *Escherichia coli* strain OP50. The following strains were obtained from the Caenorhabditis Genetics Center (Minneapolis, MN): Bristol N2, MT2426 goa-1(n1134)I, GR1321 tph-1(mg280)II, CB1339 mec-4(e1339)X, and CB1515 mec-10(e1515)X. str-33(ykp001)V was isolated using a reverse genetics method. The GOA-1 constitutively activated animal vsIs49 (10, 13) was kindly provided by Prof. Michael R. Koelle (Department of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, CT). mec-4(u231) mutant was kindly provided by Prof. Monica Driscoll (Rutgers University). Double-mutant strains were generated using standard genetics techniques (12). The goa-1(n1134) mutation is a G-to-A transition in the first codon that eliminates an Ncol site (14).

Isolation of str-33 Deletion Mutants and Phenotypic Analyses—The mutant str-33 gene was isolated by reverse genetics screening using UV-trimethylpsolaren (TMP) mutagenesis. Briefly, mutations in *C. elegans* were induced by exposure to UV irradiation in the presence of TMP (Sigma-Aldrich). Mutants were screened using a nested PCR-based method and sib selection. The following primers were designed based on the predicted N-terminal sequences of str-33: 5′-GCA TTT TGA ATG CAG TGA TCA G-3′ (outer sense) and 5′-GTG CAT TGA TGC ACC ACA AAA CAG CA-3′ (outer antisense); 5′-GAC CAG ATC TAT AGT TCC GGT CCT GT-3′ (inner sense) and 5′-GCA GGT ATG AAG CAG GCG TAA GGT TA-3′ (inner antisense). A homozygous line containing a 917-bp deletion was isolated and out-crossed with the wild type for six generations to eliminate other mutations that might have been formed by UV-TMP random mutagenesis.

For life span assays, synchronized stage L4 animals were transferred to a fresh plate and scored every 2 days. Animals were scored as dead when they no longer responded to gentle prodding with a platinum wire. Life span is defined as the time (*t*) from the L2 larval stage (*t* = 0) until the day worms were scored as dead. Death by internal hatching of progeny, desiccation on the wall of the plate, extruded vulva, or loss during handling was not scored in life span assays. The brood sizes of N2 and str-33(ykp001) hermaphrodites were determined by placing late L4 stage individual worms on OP50-seeded plates and allowing them to self-fertilize at 20 °C. The P, parental animal was then transferred to a fresh plate every 24 h for the following 5 days. The total number of F1 progeny on the plates was counted.

**Behavioral Assays**—Body bending rate assays were performed as described previously (15). Briefly, stage-synchronized young adult hermaphrodites were transferred from OP50-NGM agar plates onto a fresh, foodless NGM plate. Observation began 1 min after transfer. The number of body bends in 20-s intervals was recorded separately for each of the five animals on the assay plates. Forward run duration was counted as the time (*s*) from the initiation of forward movement to the initiation of backward movement. Backward run duration was counted using the same method. Spontaneous coiling was counted as the time (*s*) from initiation of coiling to the resumption of movement. Movement pattern was analyzed by calculating the ratio of each movement for 7 min, as described previously (16). The movement ratio for each movement was measured by transferring synchronized young adult hermaphrodites to a fresh plate and recording the time for every movement over a 1-min interval and calculating the ratio (%) 5 min after loading.

Construction of GFP-Reporter Plasmids—A polymerase chain reaction (PCR)-amplified DNA fragment encompassing the full-length str-33 gene was ligated into the GFP cassette of *E. coli* pPD114.108, kindly provided by A. Fire (Department of Pathology and Genetics, Stanford University School of Medicine). The Pstr-33::GFP construct was prepared by amplifying *C. elegans* genomic DNA by PCR to obtain a 5,064-bp promoter sequence of str-33 and ligating the resulting DNA fragment into BamHI/Smal restriction sites in the multicloning site of the pPD114.108 GFP vector. The primers used were 5′-GGA TCC CGG GCC ATG ATA ATT TCG GG-3′ (sense) and 5′-CCC GGG GCG AAG GTT CAC CGT CAT-3′ (antisense). The construct (100 ng/µl) was co-injected with the rol-6(su1006) plasmid pRF4 (50 ng/µl) into wild-type *C. elegans*, and transgenic worms were observed using an AXIO fluorescence microscope (Zeiss). The Ptph-1::GFP construct was prepared by PCR amplification of a 2,514-bp promoter sequence upstream of the open reading frame for tph-1 using *C. elegans* genomic DNA as a template. The resulting DNA fragment was ligated into HindIII/BamHI restriction sites in the multicloning site of the pPD95.79 GFP vector. The Ptph-1::GFP construct (100 ng/µl) was injected into wild-type *C. elegans* and transferred to str-33(ykp001) mutants by mating to generate str-33(ykp001);Ex[Ptph-1::GFP] transgenic animals. Synchronized L4 stage animals were transferred to fresh NGM plates and incubated at 20 °C. After 36 h, tph-1 gene expression was compared in animals with wild-type and str-33(ykp001) mutant backgrounds. Quantitative fluorescence microscopy was performed on a Zeiss AXIO fluorescent microscope, and image analysis was carried out using AxioVision software. Pmec-4::DsRED was created to confirm the expression loci of STR-33 as described previously (17). Briefly, a touch receptor neuron-specific construct was prepared by PCR, yielding a 1,140-bp promoter sequence of mec-4. The resulting DNA fragment was ligated into restriction sites within the multicloning site of the PD95.79 DsRED vector, which had been produced by substitution of a GFP-encoding region into DsRED. This final construct was co-injected into animals with the Pstr-33::GFP reporter construct.

Generation of Extrachromosomal Rescue Transgenic Animals—Extrachromosomal arrays carrying the str-33-rescuing transgene construct, Ex[5::str-33(+)] J, contained a 3.6-kb str-33 PCR

---

**Functional Characterization of STR-33**

*C. elegans* in mechanosensory neurons.
Functional Characterization of STR-33

RESULTS

Isolation and Basic Characterization of the str-33 Single Deletion Mutant—During the course of studying daunomycin signaling-associated proteins, we discovered STR-33, a novel GPCR-like protein that lacks homology to known mammalian proteins. To characterize this protein, we constructed a specific str-33 single mutant strain, termed str-33(ykp001), using UV-TMP random mutagenesis. DNA sequencing revealed that the str-33 mutant gene contained a 917-bp deletion in a region spanning from the upstream promoter (~57) to the second intronic region (~860). We anticipated that this mutation would be functionally null because it lacked a start codon (Fig. 1A and supplemental Fig. S1). We next confirmed the mutation in the str-33 gene locus using external and internal primer sets (Fig. 1B). Basic physiological features, including adult life span, total brood size, pharyngeal pumping rates, and defecation rhythm, were unchanged in str-33(ykp001) mutants (data not shown).

str-33(ykp001) Mutants Show an Abnormal Locomotor Phenotype—Unlike wild-type animals, which move forward through regular sinusoidal movements, str-33(ykp001) mutants displayed locomotor defects, exhibiting exaggerated body-bending locomotion that resulted in a steeper slope of sinusoidal movement (Fig. 1C). The bending rate of str-33(ykp001) mutant (13.9 ± 1.59, n = 15) was lower than that of wild-type animals (21.8 ± 1.62, n = 15; Fig. 1D). Moreover, the frequency of forward movement was lower, whereas that of backward movement was higher in str-33(ykp001) mutants. Adoption of a spontaneous coiling state, which was not observed in wild-type animals, was also detected frequently (Fig. 1, D–F). The net result of these properties was that str-33(ykp001) mutants showed deep body-bending movement but slower forward progress.

str-33(ykp001) Mutants Exhibit a Hyperactive Egg-laying Phenotype—str-33(ykp001) mutants showed a hyperactive egg-laying phenotype in which worms are unable to properly inhibit egg laying. Whereas wild-type animals retained an average of 12.8 ± 1.8 eggs within their uterus and laid eggs that had reached the gastrulation stage, str-33(ykp001) mutants laid early stage eggs, resulting in the retention of fewer eggs (4.6 ± 1.6; n = 30; Fig. 2, A and B), regardless of adult reproductive stage (supplemental Fig. S2).

To confirm that this abnormal egg-laying behavior was attributable to the STR-33 mutant, we performed str-33 gene rescue experiments by transforming a str-33(ykp001);Ex[str-33(+)] genomic fragment into the str-33(ykp001) mutant. As shown in Fig. 2, B and D, this maneuver corrected the hyperactive egg-laying phenotype: str-33(ykp001);Ex[str-33(+)] transgenic animals retained more eggs (9.2 ± 3.3, n = 20) than str-33(ykp001) mutants and laid many fewer early stage eggs (5%). Furthermore, the rescued transgenic animals showed an apparent recovery from the exaggerated body-bending defect, exhibiting wild-type-like sinusoidal forward locomotion (Fig. 1, C–F).

Like str-33(ykp001) mutants, goa-1(n1134) mutants bearing a GOA-1/Gαo loss-of-function mutation exhibited a strong...
hyperactive egg-laying phenotype, retaining fewer eggs (1.6 ± 0.5, n = 30) and laying predominantly (~85%) early stage eggs (Fig. 2B), suggesting that STR-33 may be involved in this known egg-laying regulatory pathway (Fig. 2, B and D). To test this, we constructed goa-1(n1134); str-33(ykp001) double loss-of-function mutants and examined their egg-laying phenotype. goa-1(n1134); str-33(ykp001) double mutants exhibited a phenotype similar to that of goa-1(n1134) mutants (Fig. 2, B and D). To clarify whether GOA-1 activation is sufficient to inhibit egg laying, we created a transgenic animal in which GOA-1 is constitutively active in the background of str-33(ykp001) mutants. The GTPase-deficient GOA-1(Q205L) mutant described previously (10) was used for this purpose. As reported, goa-1(Q205L) mutants showed an egg laying-defective phenotype opposite that of the goa-1(n1134) loss-of-function mutant, exhibiting uterine retention of many eggs (30.1 ± 6.7, n = 30) (Fig. 2B). Expression of constitutively active GOA-1(Q205L) in str-33(ykp001) mutants recapitulated the same egg laying-defective phenotype of goa-1(Q205L) single mutants (Fig. 2B), with each animal retaining an average of 27.4 ± 4.1 (n = 30) eggs in its uterus. These results suggest that the STR-33 functions upstream of GOA-1/\(\alpha\), to regulate egg laying and locomotion.

**STR-33 Is Involved in Cholinergic Signaling in Mechanosensory Neurons**—To examine STR-33 expression patterns, we injected a Pstr-33::GFP reporter construct into wild-type animals. Unexpectedly, str-33 was not expressed in HSNs or VC neurons, which are directly involved in egg-laying behavior (6, 10). However, it was detected in ALM and PLM mechanosensory neurons and head neurons (Fig. 3A). To confirm that

---

**FIGURE 1. Molecular characterization and abnormal locomotor phenotype of the str-33(ykp001) mutant.** A, the str-33 gene is composed of four exons (boxes) and three introns. A 917-bp region from before the start codon to the second intron was deleted by UV-TMP mutagenesis. a and b represent the primer set used to confirm deletion of the str-33 gene. B, PCR bands obtained from single worm PCR analyses of wild-type and str-33(ykp001) mutants using external (a) and internal (b) primers. C, wild-type animals showed normal sinusoidal locomotion (a), str-33(ykp001) mutants displayed hypersinusoidal locomotion (b). The locomotory defect of str-33(ykp001) was rescued by introducing a str-33 genetic fragment with its own promoter (c) or a mechanosensory neuron-specific promoter (d). A stage-synchronized adult animal of each genotype was placed on a plate for 5 min, and its tracks were photographed. D, the bending rate of str-33(ykp001) mutants compared with wild-type animals was measured as described under “Experimental Procedures.” Bending rates of the indicated genotypes are shown as the mean ± S.E. (error bars), *p < 0.001 versus wild-type animals; n = 15 for each genotype and three independent experiments; Student’s t test. E, movement-ratio analysis of each genotype. Error bars, S.E. F, movement-pattern analysis of each genotype. Error bars, S.E. *, p < 0.001 versus wild-type animals; n = 15 for each genotype. N.D., not detected.
Expression of str-33 was located in PLM neurons, we co-injected a str-33 reporter construct with the mechanosensory neuron-specific marker, mec-4::Ds-RED, which revealed expression in ALM and PLM neurons (Fig. 3B). The primary function of ALM (L/R) and PLM (L/R) neurons is to respond to anterior and posterior touch, respectively, via interneurons (21). However, this unexpected result suggested that some mechanosensory neurons play a role in regulating egg laying, as has been noted previously (11), and implicated STR-33 in this function. To demonstrate that str-33 acts in these mechanosensory neurons, we performed a rescue experiment using a Pmec-4::str-33 fusion construct. As shown in Fig. 3C, microinjecting Pmec-4::str-33 into str-33(ykp001) mutants rescued the hyperactive egg-laying behavior of these mutants, increasing the number of unlaid eggs in uterus from 4.6 ± 1.6 to 11.7 ± 2.1 (n = 15). This transgene was expressed in several mechanosensory neurons, including PLMs, which regulate egg laying by transiently inhibiting HSN activity (11), suggesting that STR-33 could function to regulate egg laying in PLM neurons.

MEC-4 is a component of DEG/ENaC proteins required to sense gentle mechanical touch as observed in mechanosensory neurons (22). Hyperactivation of MEC-4 protein (mec-4(d)) induces neuronal necrosis of mechanosensory neurons (23, 24).

However, mec-4(d) mutants showed a normal egg-laying phenotype (12.8 ± 1.9) (Fig. 3D) and locomotion rate (supplemental Fig. S3). To clarify whether the hyperactive egg-laying phenotype of str-33(ykp001) is linked to mechanosensory neurons, first, we prepared mec-4(d);str-33(ykp001) double mutants and counted unlaid eggs. The hyperactive egg-laying (11.6 ± 1.4) (Fig. 3D) and the locomotion defect phenotype of str-33(ykp001) mutant (supplemental Fig. S3) were suppressed by the mec-4(d) mutation, suggesting that STR-33 GPCR acts in mechanosensory neurons to regulate both egg-laying and locomotion behavior of C. elegans. Second, we performed an assay of gentle touch sensation to examine whether STR-33 GPCR is related to touch response of C. elegans. The mec-4(e1339) and mec-4(u231);str-33(ykp001) double mutants also exhibited gentle touch response defect, similar to mec-4(d) mutants, suggesting that STR-33 functions in parallel with gentle touch sensation in mechanosensory neurons (supplemental Fig. S4).

Because ACh signaling is known to mediate egg-laying behavior, we tested whether the cholinergic agonist levamisole stimulated egg laying in str-33(ykp001) mutants. Levamisole stimulated egg laying at a low concentration but exerted no...
concentration-dependent effect and failed to restore egg laying to wild-type levels (Fig. 3E). These results suggest that STR-33 may be one of the GPCRs involved in cholinergic signaling that regulate egg-laying and locomotion in *C. elegans*. Given the fact that cholinergic neurotransmission is known to affect dauer larva formation (25), and several cholinergic mutants exhibit a dauer-
Functional Characterization of STR-33

FIGURE 4. STR-33 regulates the expression of a tph-1 reporter transgene. The intensity of GFP expression from the tph-1 promoter was greater in the str-33(ykp001) mutant. A, large panels show representative images of GFP expression in HSNs of wild-type and str-33(ykp001) mutants; insets show enlarged images of tph-1 expression in a HSN neuron. The asterisk denotes the vulva. Scale bar, 50 μm. B, relative intensity of tph-1 promoter-directed GFP expression in HSNs. Error bars, S.E. *, p < 0.001 versus wild-type N2; n = 20 for each genotype; Student’s t test.

defective phenotype under certain assay conditions⁶, we anticipated that str-33(ykp001) mutants might show defects in dauer formation. However, str-33(ykp001) mutants entered the dauer state very well in standard dauer formation assays (26, 27) and showed normal dauer recovery (data not shown), suggesting that STR-33 may not be directly involved in the dauer signaling pathway, although it could be related to cholinergic signaling.

STR-33 Suppresses 5-HT Biosynthesis in HSNs through Inhibition of tph-1 Transcription—GOA-1 signaling is known to regulate egg laying by reducing the expression of the tph-1 gene in HSNs. Consistent with this, we found that tph-1 expression was increased in goa-1(n1134) mutants (data not shown), as previously reported (10). As a first step toward confirming whether the hyperactive egg-laying behavior of str-33(ykp001) mutants was due to changed tph-1 expression in HSNs, we transfected these animals with a tph-1:gfp construct. Similar to the results obtained in goa-1(n1134) mutants, tph-1 expression in HSNs was increased ~2-fold in str-33(ykp001) mutants, indicating that loss of STR-33 function up-regulates 5-HT biosynthesis in HSNs (Fig. 4). The endogenous serotonin-deficient mutant tph-1(mg280) exhibited an Egl-d phenotype in which more eggs were observed in the uterus compared to wild type, indicating that 5-HT is a critical regulator of egg laying in C. elegans (28). We constructed tph-1(mg280);str-33(ykp001) double mutants and confirmed the Egl-d phenotype of tph-1(mg280) (supplemental Fig. S5), suggesting that the egg-laying defect phenotype of str-33(ykp001) was dependent on serotonin signaling. Taken together, our results imply that STR-33 signaling strongly suppresses HSN 5-HT levels, which are dependent on GOA-1/Gαo, and are important for regulating egg laying in C. elegans.

STR-33 Mediates Egg-laying Behavior through Regulation of Endogenous 5-HT Levels—The observed increase in tph-1 expression in HSNs of str-33(ykp001) mutants suggests that these animals might release more 5-HT onto neuromuscular junctions, causing them to lay early stage eggs that would not normally be laid by wild-type animals. As the final neuromodulator in the egg-laying pathway, 5-HT, whether endogenously elevated or exogenously applied, would be expected to increase egg laying in C. elegans under conditions in which 5-HT levels are limiting. As expected, inclusion of 5-HT in hypertonic buffer, which normally suppresses egg laying (29), increased the number of eggs laid by wild-type C. elegans from fewer than 1 to 6.7 ± 1.2 (n = 50; Fig. 5, A and B). In contrast, str-33(ykp001) mutants laid more eggs in hypertonic buffer than wild-type animals and were resistant to exogenous 5-HT (Fig. 5, A and B). These results suggest that 5-HT levels in str-33(ykp001) mutants are already sufficiently elevated to maximally stimulate egg laying and suggest that wild-type STR-33 normally acts through inhibition of endogenous 5-HT biosynthesis to inhibit egg laying in C. elegans.

In wild-type animals, exogenous 5-HT treatment generally causes resistance to the paralytic effect of the acetylcholinesterase inhibitor, aldicarb, due to 5-HT-mediated inhibition of Ach release by motor neurons (20). To further characterize the role of STR-33 in the regulation of 5-HT synthesis in HSNs suggested by the above results, we assessed Ach release and accumulation in neuromuscular junctions by testing the aldicarb sensitivity of wild-type and str-33(ykp001) mutant worms pre-exposed to exogenous 5-HT. As expected, wild-type worms exhibited an aldicarb-resistant phenotype; however, 5-HT treatment did not reduce aldicarb sensitivity in str-33(ykp001) mutants (Fig. 5, C and D). These results provide additional evidence that STR-33 is involved in regulating 5-HT synthesis, suggesting that the str-33(ykp001) mutation disrupts 5-HT-induced modulation of Ach neurotransmission at neuromuscular junctions as well as 5-HT-mediated regulation of the egg-laying circuit.

DISCUSSION

Approximately 1,500 putative chemoreceptor genes or pseudogenes, or ~7% of all genes in C. elegans, are predicted to be members of the seven-transmembrane superfamily (30, 31).
The *str* family is one of the subfamilies of the Str superfamily, which is the largest superfamily, consisting of several subfamily genes, such as *str*, *srh*, *srd*, etc. Studies on the GPCR families revealed that the *str* family consists of about 200 genes and 75 pseudogenes (32). Of particular interest to us was STR-33, a previously uncharacterized nematode-specific GPCR identified in initial screening with ligand binding assays (data not shown). *str-33(ykp001)* mutants exhibited severely uncoordinated locomotion and a hyperactive egg-laying phenotype. Although other GPCRs are known to contribute to egg laying in *C. elegans*, ours is the first study to describe a GPCR involved in both locomotion and egg-laying behavior.

Numerous GPCRs expressed in sensory neurons transmit signals in response to environmental stimuli, and a variety of olfactory receptors in these neurons are able to sense volatile or water-soluble chemicals and thereby control attraction and avoidance behavior of *C. elegans*. However, we found that chemotactic responses to chemicals recognized by AWA olfactory neurons (diacetyl and pyrazine) and AWC olfactory neurons (benzaldehyde, isoamyl alcohol, butanone, and 2,3-pentanedione) were unchanged in *str-33(ykp001)* mutants (supplemental Fig. S6). Because the expression of *str-33* was observed in mechanosensory neurons, such as ALMs and PLMs, we performed standard gentle touch assays using an eyelash hair, as described previously (33). *str-33(ykp001)* mutants showed a weak defect for sensing gentle touch, but their phenotype was modest compared with that of other touch-insensitive mutants, such as *mec-4(e1339)*, *mec-4(d)*, and *mec-10(e1515)* (supplemental Fig. S4). Moreover, reversal to nose touch and avoidance of high osmolarity solution (osmosensation response) (34) characteristic of wild-type animals were fully retained in *str-33(ykp001)* mutants (data not shown). These observations suggest that STR-33 may not function as a sensory GPCR to transduce environmental stimuli. The absence of sensory defects in *str-33(ykp001)* mutants may reflect the fact that STR-33 is not expressed in chemosensory or osmosensory neurons (Fig. 3A).

The *str* family is one of the subfamilies of the Str superfamily, which is the largest superfamily, consisting of several subfamily genes, such as *str*, *srh*, *srd*, etc. Studies on the GPCR families revealed that the *str* family consists of about 200 genes and 75 pseudogenes (32). Of particular interest to us was STR-33, a previously uncharacterized nematode-specific GPCR identified in initial screening with ligand binding assays (data not shown). *str-33(ykp001)* mutants exhibited severely uncoordinated locomotion and a hyperactive egg-laying phenotype. Although other GPCRs are known to contribute to egg laying in *C. elegans*, ours is the first study to describe a GPCR involved in both locomotion and egg-laying behavior.

Numerous GPCRs expressed in sensory neurons transmit signals in response to environmental stimuli, and a variety of olfactory receptors in these neurons are able to sense volatile or water-soluble chemicals and thereby control attraction and avoidance behavior of *C. elegans*. However, we found that chemotactic responses to chemicals recognized by AWA olfactory neurons (diacetyl and pyrazine) and AWC olfactory neurons (benzaldehyde, isoamyl alcohol, butanone, and 2,3-pentanedione) were unchanged in *str-33(ykp001)* mutants (supplemental Fig. S6). Because the expression of *str-33* was observed in mechanosensory neurons, such as ALMs and PLMs, we performed standard gentle touch assays using an eyelash hair, as described previously (33). *str-33(ykp001)* mutants showed a weak defect for sensing gentle touch, but their phenotype was modest compared with that of other touch-insensitive mutants, such as *mec-4(e1339)*, *mec-4(d)*, and *mec-10(e1515)* (supplemental Fig. S4). Moreover, reversal to nose touch and avoidance of high osmolarity solution (osmosensation response) (34) characteristic of wild-type animals were fully retained in *str-33(ykp001)* mutants (data not shown). These observations suggest that STR-33 does not function as a sensory GPCR to transduce environmental stimuli. The absence of sensory defects in *str-33(ykp001)* mutants may reflect the fact that STR-33 is not expressed in chemosensory or osmosensory neurons (Fig. 3A).

It is also likely that dysfunction of STR-33 alone did not produce an overt sensory phenotype in response to mechanosen- sation, suggesting that modulation of egg laying is enacted independently or possibly downstream of mechanosen- sation. This would help explain why *str-33(ykp001)* mutants exhibited normal responses to mechanical stimuli, despite evidence that STR-33 is normally expressed in mechanosensory neurons, such as ALM and PLM.

**FIGURE 5.** *str-33(ykp001)* mutants are defective in 5-HT-mediated egg-laying behavior. A, *str-33(ykp001)* mutants were resistant to the egg-laying stimulatory effects of exogenous 5-HT in liquid. Single animals were placed into liquid M9 buffer containing 12.5 mM 5-HT for 90 min, and the number of eggs laid per individual was counted. Error bars, S.E., *p* < 0.001 versus M9-treated animals; n = 50 for each strain; Student’s t test. n.s., not significant (p = 0.48). B, wild-type animals, but not *str-33(ykp001)* mutants, were hypersensitive to stimulation of egg laying by 5-HT. Most wild-type animals laid very few eggs in M9 buffer (85%) compared with 5-HT-treated animals, which laid 1–6 eggs (40%), 7–12 eggs (38%), or more than 13 eggs (9%). There was no significant difference in egg laying by *str-33(ykp001)* mutation in either the presence or the absence of exogenous 5-HT, indicating that *str-33(ykp001)* mutants are 5-HT-resistant. C and D, the time course of aldicarb-induced paralysis of wild-type animals (C) and *str-33(ykp001)* mutants (D) pretreated with (black squares) and without (open circles) 5 mg/ml 5-HT. Error bars, S.E. (n = 3 replicates).
The fact that str-33(ykp001) mutants do not respond to treatment with the cholinergic agonist levamisole (Fig. 3E) suggests that STR-33 may be involved in cholinergic signaling. Whether STR-33 actually binds ACh and mediates ACh signaling remains to be determined. It is not clear how a response to the nicotinic cholinergic receptor agonist, levamisole, is compatible with STR-33 as a GPCR. However, previous studies reported that levamisole-activated nicotinic cholinergic receptors required G protein function (4). We suggest that the GPCR STR-33 could transmit a signal through its specific G protein that is required for nicotinic cholinergic receptors.

Among other receptors known to regulate egg laying in C. elegans is the muscarinic ACh receptor GAR-2, which signals through the GOA-1/Gαq pathway in HSNs (6). Another GPCR expressed in HSNs that regulates egg laying is EGL-47 (13). Phe-Met-Arg-Phe-NH₂ (FMRFamide) neuropeptides acting on EGL-6 in HSNs synergize with ACh to modulate egg laying in C. elegans; EGL-6, which is composed of two GPCR isoforms, also signals through GOA-1/Gαq (35). However, which molecules in PLM mechanosensory neurons regulate the egg-laying circuitry of HSNs has remained unknown. On the basis of our results, we suggest that STR-33 is a novel GPCR that regulates the egg-laying behavior of C. elegans through a mechanism different from that of other GPCRs known to regulate egg laying. Because STR-33 lacks sequence homology to other well known neurotransmitter receptors (e.g. GAR-2, GAR-3, SER-1, and EGL-47) (4, 6, 13), it is not clear which molecules bind STR-33 to transmit downstream signals.

Locomotion and egg-laying behavior are not regulated by a single signaling pathway but are affected by multiple pathways involving a variety of neurotransmitters and neuropeptides, including ACh, 5-HT, octopamine, and some Arg-Phe-NH₂ (RFamides) (6, 29, 35, 36). Prominent among these are 5-HT and ACh. 5-HT is a monoamine neurotransmitter that contributes to the regulation of locomotion, defecation, and pharyngeal pumping in C. elegans (14, 15, 29), but perhaps the best studied role of 5-HT signaling is in the control of egg laying. 5-HT released from HSNs stimulates egg laying of C. elegans via egg-laying muscle. In HSNs, ACh signaling regulates egg laying by inhibiting transcription of tph-1, which encodes the rate-limiting enzyme for 5-HT biosynthesis (6, 20). The G protein GOA-1/Gαq is known to transmit this inhibitory signal. Accordingly, we predicted that STR-33 could be one of the neurotransmitter receptors that transmit ACh signaling through GOA-1. Consistent with this, tph-1 expression was

**FIGURE 6. Proposed functional models of the putative GPCR, STR-33.** A, STR-33 in PLM mechanosensory neurons mediates regulatory signaling that suppresses egg laying by inhibiting GOA-1/Gαq signaling-dependent activity in HSNs. B, GOA-1/Gαq signaling also might regulate egg-laying behavior in PLM mechanosensory neurons, which mediate signaling directly with STR-33.
increased in str-33(ykp001) mutants compared with wild-type animals, not only in HSNs but also in ADF neurons, whereas tph-1 expression is up-regulated in goa-1(n1134) mutants (10). Taken together with other previous observations, our results suggest that STR-33 transmits signals through GOA-1/Gαo to suppress 5-HT synthesis in HSNs and thereby regulates locomotion and egg-laying behavior (Fig. 4).

Because egg laying is known to be regulated by HSNs and VC neurons, we anticipated that STR-33 would also be expressed in these neurons. Unexpectedly, STR-33 was expressed in mechanosensory neurons, such as PLMs, but was not detected in HSNs or VC neurons typically associated with regulation of egg laying. Although ALM and PLM neurons, which are mechanosensory neurons that respond to touch, are known to inhibit egg laying by sensing environmental stimuli like vibration (11), the specific mechanism by which PLM neurons regulate egg laying has been unclear. Our results suggest that STR-33 in PLM neurons regulates the egg-laying mechanism in HSNs and VC neurons by acting through an as-yet unknown inhibitory signal to down-regulate transcription of the tph-1 gene and suppress biosynthesis of 5-HT in HSNs via GOA-1/Gαo. If STR-33 acts through 5-HT in HSNs to regulate egg laying, loss of HSNs should block the constitutive egg-laying phenotype of str-33(ykp001) mutants. To test this, we used tph-1(mut)str-33(ykp001) double mutants and confirmed that hyperactive egg-laying behavior is controlled by serotonin level in the HSN (supplemental Fig. S5). Because str-33(ykp001) mutants showed a normal response to mechanical stress, STR-33 in mechanosensory neurons probably does not mediate body touch stimuli directly but rather regulates the egg laying of C. elegans via HSNs.

It is well known that both ACh and 5-HT influence various muscle functions, such as egg laying, locomotion, learning and memory, and pharyngeal pumping. These neurotransmitters can also act on muscle systems as well as neuronal systems (9, 37). str-33(ykp001) mutants showed a resistance to these neuronal transmitting compounds, suggesting that STR-33 GPCR could also be linked to muscle function, although we have not elaborated this issue in this study. This is because our studies were designed to explore the functional aspect of STR-33 in the context of egg-laying defect phenotype coupled to cholinergic signaling in which aldicarb was used to confirm the changes in the endogenous serotonin level indirectly (Figs. 3E and 5, C and D). Although we do not exclude the possibility that str-33 mutation affects muscle function, which might consequently influence egg laying as well as locomotion, we did not pursue further investigation of the consequence of muscle function change by str-33 mutation in this study. This is because the location of the str-33 expression site was seen only in the neuronal cells and not in the muscle cells. However, it deserves further investigation.

In conclusion, we show that STR-33 acts in neurons upstream of HSNs to inhibit egg-laying behavior. Furthermore, str-33 expression was observed in other neuronal cells, where it could be involved in regulating the sinusoidal locomotion of C. elegans. Our rescue studies using a transgenic strain that expresses str-33 specifically in ALM/PLM neurons confirms not only the site of str-33 expression but also the place where it acts. This result suggests that the localization of str-33 to mechanosensory neurons, which modulate the egg-laying regulatory machinery of HSNs in C. elegans, could indicate that neurotransmitters released into synapses by mechanosensory neurons act on downstream HSNs to inhibit egg laying via GOA-1/Gαo-coupled GPCRs. Taken together, our results suggest that STR-33 is a novel GPCR expressed in mechanosensory neurons that communicates with HSNs, where a signal mediated by GOA-1/Gαo inhibits tph-1 expression and regulates egg laying in C. elegans (Fig. 6).

Acknowledgments—We are grateful to Prof. Michael R. Koelle for the vos349 goa-1(Q205L) mutant and Prof. Monica Driscoll for the mec-4(d) mutant and clone. Also, we thank the Caenorhabditis Genetic Center for supplying most of the strains used for this study.

REFERENCES
1. Oliveira, I., Paiva, A. C., and Vriend, G. (1993) J. Comput. Aid. Mol. Des. 7, 649–658
2. Dittman, J. S., and Kaplan, J. M. (2008) J. Neurosci. 28, 7104–7112
3. Steger, K. A., and Avery, L. (2004) Genetcs 167, 633–643
4. Liu, Y., LeBouef, B., and Garcia, L. R. (2007) J. Neurosci. 27, 1411–1421
5. Rand, J. B., and Russell, R. L. (1984) Genetics 106, 227–248
6. Bany, I. A., Dong, M. Q., and Koelle, M. R. (2003) J. Neurosci. 23, 8060–8069
7. McIntire, S. L., Jorgensen, E., Kaplan, J., and Horvitz, H. R. (1993) Nature 364, 337–341
8. Schuske, K., Beg, A. A., and Jorgensen, E. M. (2004) Trends Neurosci. 27, 407–414
9. Shly, S. I., Kerr, R., and Schafer, W. R. (2003) Curr. Biol. 13, 1910–1915
10. Tanim, J. E., Moretto, J. L., Lindquist, R. A., and Koelle, M. R. (2008) Genetics 178, 157–169
11. Zhang, M., Chung, S. H., Fang-Yen, C., Craig, C., Kerr, R. A., Suzuki, H., Samuel, A. D., Mazur, E., and Schafer, W. R. (2008) Curr. Biol. 18, 1445–1455
12. Brenner, S. (1974) Genetics 77, 71–94
13. Moreta, J. J., and Koelle, M. R. (2004) J. Neurosci. 24, 8522–8530
14. Sélégal, L., Elkes, D. A., and Kaplan, J. M. (1995) Science 267, 1648–1651
15. Sawin, E. R., Ranganathan, R., and Horvitz, H. R. (2000) Neuron 26, 619–631
16. Tsak, I. L., and Hobert, O. (2003) J. Neurobiol. 56, 178–197
17. Royal, D. C., Bianchi, L., Royal, M. A., Lizzio, M., Jr., Mukherjee, G., Nunez, Y. O., and Driscoll, M. (2005) J. Biol. Chem. 280, 41976–41986
18. Chatzigeorgiou, M., Yoo, S., Watson, J. D., Lee, W. H., Spencer, W. C., Kindt, K. S., Hwang, S. W., Miller, D. M., 3rd, Treinin, M., Driscoll, M., and Schafer, W. R. (2010) Nat. Neurosci. 13, 861–868
19. Koelle, M. R., and Horvitz, H. R. (1996) Cell 84, 115–125
20. Govorunova, E. G., Moussafi, M., Kuliev, A., Nguyen, K. C., McDonald, T. V., Hall, D. H., and Sze, J. Y. (2010) PLoS One 5, e10368
21. Chaffie, M., Sulston, J. E., White, J. G., Southgate, E., Thomson, J. N., and Brenner, S. (1985) J. Neurosci. 5, 956–964
22. O’Hagan, R., Chaffie, M., and Goodman, M. B. (2005) Nat. Neurosci. 8, 43–50
23. Driscoll, M., and Chaffie, M. (1991) Nature 349, 588–593
24. Bianchi, L., Gerstbrein, B., Frøkjaer-Jensen, C., Royal, D. C., Mukherjee, G., Royal, M. A., Xue, J., Schafer, W. R., and Driscoll, M. (2004) Nat. Neurosci. 7, 1337–1344
25. Tissenbaum, H. A., Hawdon, J., Perreghaux, M., Hotez, P., Guarente, L., and Ruvkun, G. (2000) Proc. Natl. Acad. Sci. 97, 460–465
26. Lee, J., Kim, K. Y., Lee, J., and Paik, Y. K. (2010) J. Biol. Chem. 285, 2930–2939
27. Jeong, P. Y., Jung, M., Yim, Y. H., Kim, H. Park, M., Hong, E., Lee, W., Kim, Y. H., Kim, K., and Paik, Y. K. (2005) Nature 433, 541–545
28. Sze, J. Y., Victor, M., Loer, C., Shi, Y., and Ruvkun, G. (2000) Nature 403, 7.
560–564
29. Horvitz, H. R., Chalfie, M., Trent, C., Sulston, J. E., and Evans, P. D. (1982) Science 216, 1012–1014
30. Bargmann, C. I. (1998) Science 282, 2028–2033
31. Robertson, H. M. (2006) The putative chemoreceptor families of C. elegans in Wormbook, The C. elegans Research Community, Wormbook, doi/10.1895/wormbook.1.66.1, http://www.wormbook.org
32. Robertson, H. M. (2001) Chem. Senses 26, 151–159
33. Chalfie, M., and Sulston, J. (1981) Dev. Biol. 82, 358–370
34. Jose, A. M., Bany, I. A., Chase, D. L., and Koelle, M. R. (2007) Genetics 175, 93–105
35. Ringstad, N., and Horvitz, R. (2008) Nat. Neurosci. 11, 1168–1176
36. Trent, C., Tsung, N., and Horvitz, H. R. (1983) Genetics 104, 619–647
37. Jospin, M., Qi, Y. B., Stawicki, T. M., Boulin, T., Schuske, K. R., Horvitz, H. R., Bessereau, J. L., Jorgensen, E. M., and Jin, Y. (2009) PLoS Biol. 7, e1000265