The neuronal stomatin-like proteins UNC-1 and UNC-24 play important roles in the nervous system of *Caenorhabditis elegans*. These neuronal stomatin-like proteins are putative chaperone proteins that can modify volatile anesthetic sensitivity and disrupt coordinated locomotion. A suppressor of *unc-1* and *unc-24*, named *ssu-1* (for suppressor of stomatin uncoordination), suppresses three phenotypes of neuronal stomatin-like protein deficiency as follows: volatile anesthetic sensitivity, uncoordinated locomotion, and a constitutive alternative developmental phenotype known as dauer. Here we provide the first phenotypic characterization of *ssu-1*, predicted to be the only *C. elegans* cytosolic alcohol sulfotransferase, a family of enzymes that catalyze a sulfate linkage with the alcohol group of small molecules for the purposes of detoxification or modification of signaling. In vitro enzyme analysis of bacterially expressed *SSU-1* demonstrates sulfotransferase activity and thus confirms the function predicted by protein sequence similarities. Whereas *unc-1* is expressed in the majority of neurons of *C. elegans*, expression of *SSU-1* protein in only the two ASJ amphid interneurons is sufficient to restore the wild type phenotype. This work demonstrates that *SSU-1* is a functional sulfotransferase that likely modifies endocrine signaling in *C. elegans*. The expression of *SSU-1* in the ASJ neurons refines the understanding of the function of these cells and supports their classification as endocrine tissue. The relationship of *unc-1*, *unc-24*, and *ssu-1* is the first association of neuronal stomatin-like proteins sharing regulatory roles with a sulfotransferase enzyme.

Stomatin-like proteins (SLPs) form a large family of membrane-bound proteins that are conserved from bacteria through humans. SLPs in bacteria and yeast have well studied chaperone relationships with AAA proteases (1, 2). The molecular function of SLPs in metazoans is less well defined. The common feature of all SLPs across all phyla is a central domain of 150 conserved amino acids (NCBI conserved domain smart00244) (3). Unique carboxyl and amino ends differentiate individual SLPs. In all organisms in which SLPs have been studied, there are multiple genes encoding SLPs (2, 4–6). In several phyla, genetic and protein interactions have been described between stomatin-like paralogs (2, 4, 7). The interactions between paralogs can be explained in part by the demonstrated formation of heteromeric membrane complexes (8, 9). The contributions of multiple different stomatin-like paralogs contribute to the character of membrane complexes by altering post-translational modification (10), protein trafficking (11), and membrane ion channel function (12). Although the understanding of the function SLPs is incomplete, loss of SLPs has been associated with many adverse effects.

In humans, defects in SLPs are associated with hematopoietic, nephrotic, and neurological disorders (6, 13–16). The SLP family gets its name from the human anemia stomatocytosis. The fragile red blood cells in this disorder often resemble stoma or lips. Western blots of red blood cell protein from patients with stomatocytosis were found to be deficient in erythrocyte surface protein band 7.2, which was subsequently named stomatin (5, 15). Despite the absence of the stomatin protein in these individuals, no mutations have been found in the stomatin gene (17). In addition, a mouse knock-out for an SLP does not have red blood cell abnormalities (18). This suggests that there is a second unknown gene that needs to be mutated to cause the loss of stomatin from red blood cells and to cause stomatocytosis. Recently, a variant of stomatocytosis with a neurological component was described (16). In addition to the red blood cell abnormalities and a lack of stomatin protein in red blood cells, affected individuals experienced mental retardation and seizures. A second human SLP, podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome (6). Because the pathogenic mechanisms of podocin-associated nephropathy and stomatocytosis are uncertain, investigating the multiple stomatin-like genes in *Caenorhabditis elegans* could assist in the understanding of these human conditions.

There are 10 stomatin-like genes in *C. elegans*. Three stomatin-like genes, *unc-1*, *unc-24*, and *mec-2*, are expressed in neurons (19–21). Mutations in neuronal stomatin-like genes *unc-1* and *unc-24* alter sensitivity to volatile anesthetics (VAs) (21), linking stomatin function with a neuronal mechanism that has eluded description for 150 years. The current understanding of
neuronal SLP function in C. elegans is limited to an association between SLPs and epithelial sodium channels (ENaCs) (7, 22). The SLPs MECA-2 and UNC-24 have been shown to modulate function of the ENaCs involved in mechanosensation in C. elegans (7). In addition, the stomatin-like genes unc-1 and unc-24 have been shown to interact with the ENaC ortholog, unc-8 (22, 23). A similar stomatin and ENaC relationship has been observed in tissue-cultured mammalian cells expressing stomatin and a subset of ENaCs called acid-sensitive channels (24). However, the role of stomatin in the normal functioning of sodium channels is unknown. Because the function of SLPs is unclear, we reasoned that identification of mutations that suppress loss of stomatin function might identify the role of SLPs.

Here, we explored the function of stomatins in the nematode C. elegans through studies of genes that interact with neuronal stomatin genes, unc-1 and unc-24. Of the 10 SLPs in C. elegans, we studied unc-1 and unc-24 because of their abnormal locomotion and their ability to modify sensitivity to VAs as neuronal sites of expression of unc-24, ssu-1, and unc-1, but no changes in phenotype were seen. Because the neuronal sites of expression of unc-24, ssu-1, and unc-1 hinder RNAi efficacy, a lack of interaction between RNAi-targeted dauer-modifying genes and unc-24, ssu-1, and unc-1 mutants could be the result of failed RNAi targeting of dauer mutants. Crosses, although more time-consuming, guarantee genotype and strengthen the negative data such as the lack of observed interaction between dauer-modifying genes and the gene of interest.

**Microinjection**—PCR products were cloned into pCR2.1 vectors (Invitrogen). The promoter for the rescue and GFP constructs was amplified from DNA to include CEY113G7A 107,260 to 106,736 bp. Rescue constructs were amplified from cDNA for the two ssu-1 isoforms to include CEY113G7A 106,717 to 101,853 (3′ of the terminal stop). Promoters containing DNA and coding regions containing cDNA constructs were combined at the CEY113G7A 101,991, with GCA CCG GTC (underlined is an Agel restriction digest recognition site). Using the Agel restriction digest site, the constructs were inserted in-frame into pEGFP-1 vectors (BD Biosciences). Construct DNA was prepared with the Plasmid Miniprep kit (Qiagen, Chatsworth, CA). Mutant rescue was done as described by Mello et al. (30). The injected DNA is taken up by the developing oocytes and can form free linear arrays. “Rescued” is defined by the reversal of the phenotype of the gene of interest. Rescued progeny of unc-1(null) parents will move normally. Rescued progeny of ssu-1,unc-1(null) parents have uncoordinated locomotion. In general, the test DNA was injected at 10 μg/ml; a marker for successful microinjection, rol-6 DNA (pRF4, a plasmid containing the dominant rol-6(suu1006) mutation), was co-injected at 100 μg/ml. F1 Rollers were picked to establish stable lines.

**Microscopy**—Pictures of a single focal plane of animals were taken with a Zeiss Axioptet microscope equipped for fluorescence, using a Zeiss Axiocam digital camera.

**Amphid Staining**—Amphid cells were stained using Dil dye (Michael Koelle, New Haven, CT). Briefly, a stock solution of 2 mg of Dil (Invitrogen) per 1 ml of dimethyl formamide was diluted 1:4000. Several worms were incubated in the diluted solution at room temperature for 1 h. Worms were then transferred to a normal growth medium plate to destain for 1 h. Stained worms were immobilized on agar pads with 1 mM levamisole for fluorescent microscopy using Texas Red filter.

**Protein Purification**—cDNA for the three isoforms was cloned into pCR2.1 vector (Invitrogen). cDNA was inserted with a fusion at the amino start of the SSU-1 protein into pGEX-4T-2 protein expression vector (Amersham Biosciences) with BamHI/EcoRI, using BamHI that was incorporated in-frame into the 5′ primer and EcoRI of pCR2.1 vector. Constructs were transformed bl21 pLysS competent cells. To avoid inclusion bodies, the bacteria were grown at room temperature.
Protein was purified using the Novagen Bugbuster GST-Bind purification kit (Novagen, Darmstadt, Germany) per the manufacturer’s directions. Eluate was concentrated using a Millipore Microcon YM-50 Ultrafilter spin tube (Millipore, Billerica, MA). 4 μl of the concentrate was run in Fig. 3A. The sample was mixed to 50% glycerol and frozen at −20 °C. The GST tag was not removed.

In Vitro Enzymatic Analysis—50-μl reactions contained 100 mM, pH 7.0, potassium phosphate, 1 mM MgCl₂, 10 mM dithiothreitol, 1 mg/ml bovine serum albumin, 0.2 mM 2-hydroxypro pyl-β-cyclodextrin, 1 μM [35S]PAPS,0.1 mM 1-hydroxypropylene as an acceptor and 6 μl of enzyme glycerol mixture. 2- Hydroxy-β-cyclodextrin is used in the assay to optimize the reaction and is not targeted by the sulfotransferase. 2-Hydroxy-β-cyclodextrin is added to facilitate the exposure of the hydrophobic hydroxypropylene to the hydrophilic enzyme. Reactions were run at a nematode physiological temperature of 20 °C for 24 h to maximize formation of product. 5 μl of the reaction mixture was mixed with 10 μl of EtOH saturated with 1-hydroxypropylene as a visible marker. This mixture was spotted on a 250-μm KG silica gel 60-Å TLC plate and developed for 1 h with a solvent of chloroform/methanol/acetic acid/H₂O, 8:2:4:2:1. Plates were then either sprayed with En3hance (PerkinElmer Life Sciences) and placed on film, or lanes were scraped into Biosafe scintillation fluid, and the samples were read on a scintillation counter.

RESULTS

The Genetic Suppressor ssu-1 Was Isolated as a Suppressor of the Unc-1 Locomotion Defect—Different alleles of the pan-neuronal SLP unc-1 (23) disrupt coordination of locomotion in different patterns (25, 31). Alleles of unc-1 also have opposite effects on sensitivity to VAs. The dominant allele unc-1(n494) (31) increases VA sensitivity (21), whereas the null allele unc-1(e580) suppresses the increased VA sensitivity caused by mutations in a second gene, unc-79(e1c1) (27). To study the function of unc-1, we created genetic suppressors of unc-1(e580). unc-1(e580) has an uncoordinated locomotion phenotype (supplemental movie) and also suppresses the increased halothane sensitivity of unc-79(e1c1) (Fig. 1). We mutagenized unc-1(e580) with the mutagen ethyl methanesulfonate and selected for genetic suppressors of the Unc-1 uncoordinated locomotion phenotype. Isolated as suppressors of the locomotion defect of unc-1, the gene class was given the name suppressor of stomatin uncoordination (ssu). One of the isolates, ssu-1(fc73), suppresses the altered VA sensitivities (Fig. 1) and locomotion defects of the neuronal SLP, unc-1(e580) (Fig. 1). ssu-1(fc73) also suppressed the locomotion (supplemental movie) and VA sensitivities of the null allele unc-24(eD28) (19) (Fig. 1). Mutations in mec-2, which encodes a close homolog of unc-1, cause loss of the response to light touch in C. elegans. The double mutant ssu-1(fc73);mec-2(e75) is also defective in the response to light touch. Thus, ssu-1(fc73) is not a universal suppressor of SLP defects.

ssu-1 Suppresses a Newly Identified Dauer Modification Phenotype of unc-24 Nulls—unc-24 and ssu-1 are modifiers of dauer formation, an alternative developmental stage in C. elegans which allows larvae to survive adverse environmental conditions. Dauer larvae have distinctive morphologic characteristics and are capable of surviving several months longer than the normal 3-week life span of C. elegans at 20 °C. In normal laboratory growth conditions, N2 larvae bypass dauer formation in favor of the normal accelerated time-to-reproductive maturity developmental pathway. An increased incidence of dauer larvae to over 95% of offspring form dauer larvae. The propensity to form dauer larvae was observed in the following animals. The phenotype is not a result of the kinked phenotype in combination with mutagenic dauer larvae formation in which a small portion of dauer larvae escape to reproductive adulthood after periods of 24 h to over 6 months. Of other dauer-defective mutants, the 1–2-day transient constitutive dauer formation of unc-1(fc73) is not modified by other known dauer-modifying genes on chromosome III. The unc-24(eD28) deletion extends to include the neighboring gene ora-1 (19). To support the dauer phenotype as unc-24-specific, the nonsense mutant unc-24(e138) was combined with fc83. The double

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FIGURE 1. ssu-1(fc73) modifies volatile anesthetic sensitivity. The bars depict EC₅₀ values in halothane as measured by immobility for several worm strains. Error bars show the standard deviation from the mean EC₅₀ value. Fifty percent of wild type C. elegans are immobilized by 3.2% halothane. The sensitivity created by loss-of-function mutations in unc-79(e1c1) can be suppressed by loss-of-function mutations in unc-1(e580) and unc-24(eD28) (19, 21). The triple mutants unc-79(e1c1);ssu-1(fc73);unc-1(e580) and unc-79(e1c1); unc-24(eD28);ssu-1(fc73) have an uncoordinated locomotion phenotype, indicating that the Daf-22 dauer-constitutive gene mapped by us to chromosome III. The genetic suppressor ssu-1 was isolated as a suppressor of the locomotion defect of unc-1 (23). To suppress the increased halothane sensitivity of unc-79(e1c1), EC₅₀ value different from N2, p < 0.05; #, EC₅₀ value different from unc-79(e1c1), p < 0.05.

| Table 1 The Fc83;Unc-24 dauer phenotype is suppressed by ssu-1(fc73) and daf-12(m20) |
| --- |
| **Dauer** | * | **Not Dauer** |
| fc83; unc-24(eD28) | * | fc83 |
| daf-5(e1386); fc83; unc-24(eD28) | fc83; unc-24(eD28) | * |
| daf-16(m27); fc83; unc-24(eD28) | fc83; unc-24(eD28) | * |
| daf-22(m130); fc83; unc-24(eD28) | fc83; unc-24(eD28); daf-12(m20) | fc83; unc-24(eD28); ssu-1(fc73) | fc83; unc-1(e580) |
mutant fc83;unc-24(e138) has a similar increase of greater than 95% dauer larvae. Unlike unc-24(e138) and unc-24(eDf28), unc-1(e580) does not interact with fc83 to increase dauer formation at 20 °C. This indicates that the effect on dauer formation is not a general one for neuronal SLPs. ssu-1(fc73) suppresses the dauer phenotype of fc83;unc-24(eDf28) so that less than 5% of offspring abnormally form dauer larvae (Table 1).

A developmental pathway consisting of multiple genes controlling dauer formation has been characterized (32). fc83;unc-24(eDf28) displays a distinctive quality of dauer larva formation in which a small portion of larvae escapes to reproductive adulthood after periods of 24 h to over 6 months. For other dauer-defective mutations, the 1–2-day transient constitutive dauer formation of daf-9(m540) mutants most closely resembles the Fc83;Unc-24 phenotype. Neither ssu-1(fc73) nor unc-24(eDf28) affected the duration of transient constitutive dauer formation when paired with daf-9(m540) (Table 1). To further determine the role of ssu-1 in dauer formation, we created triple mutants of multiple dauer-defective mutations with fc83;unc-24 to determine the position of fc83;unc-24 within the dauer pathway. The dauer-defective daf-12(m20), which occupies a terminal position in this pathway, suppresses the dauer phenotype of fc83;unc-24(eDf28) so that less than 5% of offspring abnormally form dauer larvae. No other dauer-modifying mutations were found to interact with fc83, unc-24, or ssu-1 (Table 1). Sterols have been shown to play a role in the developmental decision to form dauer and occupy a similar position in the dauer pathway proximal to daf-12 as the interaction between fc83, unc-24, and ssu-1 (33).

**ssu-1 is the Predicted Gene Y113G7A.11 on Chromosome V—** ssu-1 was mapped on the end of chromosome V. Additionally, the large deletions, ozDf1 and ozDf2, which remove all of ssu-1 and several neighboring genes, suppress unc-1 when in trans with ssu-1(fc73). Sequencing identified a mutation in ssu-1(fc73) that codes for a premature termination at amino acid 284 (TGG:TGA CEY113G7A 102,754 bp) in the predicted gene Y113G7A.11 (Fig. 2A). RT-PCR of wild type cDNA identified two spliced forms of ssu-1, a 1.2-kb fragment including the five predicted exons and a shorter 1-kb spliced form that skips the second exon without disrupting the reading frame.

For biological confirmation that Y113G7A.11 is ssu-1, rescuing constructs were designed for microinjection into worms. From the identified transcripts, two constructs were created to encode the long and short spliced forms of Y113G7A.11 (Fig. 2B). To provide the native promoter, the constructs included the 543 bp of genomic DNA 5’ of the start of the first exon (CEY113G7A 107,260 to 107,717 bp). Each of the two constructs was separately microinjected into ssu-1(fc73);unc-1(e580). Scored by coordinated movement, only the longer cDNA construct eliminated the suppression of unc-1(null) by ssu-1(fc73). The shorter spliced form did not rescue the ssu-1 phenotype (0/800 F1 rollers, 0/30 stable roller lines). The longer spliced form, but not the shorter form, also rescued the change in aesthetical sensitivity associated with ssu-1 (data not shown). A second ssu-1 allele was created by the National Bioresource Project for the Experimental Animal "Nematode C. elegans" with a screen for genomic deletions. The 189-bp deletion (CEY113G7A 106,694–106,883 (189-bp deletion + 6-bp insertion)) in ssu-1(tm1117) removes the predicted protein translation start site (Fig. 2), yet worms carrying the ssu-1(tm1117) allele fail to suppress unc-1 as a homozygote or in trans to ssu-1(fc73). Larger deletions, ozDf1 and ozDf2, which remove all of ssu-1 and several neighboring genes, suppress unc-1 when in trans with fc73. ssu-1(tm1117) has no observable phenotype and expresses ssu-1 mRNA as demonstrated by RT-PCR amplification of ssu-1 products confirmed by sequencing (data not shown). B, rescuing GFP and bacterial expression constructs of ssu-1. The black bar designates the 60% of ssu-1, amino acids 86–328, that encodes protein with similarity to cytosolic alcohol sulfotransferase domain (pfam00685). The short spliced form lacks the second exon and 35 amino acids of the 242-amino acid domain with similarity to sulfotransferases. Rescuing constructs combined the 543-bp genomic DNA 5’ of the first exon with the two cDNA isoforms. GFP fusions were designed to remove the native ssu-1 stop codon. Bacterial expression constructs combine GST fusions with the amino end of ssu-1.
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PAPS donor onto potential substrates. 1-Hydroxypyrene is a universal sulfonate acceptor of sulfotransferases (36) (Fig. 3B). Because mammalian sulfotransferases can change substrate specificity with alternative splicing of the first exon (37), we predicted that the nonrescuing short splice form would be active as a sulfotransferase but would modify substrates irrelevant to the locomotion phenotype. Enzymatic activity of all three bacterially expressed isoforms was demonstrated by the radiolabeling of 1-hydroxypyrene (Fig. 3C). Although the full-length spliced form is much more active in these in vitro conditions against this specific substrate, the difference in activities could be a property of substrate affinities of the different isoforms, different activities of the enzymes, or a result of differences in enzyme stability in these in vitro conditions. Further biochemical description of the three isoforms offers the opportunity to connect separate ssu-1 substrates with different phenotypes.

SSU-1 Expression in ASJ Neurons Is Sufficient for Rescue of Phenotype—To localize ssu-1 expression, green fluorescent protein (GFP) was fused with both spliced forms of ssu-1 and the predicted ssu-1 promoter (Fig. 2B). Consistent with the previously described microinjections of the non-GFP constructs, only the full-length ssu-1::GFP fusion rescued the suppression of the locomotion defect of unc-1(null) by ssu-1(fc73). However, both constructs produced strong fluorescence in only two head neurons. Using the amphid stain DiI, these two head neurons were identified as the ASJ amphid neurons (Fig. 4B).

DISCUSSION

SSU-1 Is the Cytosolic Alcohol Sulfotransferase in C. elegans—This is the first phenotypic characterization of the only cytosolic alcohol sulfotransferase in C. elegans. Modification of neuronal phenotypes, including sensitivity to VAs, suggests relevance of ssu-1 to understanding human cytosolic alcohol sulfotransferases. The initial biochemical characterization of the full-length isoform of SSU-1 was recently published (38). Hattori et al. (38) observed strong activity toward 2-naphthol, 4-isopropylphenol, and 4-nitrophenol. Our phenotypic characterization of ssu-1 suggests sterols as the functionally relevant substrate of ssu-1. This work also provides the first phenotypic characterization and demonstration of enzymatic activity of the short spliced form of ssu-1. The shorter spliced form of the gene removes part of the conserved region of the protein similar to mammalian sulfotransferases that change substrate specificity with alternative splicing of the first exon (37). Based on the mammalian

FIGURE 3. Bacterially expressed isoforms of SSU-1 demonstrate sulfotransferase activity in vitro. A, Coomassie Blue staining of purified bacterially expressed SSU-1::GST fusion protein. The observed bands match the predicted molecular masses for the short spliced, full-length, and fc73 products, which are 67, 73, and 60 kDa, respectively, when including the 26-kDa GST fusion protein. Loaded with volumes of enzyme proportional to the reactions in C, this gel roughly estimates the relative amount of enzyme used in the in vitro reactions. B, the SSU-1 enzyme catalyzes the transfer of radiolabeled sulfonate from phosphoadenosine phosphosulfate onto hydroxypyrene. C, organic phase thin layer chromatography assay of SSU-1 isoform-catalyzed reaction of [35S]PAPS with 1-hydroxypyrene. Completed reactions are spotted along the origin of a silica TLC plate. The plates are developed in an organic solvent that mobilizes the lipophilic pyrene. The hydrophilic PAPS does not move from the origin. The three lanes are from a single plate with identical reaction conditions except for isoform of enzyme. The positions of the radiolabeled pyrene and PAPS are marked on the right of the image. The presence of activity is representative of multiple experiments on different days. The activity of the fc73 isoform is very faint, but it is active on reactions with no enzyme, which were never observed to transfer sulfonate (data not shown).

FIGURE 4. SSU-1 is expressed in only two cells, the ASJ amphid neurons. A, the GFP fluorescence pattern in animals expressing SSU-1::GFP driven by a 500-bp promoter. In normal growth conditions, these transgenic animals display rescue of the Ssu-1 locomotion phenotype. In this plane of focus, two cell bodies (white arrow) reside posterior to additional staining of the processes of these two neurons. The pair of ASJ amphid neurons sends processes anteriorly to the nerve ring and continue to the nose. B, DiI staining (red) of the six pairs of amphid neurons in the head. In this single plane of focus, six cell bodies are shown stained with DiI. One of these neurons (yellow arrows) also expresses GFP. The pair of GFP expressing neurons in the SSU-1::GFP animals co-stains with one pair of the DiI staining neurons. The relation of this pair to the other five pairs matches the mapped positions of ASJ amphids.

pounds (35). To confirm sulfotransferase activity by ssu-1, we produced bacterially expressed proteins for the full-length spliced form, short-length spliced form, and fc73 mutant forms of ssu-1 (Fig. 3A). Thin layer chromatography was employed to visualize the transfer of S35 sulfonate from the
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precedent, we propose that the two spliced forms of ssu-1 represent active forms of the enzyme with different specificities.

Although there are several other classes of sulfotransferase in C. elegans active in the Golgi, the cytosolic alcohol sulfotransferase is unique in substrate affinity and localization. Alcohol sulfotransferases target a different acceptor group than the amino sulfotransferases, which include several heparin sulfotransferases in C. elegans (39). Unlike protein-tyrosine sulfotransferases, cytosolic sulfotransferases do not target proteins. Sulfation by cytosolic sulfotransferases is involved in clearance of toxins and metabolizing small molecules, including steroid hormones, catecholamines, and drugs (35). The addition of sulfate can alter the effect of a signaling molecule on its intended target. Other species possess multiple cytosolic sulfotransferases that are classified according to substrate affinity. The SSU-1 protein sequence comparisons to the sulfotransferase protein family do not favor any of the substrate subdivisions of cytosolic alcohol sulfotransferases (34). This suggests that mammalian genomic expansion of cytosolic sulfotransferase genes occurred after the evolutionary split between mammals and nematodes.

As the genetic suppressor of unc-1 and unc-24, the predicted signaling function of ssu-1 allows the inference of a signaling function for unc-1 and unc-24. Whereas other models are plausible, these two SLPs likely function in the reception of the SSU-1-generated signal at a remote cell. As membrane proteins with direct and genetic interactions with sodium channels (22, 23), SLPs challenge a model of direct interaction with the cytosolic SSU-1. Additionally, the limited expression of SSU-1 in two ASJ neurons greatly contrasts with the pan-neuronal expression of UNC-1 (23). The present studies aim to determine whether expression of UNC-1 and UNC-24 exclusively in the ASJ neurons is sufficient to recreate normal function. The minimal expression of SLPs sufficient for function will clarify the role of SLPs in the transmission or reception of SSU-1 signaling.

The New Gene fc83 Interacts with unc-24 to Increase Dauer Larvae Formation—This is the first association of an SLP with dauer larval formation. The ability to affect dauer when paired with fc83 differentiates unc-24 from unc-1. Whereas the ability of some fc83;unc-24(eDf28) worms to escape from dauer to reproductive adult resembles the transient dauer phenotype of daf-9(m540), no genetic interactions with daf-9(m540) were observed with unc-24(eDf28) nor ssu-1(fc73) (Table 1). Further mapping of the new gene fc83 and linkage to a visible marker gene will facilitate crosses with daf-9 and other known dauer-defective genes. These crosses may refine the relationship of the Fc83;Unc-24(eDf28) dauer phenotype to known dauer-defective genes. As a double mutant, fc83;unc-24 was observed to interact with only two genes, daf-12(m20) and ssu-1(fc73) (Table 1). Current models of the dauer larvae formation pathway project daf-5, daf-9, daf-16, and daf-22 to function proximal to daf-12 such that mutations in daf-12 determine phenotype independent of the mutations in the former genes (40). The lack of interaction with the proximal components of the dauer pathway argue against dauer pheromone as a potential signaling mol-
fotransferase function of ssu-1 fits well with an endocrine model of action.

Sterols Are a Likely Substrate of SSU-1—Adding to the observations of Hattori et al. (38) of strong SSU-1 activity toward 2-naphthol, 4-isopropylphenol, and 4-nitrophenol, the phenotypes and genetic interactions of ssu-1 give some clues to the substrate affinities of the sulfotransferase. Catecholamine-deficient mutants, cat-1(e1111), cat-2(e1112), and cat-4(e1114), fail to dramatically alter the Unc-1, Unc-24, and Ssu-1 locomotion phenotypes (data not shown). These crosses argue against catecholamines as the substrate of ssu-1 locomotion phenotypes (data not shown). These crosses of neuronal SLPs in

associated phenotypes and refine the understanding of the function with minimal expression in the two ASJ amphid neurons. These observations add to the expanding list of SLP-associated phenotypes and refine the understanding of the function of neuronal SLPs in C. elegans.

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