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Enhanced neuronal RNAi in *C. elegans* using SID-1

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SUMMARY

We expressed SID-1, a transmembrane protein from *Caenorhabditis elegans* that is required for systemic RNAi, in *C. elegans* neurons. This expression increased the response of neurons to dsRNA delivered by feeding. Mutations in the *lin-15b* and *lin-35* genes further enhanced this effect. Worms expressing neuronal SID-1 showed RNAi phenotypes for known neuronal genes and for uncharacterized genes with no previously known neuronal phenotypes. Neuronal expression of *sid-1* decreased non-neuronal RNAi, suggesting that neurons expressing transgenic *sid-1(+)* served as a sink for dsRNA. This effect, or a *sid-1(−)* background, can be used to uncover neuronal defects for lethal genes. Expression of *sid-1(+)* from cell-specific promoters in *sid-1* mutants results in cell-specific feeding RNAi. We used these strains to identify a role for integrin signaling genes in mechanosensation.

INTRODUCTION

Since its discovery1, RNA interference (RNAi) has served as a powerful tool to study gene function, especially in *Caenorhabditis elegans*. *C. elegans* is unusual in that it exhibits systemic RNAi; double stranded (ds) RNA in the environment can enter and spread throughout the worm to silence the targeted gene2,3. RNAi occurs when worms are soaked in solutions of dsRNA or fed bacteria expressing dsRNA (feeding RNAi), yielding a powerful tool for reverse genetics in this organism4,5.

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AUTHOR CONTRIBUTIONS

All authors participated in the design of the experiments; DC generated the initial constructs and constructed the multiply mutant lines; XC generated the cell-specific constructs and constructed strains with the *sid-1* mutation; AC, DC, IT, and XC conducted the RNAi tests; AC, IT, and MC wrote the paper.

Editorial Summaries

AOP: Expression of the transporter SID-1 in *Caenorhabditis elegans* neurons renders the cells sensitive to systemic RNAi and permits previously unidentified neuronal phenotypes to be uncovered. This expression also reduces RNAi in non-neuronal cell types, allowing examination of neuronal functions of lethal genes.

Issue: Expression of the transporter SID-1 in *Caenorhabditis elegans* neurons renders the cells sensitive to systemic RNAi and permits previously unidentified neuronal phenotypes to be uncovered. This expression also reduces RNAi in non-neuronal cell types, allowing examination of neuronal functions of lethal genes.

The transmembrane protein SID-1 is essential for systemic RNAi in *C. elegans* because it allows the passive cellular uptake of dsRNA6. *Drosophila* possesses robust cell-autonomous RNAi but lacks both systemic RNAi and a SID-1 homolog7. *Drosophila* cells6,7 or mouse embryonic stem cells8 expressing the *C. elegans* SID-1, however, respond to dsRNA in the media.

Feeding RNAi is robust in virtually all cells in *C. elegans* except neurons5,9. In contrast, RNAi occurs in neurons when dsRNA is produced within the neurons themselves10. Thus, the lack of a neuronal response to systemic RNAi does not reflect an inability of these cells to execute RNAi. This lack does correlate, however, with the pattern of detectable SID-1; SID-1 is present in all cells outside the nervous system, but very few cells within it6,7.

We have investigated whether the lack of detectable SID-1 renders neurons refractory to systemic dsRNA. We show here that neurons expressing *sid-1* respond efficiently to feeding RNAi. This finding allowed us to produce strains that are hypersensitive to systemic neuronal RNAi. Moreover, specific expression of *sid-1* in worms otherwise missing the gene permits generation of strains that display cell-specific feeding RNAi.

RESULTS

Expression of *sid-1* in neurons enhances neuronal RNAi

We generated several strains with chromosomally-integrated arrays of the wild-type *sid-1* gene expressed from the pan-neuronal *unc-119* promoter ($P_{unc\text{-}119}$*sid-1*) to test whether SID-1 can increase neuronal RNAi. Unless noted, the TU3270 strain was used for most of the experiments described in this paper. TU3270 also contained *yfp* expressed from the *unc-119* promoter ($P_{unc\text{-}119}$*yfp*) so that RNAi for *yfp* could be assessed in all neurons, as well as $P_{mec\text{-}6}$*mec-6*. As a control we generated a strain (TU3310) with only a $P_{unc\text{-}119}$*yfp* array. We also generated additional transgenic strains carrying $P_{unc\text{-}119}$*sid-1* (TU3311, which lacks the *mec-6* transgene, and TU3356, see Methods for details). All three strains gave similar responses to feeding RNAi, did not have defects in neuronal morphology as judged by fluorescence and did not have any obvious behavioral abnormalities.

We tested for enhanced RNAi in neurons by feeding bacteria expressing dsRNA for *gfp* to $P_{unc\text{-}119}$*sid-1* and control worms. In the nerve ring region, where fluorescence is strongest, the fluorescence intensity was reduced by about 40% in $P_{unc\text{-}119}$*sid-1* worms; control worms had virtually no reduction (Fig. 1a and 2b). In the ventral cord and the anterior touch receptor neurons (TRNs) an even greater *sid-1*-dependent reduction in fluorescence intensity was seen (Fig. 1a). Interestingly, the posterior TRNs (PLML/R) showed little or no reduction in fluorescence intensity (Fig. 1a).

Neuronal RNAi in $P_{unc\text{-}119}$*sid-1* worms also produced behavioral defects. To test for specific behavioral effects in a subset of neurons (the TRNs), we fed worms dsRNA for the TRN channel gene *mec-4* and compared the touch response of $P_{unc\text{-}119}$*sid-1* worms to that of wild type and of strains with mutations known to enhance neuronal RNAi (*lin-35, lin-15b, eri-1, rrf-3,* and *nre-1; lin-15b*)11–15. *mec-4* RNAi produced a marked reduction in the anterior touch response in $P_{unc\text{-}119}$*sid-1* worms compared to wild type, *lin-35(n745),*

lin-15b(n744), eri-1(mg366), nre-1(hd20) lin-15b(hd126), and *rrf-3(pk1426)* mutants (Fig. 1b), although the reduction of response to posterior touch was much smaller (1/5 touches versus 3–4/5 touches). As noted above, a similar anterior/posterior difference occurred with *gfp* RNAi. This difference may reflect differential accessibility of dsRNA or differential expression of *sid-1(+)* in the posterior cells, or an intrinsic difference of these two types of neurons.

Importantly, we observed the same effect of *mec-4* RNAi on the touch response when $P_{unc-119}$*sid-1* worms were grown at 25°C (Fig. 1b). This is a significant advantage since all other RNAi-hypersensitive strains become either sterile or have severe morphological defects at 25°C (our unpublished observations).

Neuronal RNAi with *sid-1* is enhanced by *lin-35* and *lin-15b* mutations

Mutations in *eri-1, lin-15b, lin-35, nre-1,* and *rrf-3* improve neuronal RNAi11–15. Nonetheless, RNAi in strains with these defects cannot replicate the neuronal phenotype of many genes. For example, the touch insensitivity (Mec) phenotype has been difficult to phenocopy by RNAi; only slight effects are seen with *lin-35(n745)* and *lin-15b(n744)* (Fig. 1b; ref. 12). In contrast, YFP fluorescence and touch sensitivity were significantly reduced in strains with $P_{unc-119}$*sid-1* and mutations in *lin-15b* or *lin-35* (Fig. 2) upon treatment with *gfp* or *mec-4* RNAi, with $P_{unc-119}$*sid-1; lin-15b* showing the greater reduction. The Mec phenotype of these strains was so strong that we could see defects in posterior touch; $P_{unc-119}$*sid-1; lin-35* worms responded to 3 of 5 posterior touches, and $P_{unc-119}$*sid-1; lin-15b* worms responded only once or twice (data not shown). Mutations in *eri-1, nre-1,* and *rrf-3* did not improve the response to *gfp* RNAi (data not shown) or *mec-4* RNAi (Fig. 2c). These results suggest that mutations in *lin-35* and *lin-15b* affect RNAi independently of *sid-1*

To assess neuronal RNAi further in worms with $P_{unc-119}$*sid-1* alone and in combination with *lin-15b(n744)* and *lin-35(n745),* we screened 12 *unc* genes that are exclusively expressed in neurons (www.wormbase.org) but whose mutant phenotype had not been reproduced by feeding RNAi in wild type worms or in worms with RNAi-enhanced backgrounds15–17 and 3 genes for which RNAi phenotypes had only been obtained in *rrf-3* mutants (Table 1). RNAi in $P_{unc-119}$*sid-1,* $P_{unc-119}$*sid-1; lin-15b* and $P_{unc-119}$*sid-1; lin-35* worms showed similar phenotypes to the loss-of-function mutant phenotypes for seven of the 15 genes. $P_{unc-119}$*sid-1; lin-l5b* was consistently the most sensitive of all strains. Usually the RNAi phenotype was detectable in the adult stage (and sometimes earlier) of worms grown on RNAi bacteria from the time of hatching, although in some cases (see Table 1) only F1 progeny displayed the phenotype.

As an additional test for the efficiency of neuronal RNAi in $P_{unc-119}$*sid-1* worms, we performed feeding RNAi for 12 *mec*hanosensory abnormal (*mec*) genes needed for touch sensitivity18 (Fig. 3). Wild type worms did not show a reduction in touch sensitivity for any of the genes, and *lin-35* and *lin-15b* worms had moderate or no reduction. In contrast, a considerable loss of touch sensitivity was observed in $P_{unc-119}$*sid-1* worms for all the genes except *mec-5,* whose expression is needed in muscle cells (B. Coblitz and M.C., unpublished data), and the phenotype was even more severe in $P_{unc-119}$*sid-1; lin-15b* worms. Despite the

fact that the transgenic strains contained additional wild-type *mec-6*, bacteria expressing dsRNA for *mec-6* caused touch insensitivity.

In other work we have used DNA microarray analysis of isolated embryonic TRNs to identify 198 genes that are over-expressed in these cells (I.T. and M.C., manuscript in preparation). Using feeding RNAi and $P_{unc\text{-}119}$*sid-1; lin-15b* worms, we tested 149 of the 186 genes for which TRN phenotypes were not known and obtained partial touch-insensitive phenotypes for only five of them (*alr-1*, C03A3.3, F46C5.2, K11E4.3, Y113G7A.15). Mutations have been previously identified for two of these genes. A loss-of-function allele (*oy42*) of the *C. elegans aristaless* gene *alr-1* phenocopies the variable RNAi–induced touch insensitive phenotype (worms responded to 2–7 out of 10 touches). A deletion allele of the paraoxonase homolog K11E4.3 (*ok2266*) produces touch insensitivity in sensitized backgrounds (Y. Chen and M.C., unpublished results). Given the high efficiency of RNAi in $P_{unc\text{-}119}$*sid-1; lin-15b* worms for known *mec* genes, these results indicate that most of the 144 non-responsive genes are likely not to be essential for touch sensitivity; they are likely to function elsewhere in the TRNs or be redundant.

To test whether we could achieve RNAi by selectively expressing *sid-1* in specific neurons, we expressed *sid-1* under the control of the TRN-specific *mec-18* promoter ($P_{mec\text{-}18}$*sid-1*) from a stable extrachromosomal array in strain TU3312. Worms fed *mec-4* (Fig. 1b) and *mec-12* dsRNA (data not shown) were touch insensitive anteriorly; worms fed *mec-12* dsRNA also had reduced posterior touch sensitivity. These results indicate that neuronal RNAi can be induced by expressing *sid-1* in specific neurons with an appropriate promoter.

Expression of *sid-1* in neurons decreases RNAi in non-neuronal tissues

During these studies, we noticed a marked reduction of RNAi phenotypes in $P_{unc\text{-}119}$*sid-1* worms in response to feeding of dsRNAs expected to act in non-neuronal tissues, including *unc-22* (muscle), *rpl-3* (ubiquitous), *unc-52* (hypodermis) and *elt-2* and *nhx-2* (intestine). In all cases $P_{unc\text{-}119}$*sid*-1 worms showed little or no RNAi phenotype (Fig. 4), suggesting a generalized refractory effect to dsRNA in tissues other than neurons. The lesser effect for *unc-52* may reflect SID-1 activity from the *unc-119* promoter, which is expressed embryonically in the hypodermis19. All three $P_{unc\text{-}119}$*sid-1* strains, TU3270, TU3311, and TU3356 displayed similar refractoriness to non-neuronal RNAi (data not shown). This loss could be explained by an increased uptake of dsRNA into neurons at the expense of other tissues, causing the dsRNA in those cells to be limiting. In contrast, this block to non-neuronal RNAi did not occur in the $P_{mec\text{-}18}$*sid-1* strain (data not shown), in which *sid-1* is expressed only in the TRNs, suggesting that this limited expression does not generate a sufficient neuronal reservoir to inhibit the response of non-neuronal cells. The block also did not occur in the $P_{unc\text{-}119}$*sid-1* lines with the *lin-35* or *lin-15b* mutations (Fig. 4). suggesting that a small amount of dsRNA does enter the non-neuronal cells in $P_{unc\text{-}119}$*sid-1* worms, but that its activity needs to be amplified by the loss of *lin-15b* or *lin-35* for a phenotype to be seen.

The lack of a response in the intestine is curious since uptake of dsRNA from the intestinal lumen, which requires the *sid-2* gene (ref. 20), might be thought sufficient for intestinal RNAi. Winston et al.20, however, also found that feeding RNAi for intestinally expressed

genes did not occur without *sid-1*. These results could indicate either that SID-1 is needed for the initial transport through the intestine or that SID-1 activity is needed for RNAi in the intestine, perhaps by mediating transport of dsRNA from the pseudocoelom. The finding that RNAi is observed when *sid-1* mutants express *sid-1(+)* in muscle21 or in the TRNs (see below), suggests that SID-1 is not needed for the initial transport of dsRNA from the intestinal lumen. These data suggest that RNAi in the intestine requires *sid-1* mediated transport from the pseudocoelom.

Detection of neuronal effects of lethal genes

RNAi experiments for several genes result in a lethal phenotype, making the analysis of specific gene function in neurons difficult or impossible. Because non-neuronal RNAi is blocked in $P_{unc\text{-}119}$*sid-1* worms, neuronal phenotypes might be uncovered for lethal genes. We tested this hypothesis by examining six genes (*pat-2/α-integrin, pat-3/β-integrin, pat-4/integrin-linked kinase, pat-6/actopaxin, unc-97/PINCH,* and *unc-112/MIG2)* needed for integrin signaling that encode proteins expressed in both muscle and the TRNs and that we have previously speculated may be involved in touch sensitivity22. Mutants with defects in these genes exhibit a severe Pat (Paralyzed, arrested elongation at the two-fold embryo) phenotype, making testing of a role in touch sensitivity difficult.

Wild-type worms fed dsRNA for *pat-2, pat-4, pat-6, unc-97,* and *unc-112* became severely paralyzed and sterile; *pat-3* (RNAi) worms were arrested as larvae. Those eggs that were fertilized died, confirming the lethal phenotype. However, similarly fed $P_{unc\text{-}119}$*sid-1* worms were not paralyzed and did move when prodded with a platinum wire, so their touch sensitivity could be assessed. The worms became severely touch insensitive (Fig. 5A); moreover, they were uncoordinated, suggesting that several types of neurons were affected, and in the case of *pat-3* dsRNA, showed a developmental delay. In contrast, feeding RNAi of $P_{unc\text{-}119}$*sid-1* worms against *unc-95/paxillin, ina-1*/α-integrin, and several other genes identified as being needed for muscle dense body function, did not result in touch insensitivity (Fig. 5a). These data suggest that integrin signaling is necessary for touch sensitivity.

Because of the pleiotropic phenotype of $P_{unc\text{-}119}$*sid-1* worms fed dsRNA for integrin-signaling genes, we generated a strain in which *sid-1* is only expressed in the TRNs, by expressing $P_{mec\text{-}18}$*sid-1(+)* in *sid-1* mutant worms. When these worms were fed dsRNA for *pat-2* and *unc-112* (Fig. 5b), the only phenotype we observed was touch insensitivity, and this was slightly enhanced in worms that also contained a *lin-15b* mutation (Fig. 5b). These results demonstrate the possibility of having neuron-specific feeding RNAi. Jose et al.21 have previously shown similar results in muscle; we have extended their observations to show that selective feeding RNAi can be engineered in other cells.

DISCUSSION

Expression of *sid-1* makes neurons more susceptible to feeding RNAi, suggesting that the poor response to systemic RNAi in wild-type neurons is due to insufficient SID-1 in most of the nervous system. (Since mutations in other genes enhance feeding RNAi in some neurons, a low level of SID-1 or an equivalent protein is probably present in the wild-type

neurons.) Strains expressing SID-1 in neurons can be used to reveal neuronal phenotypes and identify neuronal functions for many genes. A significant advantage of using the $P_{unc\text{-}119}$*sid-1* strain to reveal neuronal phenotypes by feeding RNAi is that the nervous system and behavior of the worms appear to be wild type, even at 25°C, whereas other RNAi-enhancing strains would be sterile or dead at the higher temperature (our unpublished observations). The poor response to RNAi of neurons in wild type worms, however, may not be due solely to the lack of SID-1, since we were able to enhance RNAi further through loss of *lin-35* or *lin-15b*. These additive effects indicate separate roles for SID-1 and the loss of LIN-15B and LIN-35. *lin-35* and *lin-15b* mutations may increase the sensitivity of all tissues to dsRNA, whereas SID-1 expression in the nervous system enables the successful entry of dsRNA to these cells.

We expected that *eri-1* and *rrf-3* mutations would enhance neuronal RNAi in $P_{unc\text{-}119}$*sid-1* worms, but we did not find this enhancement. Moreover, despite reports showing that *eri-1* and *rrf-3* mutants are more sensitive to neuronal RNAi11,13, we have not observed touch sensitivity phenotypes upon feeding RNAi for *mec* genes in these backgrounds (A.C., D.C. and M.C., unpublished data). The lack of neuronal RNAi in *eri-1* and $P_{unc\text{-}119}$*sid-1; eri-1* worms could be due to the restricted nature of *eri-1* expression to the gonad and an unidentified subset of neurons in the head and tail13, as enhancement would be expected only in the cells expressing *eri-1*. We do not know why loss of *rrf-3* did not give a strong RNAi phenotype in our hands with or without $P_{unc\text{-}119}$*sid-1*, since *rrf-3* is more generally expressed29 and its loss is known to enhance RNAi for some neuronal genes11. Perhaps this gene is under-expressed in the TRNs.

The discovery that over-expression of *sid-1* in neurons prevents RNAi in non-neuronal tissues is consistent with the idea that the amount of dsRNA available within the animal for silencing a particular target gene is limited23. Moreover, this silencing enabled us to use the $P_{unc\text{-}119}$*sid-1* strain to uncover a requirement for integrin signaling in the TRNs. Similar results were obtained in *sid-1* mutants expressing *sid-1(+)* in the TRNs. In general, *sid-1(+)* transgenes expressed from pan-neuronal or neuron-specific promoters, especially in backgrounds such as *sid-1; lin-15b* which would enhance RNAi only in cells expressing the transgene, should be useful in uncovering neuronal phenotypes for other genes that are widely expressed or exhibit considerable pleiotropy or lethality. Other tissue-specific promoters could also be used for *sid-1(+)* expression, to allow for selective feeding RNAi in other cells.

METHODS

C. elegans growth

Wild-type *C. elegans* (N2) and strains with mutations affecting RNAi [*lin-35(n745)I* (ref. 12), *rrf-3(pk1426)II* (ref. 16), *eri-1(mg366)IV* (ref. 13), *sid-1(qt2)V* (ref. 5), *sid-1(pk3321)V* (ref. 24), *lin-15b(n744)X* (ref. 25), *eri-1(mg366)IV; lin-15b(n744)X* (ref. 17), and *nre-1(hd20) lin-15b(hd126)X* (ref. 14)], mutations causing touch insensitivity [refs. 18, 26 except were noted; *mec-1(e1496)V, mec-2(u37)X, mec-3(e1338)IV, mec-4(u253)X, mec-5(u444)X, mec-6(u450)I, mec-8(e398)I, mec-9(u437)V, mec-10(ok1104)X* (*C. elegans* Gene Knockout Consortium), *mec-12(e1605)III, mec-14(u55)III, mec-17(tm2109)IV*

(National Bioresource Project, Japan), *mec-18(u69)X*] or mutations causing uncoordination [*unc-5(e53)IV, unc-7(e5)X, unc-10(e102)X, unc-24(e138)IV, unc-30(e191)IV, unc-42(e270)V, unc-55(e1170)I, unc-79(e1068)III, unc-14(e57)I, unc-76(e911)V*]27, *vab-8(e1017)V* (ref. 28), and *unc-119(e2498)III* (ref. 29)] were grown at 20° C as previously described27.

Expression constructs and transformation

A 2.2 kb fragment 5' from the start of translation of the *unc-119* gene was amplified from genomic DNA introducing 5' Hind*III* and 3' BamH*I* sites, and cloned into TU#739 (ref. 30) to create TU#865 ($P_{unc\text{-}119}yfp$). A 7.8 kb fragment 5' from the start of translation of the *sid-1* gene was similarly amplified from genomic DNA introducing 5' BamH*I* and 3' *NotI* sites and cloned into BamH*I*/Eag*I* sites of TU#864 to create TU#866 ($P_{mec\text{-}18}sid\text{-}1$). A BamH*I*/Pvu*I* digested fragment of TU#866 containing *sid-1* was cloned into TU#865 to create TU#867 ($P_{unc\text{-}119}sid\text{-}1$). Primers used are listed in Supplementary Table 1.

We generated transgenic worms by microinjection with one or more of the above plasmids31, isolating individuals with extrachromosomal arrays, and integrating the subsequent extrachromosomal arrays with gamma rays (4,800 rads)30. All plasmids, including markers, were injected at a concentration of 25–30 ng/μl except for TU#866, which was injected at 5 ng/μl, $P_{mec\text{-}6}mec\text{-}6$, which was injected at 2 ng/μl, and pBSK (Stratagene) added to reach a concentration of 100 ng/μl.

Enhanced RNAi strains

uIs57 contains $P_{unc\text{-}119}sid\text{-}1$, $P_{unc\text{-}119}yfp$, *and* $P_{mec\text{-}6}mec\text{-}6$ integrated into wild type worms (TU3270). *uIs57* was crossed into several mutations to create a number of strains: TU3272 [*lin-35(u745)*], TU3335 [*lin-15b(u744)*], TU3337 [*eri-1(mg366)*], TU3339 [*rrf-3(pk1426)*], TU3341 [*eri-1(mg366); lin-15b (n744)*] and TU3344 [*nre-1(hd20) lin-15(hd126)*].

TU3310 contains *uIs59*, an integrated array of $P_{unc\text{-}119}yfp$, which expresses YFP in all neurons, and TU3311 contains *uIs60*, which is an integrated array of $P_{unc\text{-}119}sid\text{-}1$ and $P_{unc\text{-}119}yfp$, which expresses YFP and SID-1 in all neurons. *uEx762* is an extrachromosomal array containing $P_{mec\text{-}18}sid\text{-}1$ and $P_{sng\text{-}1}yfp$, which expresses YFP in all neurons and SID-1 in the TRNs, transformed into N2 worms to create TU3312.

TU3356 contains *uEx766*, an extrachromosomal array containing $P_{unc\text{-}119}sid\text{-}1$ and $P_{unc\text{-}4}mdm2::gfp$ (plasmid TU#703 from ref. 32); this DNA encodes a fusion of GFP and the RING domain of mammalian Mdm2 E3 ubiquitin ligase, which expresses YFP in a subset of motor neurons and SID-1 in all neurons. *uIs69* contains pCFJ90 ($P_{myo\text{-}2}$ *mCherry*) and TU#867 ($P_{unc\text{-}119}sid\text{-}1$) integrated into *sid-1(pk3321)* worms (TU3401) .

TU3403 is a strain containing *uIs71*, an integrated array of pCFJ90 ($P_{myo\text{-}2}$ *mCherry*)33 and TU#866 ($P_{mec\text{-}18}sid\text{-}1$), and *ccIs4251* (*myo-3::Ngfp-lacZ, myo-3::Mtgfp*); *sid-1(qt2)*]. The ccIs4251; *sid-1(qt2)* comes from HC75 (ref. 5). TU3568 is *uIs71; sid-1(pk3321) him-5(e1490); lin-5b(n744)*.

RNAi by feeding

Bacteria expressing dsRNA, taken from the Ahringer library4,5 were grown on LB plates supplemented with ampicillin at 37°C overnight. Next morning a large amount of bacterial lawn was inoculated in LB liquid supplemented with ampicillin and grown for 6 to 8 hours. The resulting culture was seeded onto 1-day-old NGM/IPTG/carbenicillin plates4 and allowed to dry for 24 or 48 hours. 20 to 40 embryos obtained by bleaching of gravid hermaphrodites, were added to each plate after the plates dried out and grown at 15°C. Worms treated with all dsRNA used in this work, were examined as adults five days after the embryos were added to the plates, at 15°C. Worms fed with dsRNA for *mec-4, gfp, elt-2, nhx-2* and the 15 neuronal genes, were also scored as adults in the next generation (F1). For the quantification of *mec-4* and *gfp* RNAi, F1 worms were transferred as L1 larvae and then again as L4 larvae to fresh RNAi plates and then scored 36 hours later.

Most of the fluorescent markers contained the coding region for *yfp*, which differs from *gfp* in eight nucleotides: 193 (T in *gfp* for C in *yfp*), 202 (G for C), 214 (T for G), 216 (G for C), 239 (G for A), 607 (A for T), 608 (C for A) and 609 (A for C). This changes result in five amino acid changes in from GFP to YFP (C65G, V68L, S72A, R80Q and T203Y). These differences do not prevent the targeting of *yfp* transcripts in RNAi experiments by feeding worms with *gfp* dsRNA (pPD128.110 from the Fire vector collection).

Microscopy

YFP fluorescence and differential interference contrast were observed using a Zeiss Axiophot II. All photographs of RNAi treated worms were taken with a Diagnostic Instruments Spot 2 camera using a Plan NEOFUAR 25× objective, for 300 ms at a gain of 1. YFP intensity was quantified using Image J (http://rsb.info.nih.gov/ij/). A fixed area of 200 (width) × 500 (length) pixels was measured from the tip of the nose, which covered the entire nerve ring.

Touch sensitivity

Twenty to thirty adult worms (36 hours after L4 stage at 15°C) were touched gently with an eyebrow hair26 ten times with alternative anterior and posterior touches (five each) to determine an average response. These experiments were repeated several times to obtain a mean and S.E.M. Because the effects on anterior touch were stronger in these experiments, we have usually reported only those responses. Only worms that moved when prodded with a platinum wire and that looked normal were assayed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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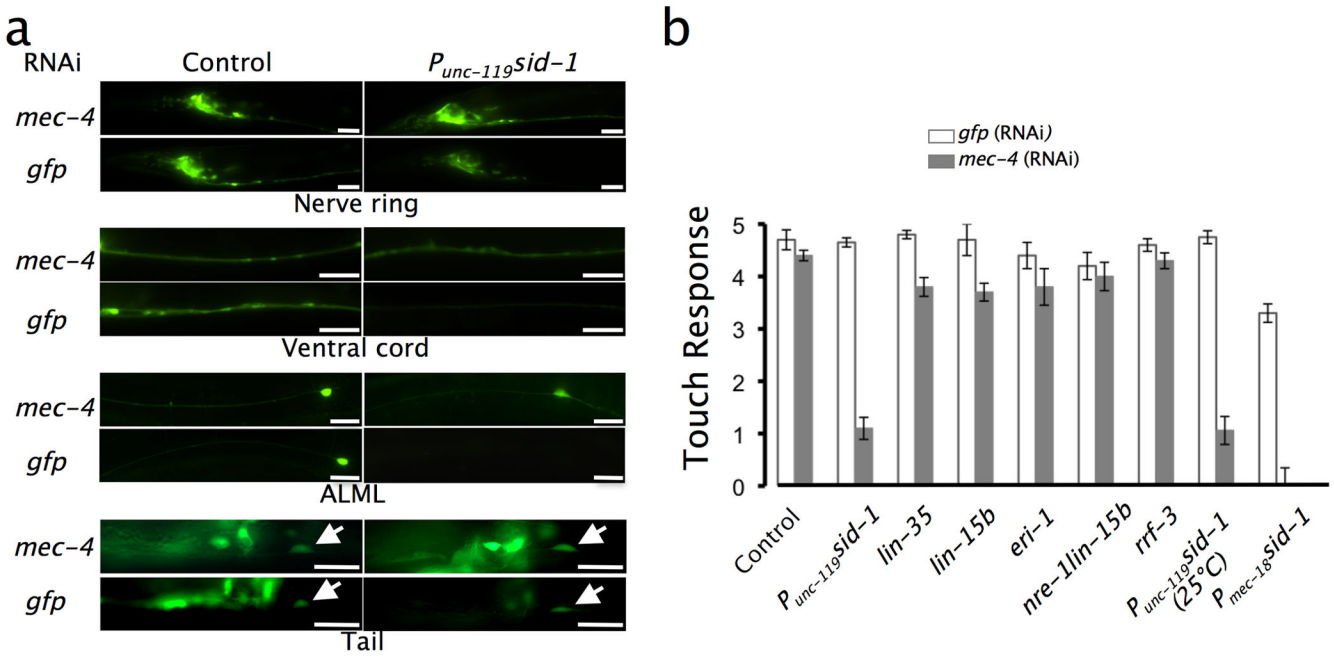

Figure 1. Expression of *sid-1* in neurons enhances neuronal RNAi

(**a**) Neuronal YFP fluorescence in different areas of $P_{unc-119}$*sid-1* (TU3270) and control worms (TU3310) fed with bacteria producing dsRNA for *gfp*. Both strains contain $P_{unc-119}$*yfp*. Worms fed *mec-4* dsRNA were used as controls. Similar results were obtained with the TU3311 strain. Arrows indicate PLML neurons. Scale bars = 25 μm, except ALML (10 μm). (**b**) Touch sensitivity of worms of the indicated genotypes or expressing the indicated transgenes, fed with either *mec-4* dsRNA (gray bars) and *gfp* dsRNA (white bars). Similar results were obtained when worms expressing P_{mec-18}*sid-1* were fed bacteria expressing dsRNA for *mec-12*. The same effect as for $P_{unc-119}$*sid-1* worms (TU3270) was obtained with TU3311. Values represent the mean ± S.E.M. of four experiments, each with 30 adults, except for the *mec-18* data, which was from nine experiments with 20 adults.

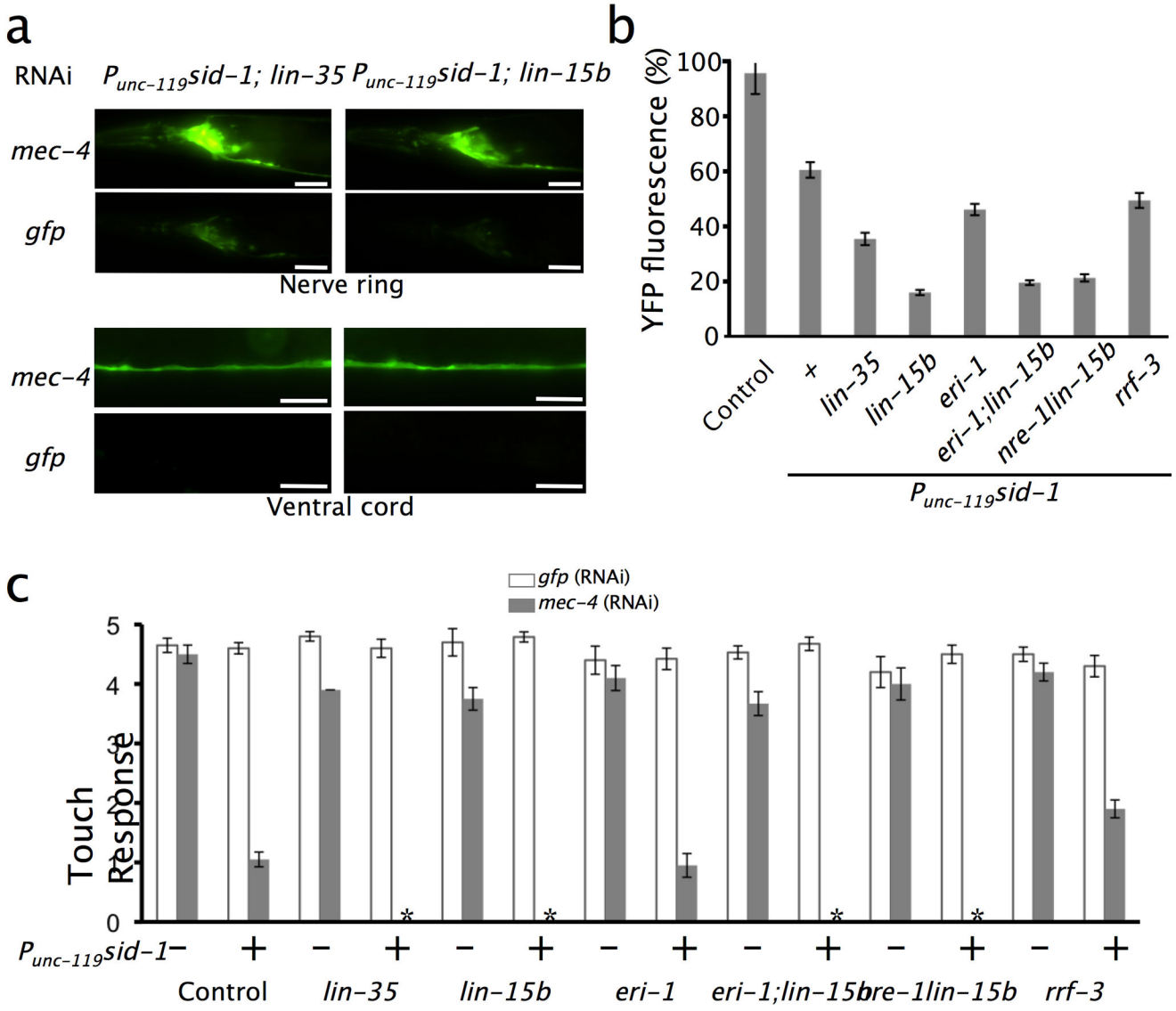

Figure 2. Mutations in *lin-35* and *lin-15b* enhance RNAi in neurons expressing *sid-1*

(**a**) YFP expression in the nerve ring and ventral cord of worms with the indicated genotypes after feeding with bacteria making dsRNA for *gfp* or for *mec-4* (compare with worms in Fig. 1). Both strains contain $P_{unc-119}$*yfp*. Scale bars = 25 μm. (**b**) YFP fluorescence in the nerve ring of the indicated strains after feeding with bacteria making *gfp* dsRNA. The control strain is TU3310, expressing $P_{unc-119}$*yfp* alone. Strains with $P_{unc-119}$*sid-1* were derived from TU3270 strain and have the *mec-6(+)* transgene. Results are presented as the mean percentage of fluorescence (± S.E.M.) measured in the same strain fed bacteria making *mec-4* dsRNA (three experiments, each with 30 adult worms). (**c**) Anterior touch response in worms of the indicated strains fed bacteria making dsRNA for *gfp* (white) or *mec-4* (gray). Values represent the mean ± S.E.M. of four experiments, each with 30 adults. The asterisk represents significance at $p < 0.05$.

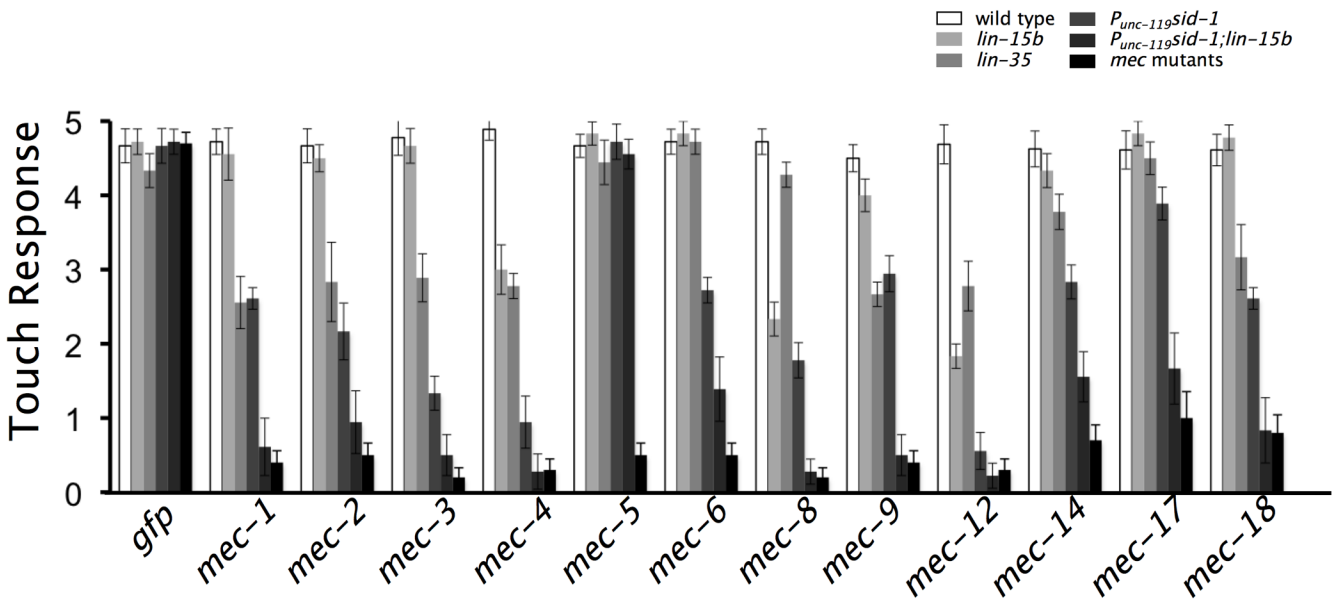

Figure 3. Enhanced RNAi for genes needed for touch sensitivity

The plots show the anterior touch response (out of five touches) in worms of the indicated genotypes fed bacteria making dsRNA for known *mec* genes (which give a touch insensitive phenotype when mutated). *mec* mutants were examined for comparison. Strains with $P_{unc-119}$*sid-1* were derived from TU3270 strain and have the *mec-6(+)* transgene. Except for the *mec* mutants, each value is the mean response (± SEM) of worms on 9 RNAi plates with 20 adult worms each. The values for the *mec* mutants represent the mean response (± SEM) of 20 adult worms.

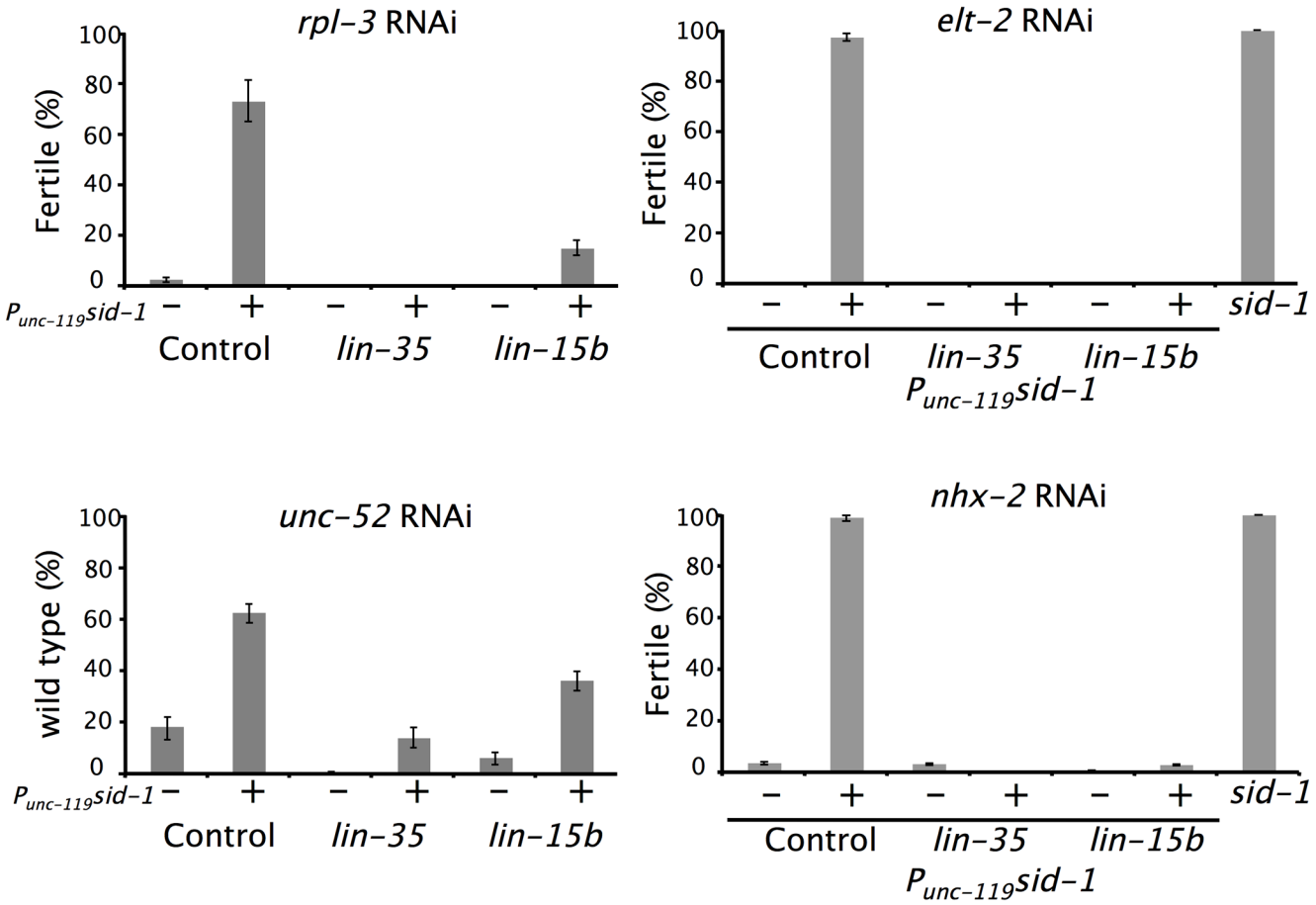

Figure 4. Expression of *sid-1* in neurons decreases RNAi responses in non-neuronal tissues

The fraction of worms of the indicated genotypes that resisted treatment with dsRNA for various genes (*rpl-3*, *unc-52*, *elt-2* and *nhx-2*) is plotted. For *rpl-3* we counted the number of worms that reached adulthood and became fertile; for *unc-52* we counted the number of paralyzed worms, and for *elt-2* and *nhx-2* we counted the number of fertile F1 worms. We used wild type (N2) as controls that do not express $P_{unc\text{-}119}$*sid-1*(+) and strain TU3270 as controls that do. *sid-1* mutants were included as negative controls. Values represent the mean ± S.E.M. of nine plates, 50 worms scored per plate.

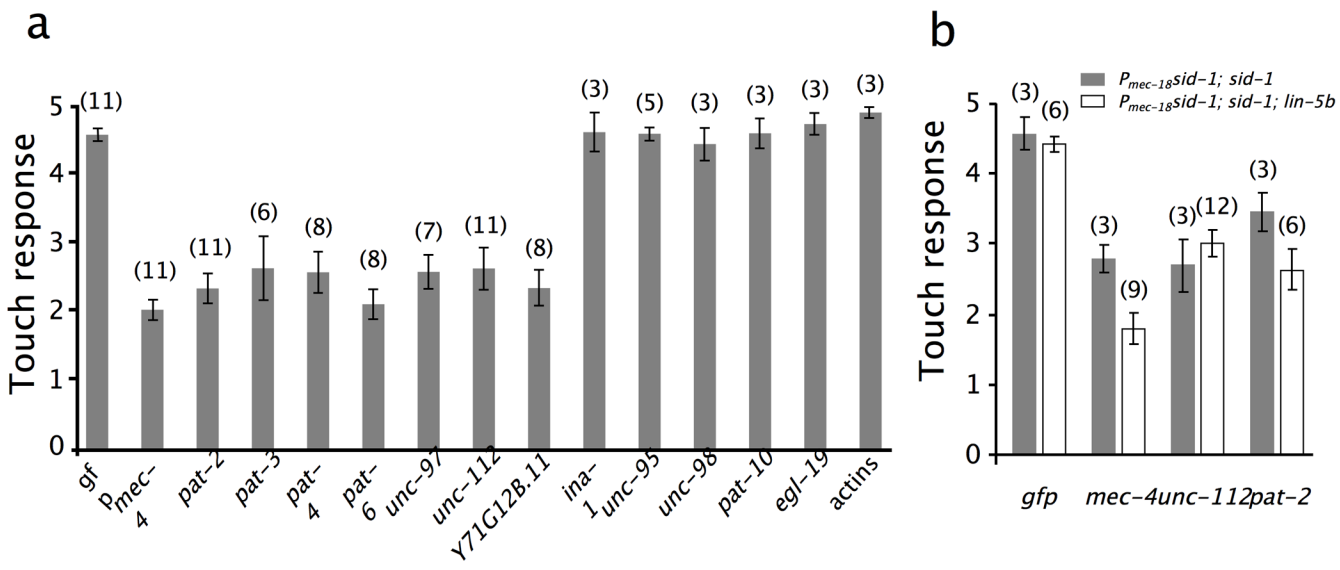

Figure 5. Eliminating integrin signaling proteins by RNAi in neurons

(**a, b**) The plots show the touch response (out of five touches) of $P_{unc\text{-}119}$*sid-1*-expressing worms (**a**), $P_{mec\text{-}18}$*sid-1; sid-1* worms (**b**, grey bars) or $P_{mec\text{-}18}$*sid-1; sid-1; lin-5b* worms (**b**, white bars) fed bacteria making dsRNA for the indicated genes. In (**a**), combined results for three strains (TU3270, TU3311, and TU3401, see Methods for details) are shown, because all gave similar results. Values are mean response ± S.E.M, 20 adult animals/plate; numbers indicate the number of plates examined.

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Table 1

Feeding RNAi for known neuronal genes

Gene	Loss-of-function Phenotype[a]	RNAi Phenotype[b]								
		$P_{unc-119}$*sid-1*		$P_{unc-119}$*sid-1; lin-35*		$P_{unc-119}$*sid-1; lin-15b*		*lin-35*[e]	*nre-1 lin-15b*	*eri-1; lin-15b*
unc-13	Let	**	++	**	++	**	++	-	-	-
unc-14[c]	Severe Unc	**	+	**	+	***	+++	-	-	-
unc-55[c]	Coiler	**	++	**[d]	++[d]	***	+++	-	-	-
unc-58	Mild Unc	*	+	*	+	*	+++	-	-	-
unc-76	Coiler	*[d]	+[d]	*	+	*	++	-	-	-
unc-119	Severe Unc; Prz	*	++	*	++	*	+++	-	-	-
vab-8	Unc	-		*	+	*	+	-	-	-
unc-5	Coiler	-		-		-		-	-	-
unc-7	Forward Kinker	-		-		-		-	-	-
unc-10	Coiler	-		-		-		-	-	-
unc-17	Let	-		-		-		-	-	-
unc-24	Kinker	-		-		-		-	-	-
unc-30[c]	Shrinker	-		-		-		-	-	-
unc-42	Kinker	-		-		-		-	-	-
unc-79	Fainter	-		-		-		-	-	-

[a] The Unc (uncoordinated) phenotype includes a broad category of movement defects, within which are Coiler, Kinker, Shrinker, and Paralyzed (Prz). Loss of some genes produced lethality (Let).

[b] The severity of the RNAi phenotype is described with regard to penetrance (*, a few, **, many; ***, most) and expressivity (+, weak phenotype compared with the loss-of-function phenotype, ++, intermediate: some worms show a weak phenotype, others have the loss-of-function phenotype; +++, phenotype is indistinguishable from the loss-of-function phenotype). RNAi for *unc-13* produced slow-growing, slow-moving coilers. Strains with no RNAi-induced phenotype are listed as –.

[c] Feeding RNAi phenotypes have been reported for these genes when an *rrf-3* mutation is present11.

[d] The phenotype was seen in the F1 progeny, but not in the worms themselves grown on the indicated bacteria.

[e] The same results were obtained when RNAi was tested in *lin-35, lin-15b, eri-1*, or *rrf-3* mutant strains.