Double-stranded RNA-mediated gene interference (RNAi) in Caenorhabditis elegans systemically inhibits gene expression throughout the organism. To investigate how gene-specific silencing information is transmitted between cells, we constructed a strain that permits visualization of systemic RNAi. We used this strain to identify systemic RNA interference deficient (sid) loci required to spread gene-silencing information between tissues, but not to initiate or maintain an RNAi response. One of these loci, sid-1, encodes a conserved protein with predicted transmembrane domains. SID-1 is expressed in cells sensitive to RNAi, is localized to the cell periphery, and is required cell-autonomously for RNAi.

One of the first reported and still mysterious aspects of RNAi is that it is systemic. Injection of gene-specific double-stranded RNA (dsRNA) into one tissue leads to the post-transcriptional silencing of that gene in other tissues and in that worm’s progeny (1). The systemic nature of RNAi also provides for initiation of RNAi by soaking animals in dsRNA (2, 3) or by cultivating worms on bacteria expressing dsRNA (4, 5). Although systemic RNAi has not been demonstrated in any other animal, systemic post-transcriptional gene silencing (PTGS) effects in plants are well established (6, 7). PTGS appears to play a role in viral defense (8), while viruses are able to inhibit systemic PTGS (9).

Genes required for RNAi have been identified in a variety of systems, as have small interfering RNAs (siRNAs) that can directly trigger RNAi and act as guide RNAs that direct sequence-specific mRNA cleavage (10, 11, 12). Among the C. elegans genes required for RNAi are rde-1 and rde-4, which have no readily detectable mutant phenotype other than resistance to RNAi (13). These mutants are resistant to dsRNA targeting both somatic and germline-specific genes and are also resistant to dsRNA produced by transgenes (13). However, these genes are not involved in systemic RNAi, as homozygous rde-1 or rde-4 mutant animals injected in the intestine with dsRNA are capable of efficiently transporting the RNAi effect to heterozygous cross progeny (13). Interestingly, rde-4 is required for the efficient production of siRNAs (14), suggesting that siRNAs are not required for systemic RNAi.

To specifically investigate systemic RNAi, we constructed a transgenic strain (HC57) that allows simultaneous monitoring of localized and systemic RNAi. HC57 expresses two Green Fluorescent Protein (GFP) transgenes, one expressed in the pharyngeal muscles (myo-2::GFP) and the other expressed in the body-wall muscles (myo-3::GFP). To initiate RNAi, a third transgene was introduced that expresses a GFP dsRNA construct under the control of the pharynx-specific myo-2 promoter (myo-2::GFP dsRNA) (15). In HC57, localized RNAi of myo-2::GFP in the pharynx was highly penetrant, but incomplete and temperature sensitive (Fig. 1B), while systemic RNAi of myo-3::GFP in body-wall muscle was position-dependent and also temperature-sensitive (Fig. 1B, C) (15). Systemic RNAi did not require expression of GFP in the pharynx, as expression of only myo-2::GFP dsRNA led to silencing of GFP in body-wall muscle (Fig. 1D). Silencing in both the pharynx and body-wall muscles was dependent on rde-1, verifying that the silencing was due to RNAi (Fig. 1G).

We used the HC57 strain to identify systemic RNAi defective (sid) mutants, by screening for animals resistant to systemic RNAi of myo-2::GFP in the body-wall muscles, but still sensitive to cell-autonomous RNAi of myo-2::GFP in the pharynx (15). To enhance the sensitivity of the screen, we incorporated bacteria-mediated RNAi of GFP to completely eliminate expression of myo-2::GFP (Fig. 1F) (15). Prospective sid mutants expressed GFP strongly in body-wall muscles, but continued to show weak GFP expression in pharyngeal cells. We identified at least 106 independent sid mutants that define three major complementation groups (sid-1, -2, -3 [48, 33, and 25 recessive alleles, respectively]). Here we describe the characterization and isolation of sid-1.

Sid-1 mutants retain cell-autonomous RNAi in the pharynx, fail to show spreading of RNAi from the pharynx into the body-wall, and are completely resistant to bacteria-mediated RNAi of myo-3::GFP (Fig. 1H). sid-1 exhibits no other obvious phenotype and produces an approximately normal-sized brood.

To further characterize systemic RNAi resistance in sid-1 mutants, dsRNAs targeting different classes of mRNAs were introduced into a single allele (qt2) by a variety of methods. To show that sid-1(qt2) is not simply deficient for RNAi in body-wall muscle cells, we introduced a transgene expressing GFP dsRNA in body-wall muscle cells. This transgene effectively silenced GFP expression in wild-type and sid-1 muscle cells, consistent with sid-1 specifically affecting systemic RNAi (15, 16). As expected for a gene required for systemic RNAi, sid-1(qt2) worms show resistance to bacteria-mediated RNAi targeting both somatic (unc-22, unc-54) and germline (mex-3, mex-6) expressed genes (Table 1A, B, (17)).

Systemic RNAi can also be assayed by injecting dsRNA into either the intestine or the syncitial germline within the gonad. Injection of a few cell volumes of mex-3 dsRNA into the intestine of adult wild-type hermaphrodites effectively targets germline mex-3 transcripts, producing an embryonic lethal phenotype (Table 1A) (15). Injections into sid-1(qt2) hermaphrodites produced only viable progeny (Table 1A), demonstrating that the RNAi response cannot spread from the intestine to the germline in sid-1 mutants. When mex-3
dsRNA was injected into sid-1(qt2) gonad arms, embryonic lethality was observed, showing that sid-1 is not required for RNAi in the germline. In contrast to wild-type hermaphrodites, which when injected with mex-3 dsRNA directly into either one or both gonad arms produced nearly 100% embryonic lethality, injection of mex-3 dsRNA into just the anterior gonad arm of sid-1(qt2) worms only produced up to approximately 50% embryonic lethality in the progeny (Fig. 2A). These experiments show that sid-1 is required to spread the RNAi effect to the germline cells in the other gonad arm.

RNAi can also be transmitted to the progeny of injected animals (1). To determine whether this requires sid-1 and therefore systemic RNAi, we analyzed silencing of unc-22 in the progeny of injected worms. Injecting either the intestine or the gonad of wild-type hermaphrodites with unc-22 dsRNA efficiently produces a twitching phenotype among their progeny (1, 15). We found by similar injections that sid-1 is required for transmission of RNAi to the progeny (Table 1B). We then asked whether supplying sid-1(+) to the embryos could restore systemic RNAi. We injected unc-22 dsRNA into the gonad of sid-1(qt2) hermaphrodites and crossed them to wild-type males to determine whether the sid-1(+) progeny were now susceptible to unc-22 RNAi. We found that the sid-1(+) progeny were susceptible (Table 1B). This suggests that embryos that inherit either dsRNA or an autonomous RNAi response require sid-1 function to transmit the effect to somatic tissues, perhaps indicating that transmission of RNAi to the progeny requires systemic spread of an amplified RNAi signal (18, 19).

The identity of sid-1 was determined by genetic mapping and DNA transformation rescue and corresponds to the predicted open reading frame C04F5.1 (Web figs. 1, 2) (15). To confirm the structure of the predicted gene and to verify the identity as sid-1, we isolated and sequenced wild-type complementary DNA (cDNA) and mutant genomic DNA (gDNA) (15). cDNA sequencing revealed an extra 387 bp (exon 4) not found in the reported genomic sequence of C04F5.1. Sequencing gDNA confirmed that 1014 nucleotides are missing from coordinate 5122952 of the curated genomic sequence. exon/intron structure and predicted domains of the revised gene are shown in Fig. 2B. Sequencing gDNA from 10 mutant alleles confirmed that C04F5.1 corresponds to sid-1 (Fig. 2B). SID-1 is predicted to encode a 776 amino acid protein with 11 transmembrane domains and localizes a GFP protein to the cell periphery. These observations suggest that sid-1 may act as a channel for dsRNA, siRNAs, or some undiscovered RNAi signal. An additional possibility is that sid-1 may be necessary for endocytosis of the systemic RNAi signal, perhaps functioning as a receptor. Consistent with the cell-autonomous requirement for sid-1 function and the global nature of systemic RNAi, we detected sid-1::GFP in most non-neuronal cells. The failure to detect sid-1::GFP in the majority of neuronal cells is consistent with the observation that neuronal cells are generally resistant to systemic, but not autonomous RNAi (20). Notably, the few neurons that strongly express sid-1 have externally exposed axons (phasmids and male rays (17)). Similarly, the non-neuronal cells with the strongest sid-1::GFP levels are also the cells and tissues exposed to the environment. This suggests that sid-1 may be involved in responding to environmental cues that may include viral and microbial pathogens. Finally, the absence of a detectable sid-1 homolog in Drosophila is consistent with the apparent lack of systemic RNAi in Drosophila (21, 22), while the strong similarity to predicted human and mouse proteins suggests the possibility that RNAi is systemic in mammals and that the mechanism may share some components found in C. elegans.

References
13. Thirty-four F1 transformed sid-1(qt2) worms showing RNAi of myo-3::GFP were recovered, and 27 of these produced transformed, myo-3::GFP RNAi F2s.
16. Supplementary figures and details of experimental procedures are available at Science Online at www.sciencemag.org/cgi/content/full/1068836/DC1.
21. Acknowledgements. Some nematode strains used in this work were provided by the Caenorhabditis Genetics Center, which is funded by the NIH National Center for Research Resources. The GenBank accession number for sid-1 cDNA nucleotide sequence is AF478687. We acknowledge the Washington University Genome Sequence Center for the unpublished C. briggsae information. We thank S. Wicks for sharing snp-SNP information prior to publication. A. Fire for plasmids, S. Mou for critical reading, and D. Moottz and A. Kay for critical reading of the manuscript. A Beckman Young Investigator award and a National Science Foundation award to C.P.H supported this work.

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Figure 1. Visualization of systemic RNAi. (A) HC46 strain expressing GFP in pharynx (ph) and body-wall muscle (bm) (25°C). (B, C) HC57 strain in which the pharynx driven GFP dsRNA hairpin has been introduced into the HC46 background at 25°C (B) and 20°C (C). (D) HC59 strain expressing GFP dsRNA in the pharynx and GFP in only the body-wall muscles. (E, F) Bacteria-mediated RNAi of GFP in HC46 (E) and HC57 (F) at 20°C. (G) rde-1(ne219) in the HC57 background (20°C). (H) sid-1(qt2) in the HC57 background at 20°C. All images are of adult hermaphrodites taken at equal exposures. Insets are white-light images of the corresponding worm(s). Anterior is to the left. Scale bars indicate 0.1 mm.

Figure 2. Autonomy and dose dependence of RNAi in sid-1 and wild-type gonads and sid-1 predicted gene structure. (A) sid-1 is required for systemic RNAi between gonad arms. Progeny of sid-1(qt2) and wild-type hermaphrodites injected with mex-3 dsRNA (1 mg/ml) in one or both gonad arms were scored for viability. Injection of 100, 10, and 1 μg/ml mex-3 dsRNA into both gonad arms showed similar effects at a given concentration (17). (B) Predicted sid-1 gene structure, protein domains, and sequenced mutations. The qt12 5' splice-site mutation following exon three is predicted to terminate translation seven amino acids after amino acid 142.

Figure 3. Mosaic analysis of sid-1 function. (A) sid-1(+)/sid-1(+) body-wall muscle cells are marked by the co-expression of DsRED2 (red). (B) Two muscle cells resistant to bacteria-mediated RNAi retain expression of GFP (green). (C) Only sid-1(+) muscle cells lose GFP expression. Scale bars indicate 10 μM.

Figure 4. SID-1::C-GFP reporter expression patterns. SID-1::C-GFP is expressed in (A) the procorpus (pc), metacorpus (mc), and terminal bulb (tb) of the pharynx; (C) intestine (i); (E) spermatheca (sp) and proximal somatic gonad (g); (G) sphincter muscles (sm), phasmid neurons (pn), and extreme posterior intestinal cells (ip), which is stronger compared to more anterior intestinal cells (i); and in (I) the excretory cell where it is localized throughout the tubular processes. Since the SID-1::C-GFP expression array is not integrated, mosaic expression patterns are observed (In (C) a non- or poorly-expressing intestinal cell is marked (i-).). (K) Cell periphery localization (arrow heads) of SID-1::C-GFP compared to (L) cytoplasmic GFP (myo-2::GFP) in deconvolved images representing 0.9 μM section of the pharynx. (B, D, F, H, J) Accompanying DIC images. Scale bars indicate 10 μM.

Table 1. Characterization of sid-1 systemic RNAi resistance. Progeny of sid-1(qt2) and wild-type worms exposed to unc-22 and mex-3 dsRNA by various methods were scored for RNAi phenotypes. Asterisks indicate that only cross progeny were scored. N.A. = not applicable. n = number of progeny scored.
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Table 1

### A. *mex-3* RNAi

<table>
<thead>
<tr>
<th>dsRNA delivery (Hours after injection)</th>
<th>wild-type N2</th>
<th><em>sid-1 (qt2)</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria-mediated (N.A.)</td>
<td>100% (615)</td>
<td>1% (535)</td>
</tr>
<tr>
<td>Intestine (12.5 to 24.5)</td>
<td>86% (665)</td>
<td>2% (782)</td>
</tr>
</tbody>
</table>

### B. *unc-22* RNAi

<table>
<thead>
<tr>
<th>dsRNA delivery (Hours after injection)</th>
<th>wild-type N2</th>
<th><em>sid-1 (qt2)</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria-mediated (N.A.)</td>
<td>100% (394)</td>
<td>0% (363)</td>
</tr>
<tr>
<td>Intestine (11 to 23)</td>
<td>68% (701)</td>
<td>0% (563)</td>
</tr>
<tr>
<td>Intestine crossed to wild-type males (7.5 to 31.5)</td>
<td>70% (497)*</td>
<td>14% (571)*</td>
</tr>
<tr>
<td>Anterior gonad arm (15.5 to 42.5)</td>
<td>89% (688)</td>
<td>0% (981)</td>
</tr>
<tr>
<td>Both gonad arms (7 to 40.5)</td>
<td>80% (886)</td>
<td>2% (1050)</td>
</tr>
<tr>
<td>Both gonad arms crossed to wild-type males (12 to 24)</td>
<td>99% (206)*</td>
<td>63% (380)*</td>
</tr>
</tbody>
</table>

*sid-1 (qt2), dpy-11/+ (Dpy-11 progeny scored) (n)*

| Intestine (9.5 to 24.5) | 96% (147) |
| Both gonad arms (9.5 to 24.5) | 98% (127) |
Systemic RNAi in *C. elegans* Requires the Putative Transmembrane Protein SID-1

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