Images in 14 bits were converted to 8 bits with NIH Image 1.6, and all images were composed with Adobe Photoshop 5.5.

34. We thank A. Dernburg and members of the Heald and Weis labs for helpful discussions and/or comments on the manuscript. M. Welch for help with fluorimetry, Y. Azuma for help with RCC1 purification, M. Ignatius for help with microscopy, and G. O. Nads for sperm nuclei. Funded by grants from NIH and the Pew Scholars Program (R.H.) and the Searle Scholars Program (K.W.).

7 December 2001; accepted 25 February 2002

**Systemic RNAi in C. elegans Requires the Putative Transmembrane Protein SID-1**
William M. Winston, Christina Molodowitch, Craig P. Hunter*

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 systemic RNAi.

One of the first reported and still mysterious aspects of RNAi in C. elegans is that it is systemic. Injection of gene-specific double-stranded RNA (dsRNA) into one tissue leads to the posttranscriptional 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 posttranscriptional gene silencing (PTGS) effects in plants are well established (6, 7). PTGS appears to play a role in viral defense (8); at the same time, 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 (10–12). 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 germ line–specific genes and are also resistant to dsRNA produced by transgenes (13). However, these genes are not involved in systemic RNAi, because 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). It is noteworthy that 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, compare with 1A), whereas systemic RNAi of myo-3::GFP in body-wall muscle was position-dependent and also temperature-sensitive (Fig. 1, B and 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 RNA interference defective (sid) mutants, by screening for animals resistant to systemic RNAi of myo-3::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-3::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 reference 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 germ line (mex-3, mex-6) expressed genes (Table 1 (17)).

Systemic RNAi can also be assayed by injecting dsRNA into either the intestine or the syncitial germ line within the gonad. Injection of a few cell volumes of mex-3 dsRNA into the intestine of adult wild-type hermaphrodites effectively targets germ line 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 germ line 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

### Table 1. Characterization of sid-1 systemic RNAi resistance.

<table>
<thead>
<tr>
<th>dsRNA delivery (hours after injection)</th>
<th>Percent embryonic lethal</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) mex-3 RNAi</td>
<td>Wild-type N2</td>
</tr>
<tr>
<td>Bacteria-mediated (NA)</td>
<td>100 (615)</td>
</tr>
<tr>
<td>Intestine (12.5 to 24.5)</td>
<td>86 (665)</td>
</tr>
<tr>
<td>(B) unc-22 RNAi</td>
<td></td>
</tr>
<tr>
<td>Bacteria-mediated (NA)</td>
<td>100 (394)</td>
</tr>
<tr>
<td>Intestine (11 to 23)</td>
<td>68 (701)</td>
</tr>
<tr>
<td>Intestine crossed to WT males (7.5 to 31.5)</td>
<td>70 (497)*</td>
</tr>
<tr>
<td>Anterior gonad arm (15.5 to 42.5)</td>
<td>89 (688)</td>
</tr>
<tr>
<td>Both gonad arms (7 to 40.5)</td>
<td>80 (886)</td>
</tr>
<tr>
<td>Both gonad arms crossed to WT males (12 to 24)</td>
<td>99 (206)*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Percent twitching progeny</th>
<th>Wild-type N2</th>
<th>sid-1 (qt2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestine (9.5 to 24.5)</td>
<td>96 (147)</td>
<td></td>
</tr>
<tr>
<td>Both gonad arms (9.5 to 24.5)</td>
<td>98 (127)</td>
<td></td>
</tr>
</tbody>
</table>

sid-1(qt2) dpy-11/++ (Dpy-11 progeny scored)
germ line. 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 germ line 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 and 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. The 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 with nematode, human, and mouse homologs [Web fig. 3 (15)].

Because sid-1 is required for systemic RNAi and is predicted to be transmembrane, it is reasonable to predict that it is required for the import or export of a systemic RNAi signal. To determine whether SID-1 is required autonomously to import a bacteria-mediated RNAi signal or whether it can function nonautonomously to deliver a signal from a neighboring cell, we analyzed sid-1 genetic mosaics. A sid-1(qt2) strain expressing myo-3::GFP was injected with sid-1 gDNA and myo-3::DsRED2 to produce an extrachromosomal array that both rescues sid-1(qt2) and expresses the red fluorescent protein DsRED2 in body-wall muscle cells (15). Because extrachromosomal arrays are mitotically unstable, these lines produce mosaic worms composed of both DsRED2(+) sid-1(+) cells and DsRED2(-) sid-1(-) cells. If sid-1 functions cell-autonomously, then

Fig. 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 selected mutations. The qt12 5’ splice-site mutation following exon 3 is predicted to terminate translation seven amino acids after amino acid 142.
DsRED2(+), sid-l(+) muscle cells will be sensitive to systemic RNAi of GFP, whereas DsRED2(-), sid-l(-) cells will be resistant. If sid-l functions nonautonomously, then DsRED2(-), sid-l(-) cells may be sensitive to systemic RNAi if they are adjacent to DsRED2(+), sid-l(+) cells. To distinguish between these possibilities, we exposed sid-l mosaic worms to bacteria expressing GFP dsRNA and examined DsRED2 expression at the boundaries between GFP-silenced muscle cells and those that were not silenced. In all 35 scored boundaries, cells that were sensitive to systemic RNAi (GFP silenced) were sid-l(-) (DsRED2 expressed), and cells that were resistant (GFP expressed) were sid-l(-) (DsRED2 not expressed) (Fig. 3). Although these results do not address the possibility that sid-l may function in the export of a systemic RNAi signal, they do show that sid-l is required to import or process one.

To determine when and where sid-l is expressed, we introduced transcriptional and translational sid-l::C-GFP transgenes into wild-type animals. A transgene that includes only the sid-l promoter region fused to GFP (sid-l pro::GFP), is expressed in late embryos and continues to be expressed at the boundaries between GFP-silenced muscle cells and tissues in direct contact with the environment. These environment-exposed cells also show expression of a transgene construct in which GFP was fused to the COOH-terminus of full-length SID-1 (sid-l::C-GFP) (Fig. 4 (15)). This construct was capable of rescuing sid-l(q2) animals, suggesting that localization of this reporter is representative of SID-1 localization. In addition to cytoplasmic expression, significant enrichment of subcellular localization of sid-l::C-GFP was seen at the cell periphery, consistent with the presence of predicted transmembrane regions in SID-1 (Fig. 4, K and L). Expression of sid-l::C-GFP was only seen in these environment-exposed cells, perhaps because of the significantly lower expression of the translational reporter relative to the transcriptional reporter.

sid-l functions cell-autonomously for systemic RNAi; encodes a protein with a signal peptide sequence and 11 putative transmembrane domains, and localizes a GFP protein fusion to the cell periphery. These observations suggest that sid-l may act as a channel for dsRNA, siRNAs, or some undiscovered RNAi signal. An additional possibility is that sid-l may be necessary for endocytosis of the systemic RNAi signal, perhaps functioning as a receptor. Consistent with the cell-autonomous requirement for sid-l function and the global nature of systemic RNAi, we detected sid-l::GFP in most nonneuronal cells. The failure to detect sid-l::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-l have externally exposed axons (phasmids and male rays (17)). Similarly, the nonneuronal cells with the strongest sid-l::GFP levels are also the cells and tissues exposed to the environment. This suggests that sid-l may be involved in responding to environmental cues that may include viral and microbial pathogens. Finally, the absence of a detectable sid-l homolog in Drosophila is consistent with the apparent lack of systemic RNAi in Drosophila (21, 22), whereas 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.

Note added in proof: Transmission of RNAi to progeny has recently been reported in Tribolium (Coleoptera) (23).

References and Notes

15. Supplementary figures and details of experimental procedures are available on Science Online at www.sciencemag.org/cgi/content/full/1068836/DC1.
16. Of 34 F1 transformed sid-l(q2) worms showing RNAi of myo-2::GFP that were recovered, 27 produced transformed, myo-2::GFP RNAi F2 offspring.
24. 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. We acknowledge the Washington University Genome Sequence Center for the unpublished C. briggsae information. We thank S. Wicks for sharing snp-SNP information before publication, A. Fire for plasmids, S. Mou for technical help, and D. Mootz and A. Kay for critical reading of the manuscript. A Bednarz Young Investigator award and a National Science Foundation award to C.P.H supported this work. The GenBank accession number for sid-l cDNA nucleotide sequence is AF478687.

10 December 2001; accepted 25 January 2002
Published online 7 February 2002; 10.1126/science.1068836
Include this information when citing this paper.