Minireview

Spreading silence with Sid

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Abstract

RNA interference (RNAi) has been shown to spread from cell to cell in plants and in *Caenorhabditis elegans*, but it does not spread in other organisms, such as *Drosophila*. A recent report demonstrates that a membrane channel, encoded by the gene *sid-1*, is responsible for the spreading of RNAi between cells.

RNA interference (RNAi) is the phenomenon by which double-stranded RNA (dsRNA) sequences trigger the sequence-specific degradation of homologous endogenous mRNAs. RNAi was discovered serendipitously in 1998, when Andrew Fire and colleagues [1] were attempting to block gene expression by injection of antisense RNA into adult *Caenorhabditis elegans*. They discovered that the double-stranded RNA side-products of their RNA synthesis reactions were more effective inhibitors than single-stranded antisense RNA. Concentrated solutions of dsRNA have since become a potent experimental tool for inhibiting gene expression in *C. elegans* and other model organisms, including *Drosophila*.

RNAi in *C. elegans* has two striking characteristics. First, it is extremely specific and only targets mRNA sequences that are identical, not those that are closely related or highly homologous. Second, it is systemic: injection of dsRNA into the gut of a hermaphrodite individual allows gene suppression in most tissues of the animal, as well as effective suppression in most tissues of the animal's progeny. This 'spreading' characteristic underlies some of the most surprising observations in the short history of RNAi: namely that simply soaking worms in a solution of dsRNA [2], or feeding them transformed bacteria expressing dsRNA encoding a gene of choice [3], selectively suppresses the function of that gene in all of the individual's progeny. The latter 'feeding' induction technique has enabled successful large-scale genome-wide screens, in which banks of transgenic strains of *Escherichia coli*, each engineered to produce dsRNA for a single gene of the *C. elegans* genome, have been used to screen *C. elegans* genes for roles in embryonic development, genome stability, fat metabolism, longevity, and other biological processes [4-8].

As befits a pathway with such basic biological significance and such tremendous experimental potential, a great deal of recent work has gone into understanding the molecular mechanisms of RNAi. In the last five years great strides have been made in understanding the mechanisms by which dsRNA targets mRNA transcripts. The current picture (reviewed in [9,10]) is that dsRNA is cleaved into fragments of 21-23 nucleotides by the Dicer family of RNAse III enzymes. These short dsRNA fragments are then incorporated into another enzyme complex, called the RNA-induced silencing complex (RISC). The antisense strand of the dsRNA fragment targets the homologous mRNA for cleavage. In contrast, however, surprisingly little is known about the mechanisms that allow the spreading of RNAi from cell to cell; for instance, it is not known what kind of molecule conveys the systemic RNAi signal, nor why some tissue types in *C. elegans*, such as the nervous system, are more resistant to systemic RNAi than others. Two recent publications from the Hunter lab [11,12] have now made significant progress in

this direction. The first contribution, from 2002 [11], describes a successful screening strategy for identifying genes involved in this non-autonomous spreading of RNAi. The second, appearing in September 2003 [12], characterizes one of these genes and shows that it encodes a putative channel protein that functions in the uptake of dsRNA across cell membranes.

Hunter and colleagues took a clever approach to identify genes supporting the non-autonomous effects of RNAi. They constructed a strain of *C. elegans* that visibly demonstrates both cell-autonomous and non-autonomous RNAi and screened for mutants in which non-autonomous RNAi fails but cell-autonomous RNAi persists. The strain, described by Winston *et al.* [11], is one in which expression of green fluorescent protein (GFP) is driven in the muscles of both the pharynx and the body wall. Expression of a dsRNA that targets and silences the GFP gene is then driven by a transgene construct that expresses a hairpin (double-stranded) RNA only in pharyngeal muscles. This dsRNA triggers suppression of GFP in pharyngeal muscles, demonstrating that cell-autonomous RNAi is still functional, but it also triggers partial suppression of GFP expression in body-wall muscles, demonstrating systemic spreading of RNAi. The authors [11] completely silenced all GFP expression in this strain by additionally feeding these worms on transformed *E. coli* expressing *GFP* dsRNA, further demonstrating non-autonomous RNAi. They then mutagenized the strain to identify progeny in which GFP expression was suppressed in pharyngeal muscles but retained body-wall muscles. In such strains, the loss of GFP in pharyngeal muscles should confirm that cell-autonomous RNAi mechanisms remain intact, while the persistent expression of GFP in body-wall muscles suggests that the RNAi no longer spreads systemically to neighboring cells. Three complementation groups of 'systemic RNAi-deficient' animals were identified using this approach, and the mutations were mapped to genetic loci called *sid-1, sid-2*, and *sid-3*.

The *sid-1* gene encodes a 776-amino-acid protein with multiple stretches of hydrophobic amino acids, and Sid1-GFP fusion proteins concentrate at cell membranes [11]. Together, these data suggest that the Sid-1 gene product functions as a membrane channel for an RNAi-inducing signal. Using a β-galactosidase protein-fusion assay, Feinberg and Hunter [12] have now shown that the amino terminus of Sid-1 is extracellular and that multiple hydrophobic sequences in Sid-1 indeed span the plasma membrane of expressing cells. A membrane channel for RNAi could conceivably function either in the export and/or in the import of an RNAi signal: demonstration that *sid-1* is required cell-autonomously would indicate a function for the protein in the import of an RNAi signal, whereas demonstration that the protein functions non-cell-autonomously would be consistent with a role in export of RNAi. Hunter and colleagues addressed this issue in the original report describing the identification of *sid-1* [11]. Using an extrachromosomal transgene array driving mosaic expression of a *sid-1*-rescue transgene and a reporter construct encoding the red fluorescent protein DsRed in *sid-1* mutants, they tested the ability of GFP expression in body-wall muscles to be repressed by exogenous GFP dsRNA. In these *sid-1* mosaic animals, cells that were observed to have silenced GFP expression (indicating effective systemic RNAi) were found to be marked by DsRed, indicating that they also expressed functional Sid-1 from the extrachromosomal array, whereas neighboring cells in which GFP was not silenced (those resistant to systemic RNAi) all lacked Sid-1 expression [11]. Thus, Hunter and colleagues [11] inferred that *sid-1* is required for import of an RNAi signal. (The authors do not, however, exclude the possibility that *sid-1* may also be required for export of an RNAi signal.)

In their more recent work, Feinberg and Hunter [12] have shown that *sid-1* is not only required for systemic RNAi but is also sufficient when ectopically expressed to promote uptake of dsRNA in cultured cells of *Drosophila*. Flies lack an endogenous *sid-1* ortholog, but despite this, *Drosophila* cells (such as the S2 cell line) are known to be susceptible to RNAi induced by dsRNA in their culture medium [13,14]. Feinberg and Hunter [12] have shown, however, that S2 cells expressing Sid-1 showed equivalently effective RNAi of a luciferase transgene at dsRNA concentrations 10,000 times less than those required by cells lacking Sid-1 function. This effect appeared to be dependent on dsRNA length, as Sid-1 facilitated RNAi much more when cells were soaked in a solution of 500 base-pair (bp) dsRNAs than when they were soaked with 50 bp dsRNAs or 21 bp small interfering RNAs (siRNAs). Using a similar cell-culture assay, the authors [12] went on to show that Sid-1-mediated uptake of dsRNA probably takes place by a passive-diffusion mechanism: if transport were active it would be reduced in cold or energy-depleted conditions, but in fact the uptake of radiolabeled dsRNA by Sid-1-expressing S2 cells was affected by cold or ATP depletion less than the uptake of dsRNA by cells lacking functional Sid-1. Thus, the authors arrived at a model by which Sid-1 functions as a transmembrane channel protein, allowing passive diffusion of dsRNA into cells.

If RNAi functions as a primitive adaptive immune response, as has been postulated (discussed in [15]), then Sid-1 may clearly have a function in supplying cells with the dsRNA trigger for such a response. Consistent with such a role, endogenous Sid-1 in *C. elegans* appears to be expressed maximally in the cells that are exposed to the environment, such as those of the gut [11]. Beyond natural roles for the protein, however, as Feinberg and Hunter [12] concluded, Sid-1-mediated dsRNA uptake may have numerous therapeutic and experimental applications. Experimentally, high-throughput dsRNA 'soaking' screens of cell lines [16] may be made more efficient by transgenic expression of Sid-1. Cell lines that are otherwise refractory to such a soaking approach may be made responsive by expression of Sid-1.

Although RNAi induced by dsRNA transgenes in *Drosophila* is cell-autonomous and non-systemic [17], there is some evidence for the susceptibility of fly larvae to RNAi by soaking or ingesting dsRNA solutions [18] and for the susceptibility of adults to intra-abdominal dsRNA injection [19]. Soaking or injection screens in *Drosophila* or other organisms that lack effective systemic RNAi, such as vertebrates, may be dramatically improved by creation of strains ubiquitously expressing exogenous Sid-1. Feinberg and Hunter [12] postulate that manipulating levels of Sid-1 in vertebrates may facilitate the future therapeutic use of RNAi to modulate gene expression *in vivo*. Such applications may depend on the size-dependence of dsRNA transport mediated by Sid-1, however, as longer dsRNA sequences provoke non-specific responses in vertebrate cells. Potential RNAi-based therapies have focused on the application of short siRNA sequences [20,21]. Nonetheless, the description of *sid-1* makes an extremely important contribution to our understanding of the systemic nature of RNAi in *C. elegans*, an under-researched aspect of this fundamentally important biological phenomenon. Characterization of Sid-2 and Sid-3 is eagerly awaited.

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