

Extracellular RNA is transported from one generation to the next in *Caenorhabditis elegans*

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Experiences during the lifetime of an animal have been proposed to have consequences for subsequent generations. Although it is unclear how such intergenerational transfer of information occurs, RNAs found extracellularly in animals are candidate molecules that can transfer gene-specific regulatory information from one generation to the next because they can enter cells and regulate gene expression. In support of this idea, when double-stranded RNA (dsRNA) is introduced into some animals, the dsRNA can silence genes of matching sequence and the silencing can persist in progeny. Such persistent gene silencing is thought to result from sequence-specific interaction of the RNA within parents to generate chromatin modifications, DNA methylation, and/or secondary RNAs, which are then inherited by progeny. Here, we show that dsRNA can be directly transferred between generations in the worm *Caenorhabditis elegans*. Intergenerational transfer of dsRNA occurs even in animals that lack any DNA of matching sequence, and dsRNA that reaches progeny can spread between cells to cause gene silencing. Surprisingly, extracellular dsRNA can also reach progeny without entry into the cytosol, presumably within intracellular vesicles. Fluorescently labeled dsRNA is imported from extracellular space into oocytes along with yolk and accumulates in punctate structures within embryos. Subsequent entry into the cytosol of early embryos causes gene silencing in progeny. These results demonstrate the transport of extracellular RNA from one generation to the next to regulate gene expression in an animal and thus suggest a mechanism for the transmission of experience-dependent effects between generations.

circulating RNA | parental RNAi | epigenetics | transgenerational inheritance | endocytosis

The impact of ancestral experiences on descendants in animals has been evaluated and reevaluated for more than a century. Recent studies in animals have focused on changes in diet and stress as triggers in ancestors and found that such experiences correlate with changes in descendants (reviewed in refs. 1–4). Changes in diet, for example, are correlated with mortality of grandprogeny in humans (5), altered metabolism of progeny in mice (6), and longevity of descendants in the worm *Caenorhabditis elegans* (7). Maternal separation (8), social defeat (9), and chronic variable stress (10) are correlated with hypersensitivity to similar stresses in descendants in mice. Molecules that transmit gene regulatory information from one generation to the next generation in response to somatic cells that experience the effects of diet or stress could provide a mechanistic explanation for the observed correlations. Extracellular RNAs are candidates for transmitting gene-specific information from somatic cells to the germline and thus to the next generation because they can be detected in circulation (e.g., ref. 11), their composition is altered in disease states (e.g., ref. 12), and they can enter cells to regulate genes of matching sequence (e.g., ref. 13) (reviewed in ref. 14).

Studies in the worm *C. elegans* have provided some of the clearest evidence for RNA acting as a carrier of gene-specific information from somatic cells to germ cells in an animal. Expression of double-stranded RNA (dsRNA) in *C. elegans* neurons generates mobile RNAs that can silence a gene of matching sequence through RNA interference (RNAi) within the germline, and this silencing can persist for more than 25 generations (15). Similar persistent silencing also occurs when dsRNA is delivered into worms by

injection (16), by soaking (17), or through expression within bacteria that worms ingest as food (18). Silencing of somatic genes typically persists for one generation, but silencing of germline genes can persist for many more generations (see Fig. S1 for a summary of previous studies). Silencing by extracellular dsRNA requires entry into the cytosol, which is the aqueous component of the cytoplasm within which various organelles and particles are suspended. In all cases, entry of extracellular dsRNA into the cytosol of *C. elegans* cells requires the dsRNA-selective importer SID-1 (19–21). Upon entry into the cytosol, dsRNA is processed to generate small RNAs that are used as guides to identify mRNA of matching sequence. The target mRNA is then used as a template to generate numerous secondary small RNAs that can direct the deposition of repressive chromatin marks (reviewed in ref. 22). Although secondary small RNAs and chromatin marks have been detected in progeny upon parental exposure to dsRNA (23, 24), it is unknown where extracellular dsRNA needs to interact with intracellular RNA or DNA to cause gene silencing in progeny.

Here, we show that extracellular dsRNA can be transported to progeny without entry into any cytosol in the parent and that, upon entry into the cytosol in embryos, it can silence genes of matching sequence. Processing of ingested dsRNA within the parental germline or in early development of progeny generates additional forms of dsRNA that spread between cells in progeny to cause potent gene silencing. Use of fluorescently labeled RNA reveals that the dsRNA is imported into oocytes via the yolk endocytosis pathway.

Results

Silencing Signals Are Transported to Progeny Through Oocytes. To evaluate gene silencing in progeny upon ingestion of dsRNA, we fed worms bacteria that express dsRNA, removed the bacteria,

Significance

Changes in the environment or in physiology can induce an animal cell to secrete signaling molecules that move through circulation to regulate distant cells. Extracellular RNAs can accumulate in circulation in humans, can change during disease, and can potentially act as signaling molecules, but their source and destination are typically unclear. When extracellular RNAs enter cells, they can regulate genes of matching sequence. We show that double-stranded RNA introduced into the circulation of the animal *Caenorhabditis elegans* can be transported to the next generation through oocytes and can silence matching genes in progeny. These results demonstrate that extracellular RNA can carry gene regulatory information between generations. Such intergenerational messages could transmit effects of ancestral experience to descendants in animals.

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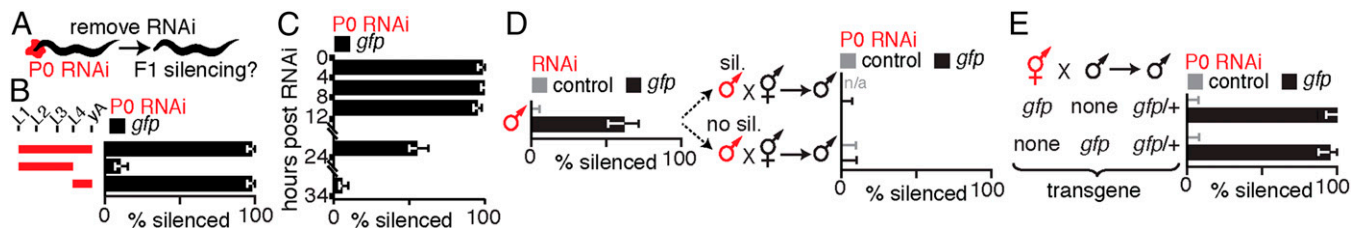


Fig. 1. Ingested dsRNA or dsRNA-derived silencing signals can be transported to progeny through oocytes by parents that lack DNA of matching sequence. (A) Schematic of assay to assess silencing in progeny (F1) by parental ingestion of dsRNA (P0 RNAi, red). Also see Fig. S2. (B and C) Silencing of multicopy *gfp* transgenes in progeny by ingested dsRNA. (B) Robust silencing of *Psur-5::sur-5::gfp* in intestinal cells required parental ingestion of *gfp*-dsRNA during adulthood. L1 to L4, larval stages; yA, young adult. (C) Silencing of *Pmyo-3::gfp* in muscle cells after parental ingestion of *gfp*-dsRNA was detectable in all early progeny (0–12 h post-RNAi) but only in diminishing fractions of later progeny (12–34 h post-RNAi). (D) Males showing silencing of *gfp* (*Pgtbp-1::gtbp-1::gfp*) within the germline (sil.) did not transmit silencing to any cross-progeny (Right). Males fed control RNAi did not show any silencing. n/a, not applicable. (E) Silencing of a single-copy *gfp* transgene (*Peft-3::gfp*) in cross-progeny was detected in somatic cells even when only hermaphrodites (red) that lack *gfp* (none) ingested *gfp*-dsRNA (black bars). Error bars indicate 95% confidence interval (CI); L4-staged animals were assayed [$n > 80$ (B), $n > 84$ (C), $n > 40$ (D), $n > 56$ (E)]; and gray bars indicate silencing in progeny of animals that ingested control dsRNA (D and E).

and examined silencing in progeny (Fig. 1A). Using this assay, all silencing of endogenous genes, as well as transgenes detected in progeny, was due to the inheritance of a silencing signal from parents to progeny (Fig. S2). We found that ingestion of dsRNA by animals from hatching until their fourth larval (L4) stage resulted in silencing in only ~10% of progeny, but ingestion beyond the L4 stage for a 24-h period resulted in silencing in ~100% of progeny (Fig. 1B). Silencing occurred in all animals among early progeny but was observed in progressively fewer animals among later progeny (Fig. 1C), as is the case when limiting amounts of dsRNA are introduced by injection into the germline (25). This reduction of inherited silencing is consistent with the dilution of silencing signals by two known processes: cytoplasmic streaming within the germline (26) and the flow of material from the intestine into oocytes (e.g., yolk) (27). Such dilution is expected to be progressive in oocytes, which are made continuously during adulthood, but not in sperm, which are made in a single batch during the fourth larval stage (Fig. S3) (28). Furthermore, unlike the ~100% silencing that could be observed in progeny of hermaphrodites that ingested dsRNA, silencing was not detectable in any progeny of males that ingested dsRNA (Fig. 1D), despite the detection of SID-1–dependent silencing within the germline of male parents (Fig. 1D and Fig. S4). Together, these results suggest that ingested dsRNA or dsRNA-derived silencing signals that can be progressively diluted are transported to progeny through oocytes.

Silencing in Progeny Does Not Require Parents That Ingest dsRNA to Have DNA of Matching Sequence. The progressive dilution of silencing in progeny is consistent either with the inheritance of

small RNAs synthesized using mRNA templates, as was previously proposed in response to injected dsRNA (29) and ingested dsRNA (23), or with the inheritance of ingested dsRNA or its derivatives independent of any homologous sequence. To test whether homologous sequences are required in animals that ingest dsRNA for silencing in progeny, we exposed hermaphrodites lacking target sequences that match ingested dsRNA and examined silencing in cross-progeny after introducing the target sequence through males. Silencing was detected in ~100% of progeny when *gfp*-dsRNA was ingested by hermaphrodite animals lacking a *gfp* transgene (Fig. 1E and Fig. S5). Thus, for ingested dsRNA to cause silencing of a matching gene in progeny, that gene need not be present in the parent that ingests the dsRNA.

Forms of dsRNA Reach Progeny and Spread Between Cells in the Embryo. The simplest hypothesis explaining the ability of ingested dsRNA to cause silencing in progeny even when parents lack matching sequences is that either the ingested dsRNA itself or a processed derivative is delivered into progeny. Processing of dsRNA can begin upon entry of dsRNA into the cytosol through SID-1 (Fig. 2A and reviewed in ref. 22). The dsRNA is bound by the dsRNA-binding protein RDE-4 and recruited to the endonuclease Dicer, which processes it into primary double-stranded short-interfering RNAs (1° ds siRNAs). One of the strands of 1° ds siRNAs is eliminated by the Argonaute RDE-1 to generate primary single-stranded short-interfering RNAs (1° ss siRNAs), which are used as guides to identify mRNAs of matching sequence. Subsequent recruitment of RNA-dependent RNA polymerases

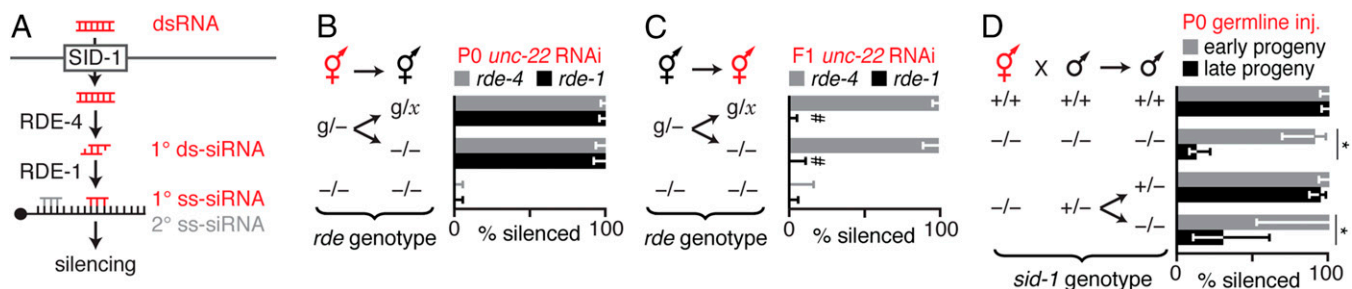


Fig. 2. Inherited dsRNA spreads between cells in the embryo to cause silencing. (A) Model of RNA silencing in *C. elegans*. Extracellular long dsRNA (red) enters the cytosol of cells through SID-1 and is processed by proteins (RDE-4 and RDE-1) into primary RNA species (1° ds siRNA and 1° ss siRNA, red) that are used to find target mRNA and trigger the synthesis of secondary RNA species (2° ss siRNA, gray), which results in gene silencing. (B and C) Silencing of *unc-22* in progeny of animals with *rde-4* (gray bars) or *rde-1* (black bars) expressed within the germline and the intestine under the *mex-5* promoter [*g*, *Pmex-5::rde(+)*] progeny genotypes (–/– or *g/x*, where *x* = + or *g*) and type of feeding RNAi (P0 *unc-22* RNAi or F1 *unc-22* RNAi) are indicated. #, much weaker silencing in all animals (Movies S1–S3). (D) Presence (+) of *sid-1* was necessary in late progeny (laid 72 h postinjection, black) for ~100% silencing of *unc-22* when *unc-22*-dsRNA was injected into the germline of hermaphrodite parents (red, P0 germline inj.). Asterisks indicate $P < 0.05$ (Student's *t* test); error bars indicate 95% CI (B–D); and L4-staged animals were assayed [$n > 56$ (B), $n > 42$ (C), and $n > 22$ (D)].

generates numerous secondary small RNAs (2° ss siRNAs), which are used for silencing. Thus, although secondary RNAs require mRNAs of matching sequence for synthesis, all primary RNAs can be made independent of any homologous sequence.

To determine the requirements for dsRNA processing in parent and in progeny to silence genes in response to ingested dsRNA, we examined silencing when SID-1, RDE-4, or RDE-1 was each present in either the parent or the progeny (Figs. S6 and S7 and *Materials and Methods*). Analysis of *sid-1* mutants revealed that the entry of dsRNA into cells in parents or during early development in progeny is sufficient for silencing in progeny (Fig. S6 A, B, and E). Analysis of *rde-4* mutants revealed that recruitment of ingested dsRNA into the RNAi pathway can occur in animals that ingest dsRNA or in their progeny at any stage during development (Figs. S6 C and F and S7) (29, 30). However, analysis of *rde-1* mutants revealed that the production of 1° ss siRNAs must occur in animals that ingest dsRNA or in their progeny before larval development for silencing in progeny (Fig. S6 D and G) (30). Therefore, ingested dsRNA and all primary RNAs derived from it can be processed in the animal that ingests dsRNA or in its progeny during early development for silencing in progeny.

If dsRNA is processed in a parental cell containing homologous mRNA—e.g., muscle cells for *unc-22*-dsRNA—processed derivatives of dsRNA could interact with mRNA to generate secondary small RNAs (Fig. 2A). But, if *unc-22*-dsRNA is processed in cells without homologous mRNA, such as the germline, the dsRNA can be processed only to primary single-stranded siRNA (1° ss siRNA) (Fig. 2A). To allow processing only in cells that lack matching mRNA and to allow subsequent inheritance of silencing signals to progeny, we expressed RDE-4 or RDE-1 under the control of a germline promoter (*Pmex-5*) and examined silencing in response to ingestion of *unc-22*-dsRNA. This promoter enabled expression within the germline and additionally within the intestine, but not within the muscle or hypodermis (Fig. S8). Expression of RDE-4 under the control of *Pmex-5* enabled silencing of *unc-22* in *rde-4(-)* progeny when either the parent with RDE-4 expression or progeny lacking RDE-4 expression ingested dsRNA, reflecting the persistence of parental RDE-4 in progeny (Fig. 2 B and C). However, such expression of RDE-1 under the control of *Pmex-5* enabled silencing in *rde-1(-)* progeny only when the parent with RDE-1 ingested dsRNA (Fig. 2 B and C). These results suggest that processing of ingested dsRNA by RDE-4 and subsequent processing by RDE-1 within cells that lack target mRNA in parents or within progeny during early development is sufficient for silencing in progeny. Thus, some primary RNAs, which could include long dsRNA, 1° ds siRNA, and 1° ss siRNA, are inherited from parents to progeny.

When radioactively labeled dsRNA is injected into the germline, a large fraction of the dsRNA remains as high molecular weight material in progeny (31, 32), consistent with substantial delivery of long dsRNA into progeny. When *unc-22*-dsRNA was similarly delivered directly into the parental germline, and thus into the cytosol of cells in the embryo, potent silencing occurred in early-born progeny in WT and in *sid-1(-)* animals (Fig. 2D), suggesting that sufficient dsRNA was delivered into all cells in early progeny. In contrast, later-born progeny, which are expected to receive smaller doses of dsRNA because of dilution within the germline (26), required SID-1 for efficient silencing (Fig. 2D). This need for SID-1-dependent spread of dsRNA between cells for efficient silencing in later progeny is consistent with forms of dsRNA (long dsRNA and/or 1° ds siRNA) being inherited from the injected parents to progeny.

Taken together, these results suggest that intracellular primary small RNAs—including forms of dsRNA—can be inherited from parent to progeny and explain the surprising observation that, when animals that lack DNA of matching sequence ingest dsRNA, they can still transmit a silencing signal to progeny.

Extracellular dsRNA Can Cause Silencing in Progeny Without SID-1-Dependent Entry in Parents. Ingested dsRNA can cross the intestine into the fluid-filled body cavity that surrounds all tissues, including the germline, without SID-1-dependent entry into the cytosol of intestinal cells (33–35). To determine where subsequent entry into the cytosol in parents is required for silencing in progeny, we delivered dsRNA into the extracellular space by injecting into the body cavity beyond the bend of the posterior gonad arm (Fig. 3A and Fig. S9A). Such injection resulted in the immediate spread of the injected material by diffusion throughout the entire body cavity, as evidenced by injection of fluorescently labeled dextran (Fig. S9B). Entry of dsRNA from the body cavity into the cytosol of a cell is expected to occur only in cells that have the dsRNA importer SID-1 (19). Consistent with results from feeding RNAi, presence of SID-1 in parents was sufficient for silencing in progeny in response to dsRNA injected into the body cavity (Fig. S9 C and D). However, when SID-1 was restricted to either sperm [*sid-1(+)* male] or to all cells except sperm [*sid-1(+)* hermaphrodite], injection of dsRNA targeting the muscle gene *unc-22* (*unc-22*-dsRNA) into the body cavity of hermaphrodites enabled *unc-22* silencing in cross-progeny (Fig. 3B and Fig. S9E), as was reported earlier (19). Taken together with the dynamics of silencing by ingested dsRNA (Fig. 1 B–D), these results suggest that injected extracellular dsRNA can be transported to the next generation through oocytes, presumably within intracellular vesicles, from which the entry of dsRNA into the cytosol through SID-1 occurs in progeny. Consistently, any paternal SID-1 protein or mRNA present in sperm

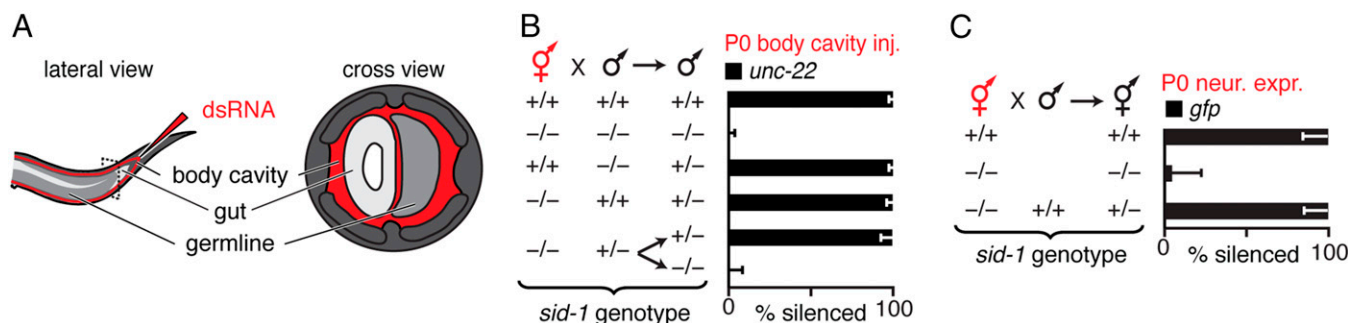


Fig. 3. Extracellular dsRNA does not need to enter the cytosol of any cell in parents to cause silencing in progeny. (A) Schematic showing injection of dsRNA into the body cavity. (B) Presence (+) of *sid-1* in progeny was sufficient for silencing of an endogenous gene (*unc-22*) in the muscles of progeny when dsRNA matching the gene (*unc-22*-dsRNA, red) was injected into the body cavity of hermaphrodite parents. Also see Fig. S9. (C) Presence (+) of *sid-1* in hermaphrodite parents was not required for silencing of a single-copy *gfp* transgene (*Pmex-5::gfp*) in the germline of progeny when *gfp*-dsRNA was expressed within parental neurons (P0 neur. expr., red) of hermaphrodite parents. Error bars indicate 95% CI (B and C), and L4-staged animals were assayed [$n > 49$ (B), $n > 38$ (C)].

(36) because fluorescently labeled dextran (10 kDa) also accumulated in punctate structures within oocytes (Fig. 5B). Consistent with the results from injected dsRNA, RME-2 was required for silencing in progeny when parents ingested dsRNA (Fig. 5C). Together, these results suggest that the import of dsRNA into oocytes occurs through the same pathway that imports yolk in *C. elegans*—RME-2-mediated endocytosis.

Extracellular dsRNA Can Reach Embryos Without SID-1-Dependent Entry. Despite import into oocytes through RME-2-mediated endocytosis, dsRNA is expected to require SID-1 for entry into the cytosol and subsequent silencing. Injection of fluorescent dsRNA into WT animals resulted in silencing in embryos (Fig. 4), consistent with cytosolic entry either in oocytes or in the embryo. To determine whether extracellular dsRNA can reach embryos without cytosolic entry, as predicted by genetic analyses (Fig. 3B), we injected fluorescently labeled *gfp*-dsRNA into the body cavity of *sid-1(-)* animals that express GFP in embryos and imaged the germline of injected animals, as well as embryos laid by the injected animals (as schematized in Fig. 4D). The dsRNA accumulated in proximal oocytes of *sid-1(-)* adults (Fig. 6A), as was observed in WT animals. The dsRNA also accumulated in embryos, but, unlike in WT embryos, such accumulation was not accompanied by silencing of *gfp* expression in *sid-1(-)* embryos (Fig. 6B–E). Thus, extracellular dsRNA can be imported into oocytes and can reside in presumed intracellular vesicles that reach embryos without SID-1-dependent entry into the cytosol.

In *C. elegans* embryos, yolk is secreted by blastomeres and reimported by intestinal cells, resulting in enrichment within the intestinal primordium (37, 38). We did not observe a similar intestinal enrichment of labeled dsRNA in embryos (e.g., Fig. 4H and Fig. 6D), suggesting that yolk and dsRNA are trafficked

differently in progeny. Consistent with this possibility, the colocalization between fluorescently labeled dsRNA and VIT-2::GFP is greatly reduced in embryos that are beyond the four-cell stage (Fig. 6F). These results raise an intriguing hypothesis that is worthy of further investigation: *C. elegans* embryos have mechanisms to distinguish inherited nutrition (yolk) from inherited information (dsRNA) when both reside within the same putative intracellular vesicle.

Discussion

Using genetic analyses and fluorescently labeled RNA, we have established that extracellular dsRNA is imported into oocytes along with yolk and can reach embryos with or without entry into the cytosol (Fig. 6G). Cytosolic entry in embryos of dsRNA from parental circulation and spread between cells of dsRNA processed within the parental germline or during early development in progeny results in robust gene silencing.

Implications for the Inheritance of RNA Silencing. The direct transfer of extracellular and intracellular dsRNA from parents to progeny when parents ingest dsRNA, when dsRNA is injected into parents, or when dsRNA is expressed within neurons in parents demonstrates that the trigger for RNAi is transported between generations in *C. elegans*. Therefore, when multigenerational silencing is observed for genes expressed within the germline (Fig. S1), the mechanisms that are required for transgenerational stability of silencing could be initiated in progeny—potentially during germline development. Thus, the production of secondary small RNAs and deposition of chromatin modifications proposed to be required for transgenerational gene silencing (15, 24, 29, 39–41) could be initiated in progeny when parents encounter dsRNA. These considerations also impact the interpretation of experiments evaluating the duration of transgenerational inheritance in response to RNAi (25, 42).

Inherited silencing of genes expressed in somatic cells in *C. elegans*, which typically lasts for precisely one generation (23) (Fig. S1), could simply reflect silencing triggered by dsRNA in progeny without engaging any transgenerational gene silencing machinery within the parent germline. Similar direct delivery to progeny could underlie parental RNAi in insects (reviewed in ref. 14), when dsRNA is delivered into the hemocoel (e.g., ref. 43) or through ingestion (e.g., ref. 44) to initiate RNAi. Additional studies are required to discover the evolutionarily selected function, if any, for the delivery of ingested material—including regulatory RNA—directly into progeny.

RNAs in Circulation as Carriers of Gene-specific Information Between Generations.

Molecules that can cross generational boundaries can cause apparent intergenerational effects. Exposure to some chemicals can cause multigenerational effects in mammals [e.g., endocrine disruptors (45) or odors (46)]. Examination of how many generations the molecules used to trigger a response persist within an animal could inform mechanisms underlying multigenerational effects. Alternatively, intergenerational effects could result if RNAs carry sequence-specific information to gametes through circulation from distant tissues that experience chemicals, changes in diet, or stress. In support of this possibility, studies focused on intergenerational and transgenerational effects in mammals implicate RNA in the inheritance of gene expression states across generations (47), report changes in small RNAs in gametes (48, 49), and report changes in RNAs acquired during gamete maturation from surrounding epithelia (50, 51). However, in all these cases, direct effects of a treatment—e.g., diet—on gametes and surrounding support tissues that alter RNA composition in gametes have not been ruled out. Furthermore, although extracellular RNAs have been detected in mammals, their biology is not well-understood and is under intense investigation (see ref. 52 for a recent review). Using genetic mutants and fluorescently labeled RNAs to control and follow the traffic of extracellular RNAs, our results demonstrate their direct

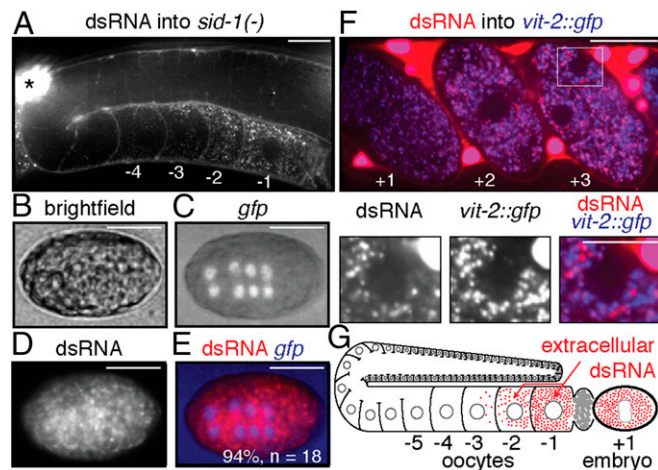


Fig. 6. Extracellular dsRNA can accumulate without cytosolic entry in proximal oocytes and subsequently within embryos. (A) In injected *sid-1(-)* animals, dsRNA concentrated in proximal oocytes (-1 and -2). Slices from Z-series (Movies S8 and S9) were spliced. Asterisk indicates brightly fluorescent coelomocyte. (B–E) The *sid-1(-)* embryos inherited the *gfp*-dsRNA, but no silencing of *Pend-1::gfp* occurred. (Scale bars: 20 μ m.) Proximal oocytes are numbered as in Fig. 4. Multiple adults ($n = 4$ in A) and embryos ($n = 18$ in B–E) were imaged for each experiment. (F) Colocalization of fluorescently labeled dsRNA and VIT-2::GFP is reduced as the embryo develops. Single confocal slice of an adult animal showing accumulation of fluorescently labeled dsRNA that was injected into the body cavity of a strain with *vit-2::gfp* in embryos held in utero ($n = 6$ <4-cell embryos and $n = 10$ \geq 4-cell embryos). (Top) Merged image showing +1, +2, and +3 embryos after fertilization. (Scale bar: 20 μ m.) (Bottom) Zoomed image of highlighted region (white box) in Top image for individual channels of dsRNA and VIT-2::GFP fluorescence and merge. (Scale bar: 10 μ m.) (G) Model illustrating that extracellular dsRNA can be transported through oocytes to progeny with or without entry into the cytosol.

transfer between generations in an animal—an inheritance that can potentially vary based on parental experience.

Materials and Methods

All *C. elegans* strains (Table S1) were maintained at 20 °C and were fed *Escherichia coli* OP50 (53). Oligonucleotides were used for injections and genotyping as necessary (Table S2). Transgenic strains with RDE-4 and RDE-1 restricted to the germline were generated using Mos1-mediated single copy insertion (MosSCI) (54). Transgenes were moved into different genetic backgrounds using genetic crosses and were either balanced with visible markers or sequenced for verification. Parent (P0) and progeny (F1) RNAi experiments (Table S3) were performed using RNAi *E. coli* clones for endogenous genes from the Ahringer library (55). Feeding RNAi and soaking animals in gfp-dsRNA are expected to cause similar silencing (56). RNAi clones and bacteria expressing gfp-dsRNA provided by the Hamza laboratory, University of Maryland, College Park, MD. Fluorescent transgenes were imaged using a Nikon AZ100 microscope using exposure times just under

saturation upon control RNAi of each genetic background. dsRNA that was injected into animals was transcribed *in vitro* or purchased as single-stranded oligos that were then annealed. Worms injected with fluorescently labeled dsRNA were imaged using a Nikon Eclipse Ti spinning disk confocal microscope and processed using ImageJ (NIH). Detailed procedures are provided in *SI Materials and Methods*.

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