dehydration before the experiment.

Tardigrades are composed of about 40,000 cells, which survive not only high-speed compression under a hydrostatic pressure of 600 MPa (equivalent to six times the pressure of sea water at a depth of 10,000 metres), but also being maintained at this pressure, and high-speed decompression as well. With perfluorocarbon as the pressure medium, we have demonstrated the viability of tardigrades after keeping them in an anhydrobiotic state. This viability is influenced not just by pressure but by the absolute amount of water in the organism's body, enabling us to exploit its dehydration/water-absorption mechanism for preservation purposes.

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Specific interference by ingested dsRNA

A genetic interference phenomenon in the nematode *Caenorhabditis elegans* has been described in which expression of an individual gene can be specifically reduced by microinjecting a corresponding fragment of double-stranded (ds) RNA[1]. One striking feature of this process is a spreading effect: interference in a broad region of the animal is observed following the injection of dsRNA into the extracellular body cavity. Here we show that *C. elegans* can respond in a gene-specific manner to dsRNA encountered in the environment. *C. elegans* normally feed on bacteria, ingesting and grinding them in the pharynx and subsequently absorbing bacterial contents in the gut. We find that *Escherichia coli* bacteria expressing dsRNAs can confer specific interference effects on the nematode larvae that feed on them.

Three *C. elegans* genes were used for this analysis. For each gene, a corresponding dsRNA was expressed in *E. coli* by inserting a segment of the coding region into a plasmid vector designed for bidirectional transcription by bacteriophage T7 RNA polymerase. The dsRNA segments used for these experiments were the same as those used previously[1]. We then observed the results of feeding these bacteria to *C. elegans*, and compared the effects with those of loss-of-function mutations and to animals microinjected with dsRNA.

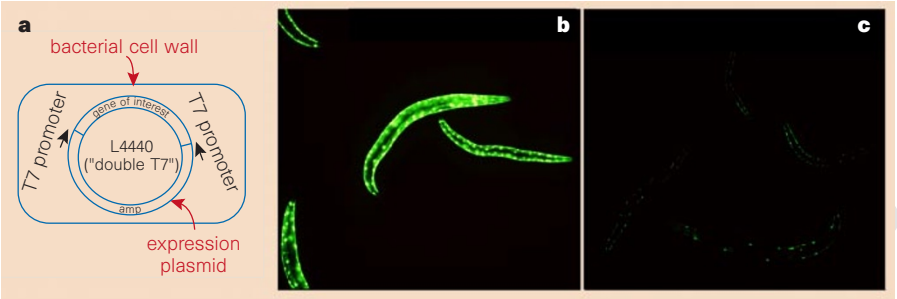

Figure 1 Genetic interference following ingestion of dsRNA-expressing bacteria by *Caenorhabditis elegans*. **a,** General scheme for dsRNA production. Segments were cloned between flanking copies of the bacteriophage T7 promoter into a bacterial plasmid vector (pPD129.36; J. Fleenor and A. F., unpublished). A bacterial strain (BL21/DE3; ref. 7) expressing the T7 polymerase gene from an inducible (Lac) promoter was used as a host. As an alternative strategy, we used a single copy of the T7 promoter to drive expression of an inverted duplication for a segment of the target gene (*unc-22* or *gfp*). A nuclease-resistant dsRNA was detected in lysates of these bacteria. The two bacterial expression systems gave similar interference results. **b,** A GFP-expressing *C. elegans* strain (PD4251)[1] fed on a naive bacterial host. Animals show high GFP fluorescence in body muscles. **c,** PD4251 animals reared on bacteria expressing dsRNA corresponding to the *gfp* coding region. Under the conditions of this experiment, 12% of these animals show a dramatic decrease in GFP.

The *C. elegans* gene *unc-22* encodes an abundant muscle filament protein[2]. Null mutations[3] or injection of *unc-22* dsRNA[1] produce a characteristic and uniform twitching phenotype in which the animals can sustain only transient muscle contraction. When wild-type animals were fed bacteria expressing a dsRNA segment from *unc-22*, 85% exhibited a weak but distinct twitching phenotype characteristic of partial loss of function for the *unc-22* gene.

The gene *fem-1* encodes a late component of the *C. elegans* sex-determination pathway[4,5]. Null mutations[4] or injection of dsRNA[1] prevent the production of sperm and lead euploid (XX) animals to develop as females (wild-type XX animals develop as hermaphrodites). When wild-type animals were fed bacteria expressing dsRNA corresponding to *fem-1*, 43% exhibited a sperm-less (female) phenotype and were sterile.

We then assessed the ability of dsRNA to interfere with a transgene target. When animals expressing a green fluorescent protein (GFP) transgene were fed bacteria expressing dsRNA corresponding to the *gfp* reporter[1,6], a decrease in GFP fluorescence was observed in about 12% of the population (Fig. 1).

The effects of bacteria carrying different dsRNAs were fully gene specific: dsRNAs from *fem-1* and *gfp* produced no twitching; dsRNAs from *unc-22* and *gfp* did not produce females; and dsRNAs from *unc-22* and *fem-1* did not reduce GFP expression. These interference effects were evidently mediated by dsRNA, as bacteria expressing only the sense or antisense strand (for *gfp* or *unc-22*) caused no evident phenotypic effects (data not shown).

As with injected dsRNAs, the effects of feeding dsRNA to *C. elegans* are reversible and do not reflect a stable genetic change, as transfer of affected animals back to non-engineered bacterial food led within a generation to loss of the Unc-22 or faint-GFP phenotypes. From an engineering perspective, bacterial-mediated delivery of dsRNA is less effective than direct microinjection. This is evident from the frequency and severity of the interference phenotypes discussed above, and from observations that, for several genes known to be inhibited by injected dsRNA, bacterially mediated interference was marginal or not evident. Differences in susceptibility could reflect resistance of some cells or stages to the consequences of ingested dsRNA.

This work provides an example of RNA-mediated transfer of information between organisms and between species. It is not yet known whether such RNA-mediated interference–transfer mechanisms participate in natural ecological interactions, such as antiviral defence or communication during biological symbiosis.

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