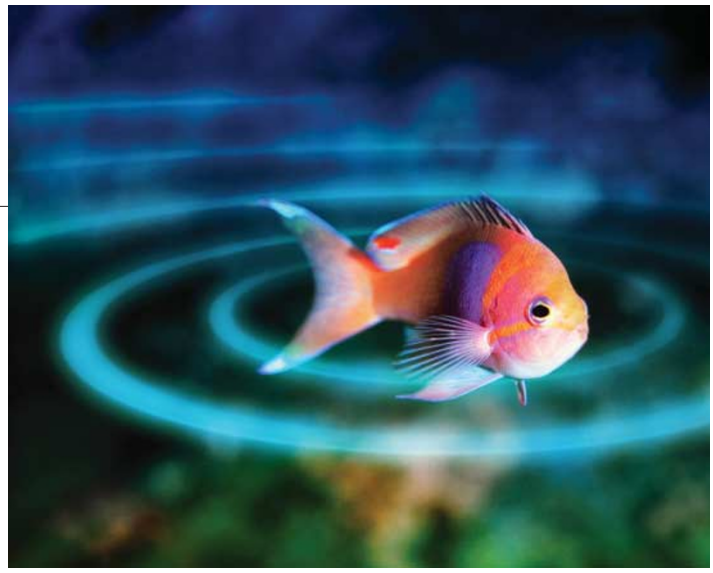

SID1
http://ca.expasy.org/cgi-bin/niceprot.pl?Q9GZC8

RNA

Spreading silence

How RNA interference (RNAi) spreads through plants and worms, away from the site of initiation, is not known. But an important clue has now come from work by Feinberg and Hunter who report a newly identified transporter protein — SID1 — that mediates the cellular uptake of dsRNA.

Hunter and colleagues had previously identified a gene, *sid1*, that is required for systemic, but not cell-autonomous, RNAi in *Caenorhabditis elegans*. To determine the global membrane structure of this putative transmembrane protein, the authors created a series of fusion proteins, consisting of SID1 truncated after each of the predicted transmembrane domains, fused to β-galactosidase (β-Gal) with or without a leading synthetic transmembrane domain. By measuring β-Gal activity (only those constructs that express cytoplasmic β-Gal produce active β-Gal), Feinberg and Hunter showed that the amino- and carboxyl-termini are extracellular and intracellular, respectively, and that the protein is indeed a multispan transmembrane protein.

To study the function of SID1, the authors chose *Drosophila* S2 cells — which lack systemic, but not cell-autonomous, RNAi — and co-transfected them with two plasmids, one encoding luciferase and the other encoding either SID1 or a SID1 functional mutant. Cells were soaked in low concentrations of luciferase dsRNA for a short time (to distinguish SID1-dependent activity from endogenous RNAi, which requires high concentrations and longer soaking), and luciferase activity was measured. SID-expressing cells showed a dsRNA-dependent silencing response. Interestingly, the response was dependent on dsRNA length, which was confirmed *in vivo*, as the injection of longer dsRNAs in *C. elegans* caused more potent systemic RNAi than shorter ones.

When Feinberg and Hunter used *Drosophila* cl-8 cells — which are resistant to dsRNA soaking — for similar luciferase-silencing experiments, cells that expressed SID1 showed dose-dependent luciferase silencing when soaked in luciferase dsRNA, whereas cells expressing mutant SID1 were resistant to soaking. So, the authors concluded that SID1 must facilitate dsRNA uptake into cells.

But how is dsRNA uptake mediated? Feinberg and Hunter found that SID1-mediated dsRNA uptake is far less sensitive to both ATP depletion and low temperature (4°C) than the endogenous uptake mechanism that is used by S2 cells. This ruled out active transport by a pump, endocytosis or phagocytosis as possible mechanisms. They concluded that SID1 passively transports dsRNA into cells in a diffusion-limited manner, possibly by forming a channel.

Feinberg and Hunter predict that "SID1-mediated transport may have numerous functional genomic and therapeutic applications". Much of the hope for therapeutic applications is likely to depend on the outcome of the functional characterization of *sid1* homologues in mouse and humans.

Arianne Heinrichs

References and links

ORIGINAL RESEARCH PAPER Feinberg, E. H. & Hunter, C. P. Transport of dsRNA into cells by the transmembrane protein SID-1. *Science* **301**, 1545–1547 (2003)

WEB SITE

Craig Hunter's laboratory:
http://www.mcb.harvard.edu/Faculty/Hunter.html