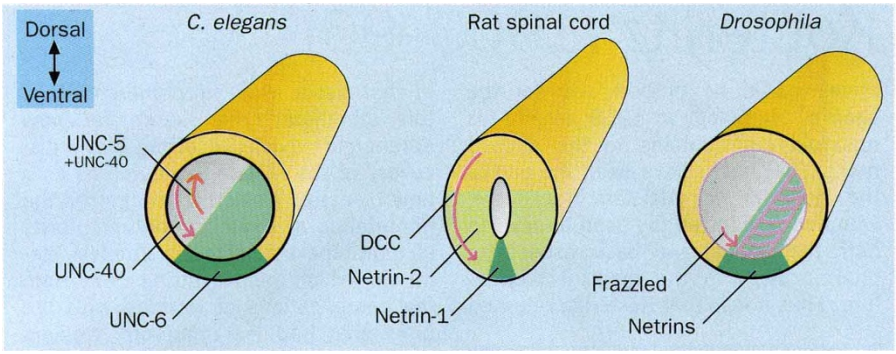

Cross-sections through *C. elegans*, rat spinal cord and *Drosophila* at early stages of development. The diagram shows the expression pattern of netrin receptors on various axonal trajectories, in relation to ventrally expressed netrins, as identified by Chan *et al.*[7], Keino-Masu *et al.*[8] and Kolodziej *et al.*[9].

tein, the *Drosophila* homologue of UNC-40, by using an enhancer trap screen for mutations affecting the nervous system. In the *frazzled* mutant, posterior and, to a lesser extent, anterior commissures in abdominal segments are thinner or are absent, and breaks in the longitudinal tracts are occasionally observed. This phenotype closely resembles that seen for null mutations of the two *Drosophila* netrin genes[16,17], which parallels the similarity of the *unc-6* and *unc-40* mutant phenotypes[11].

In wild-type flies, Frazzled protein is expressed at high levels on commissural and longitudinal axons in the developing central nervous system (see figure), and at lower levels on peripheral motor axons that extend outward in the intersegmental and segmental nerves. It exists in two isoforms, which differ in an insertion of about 150 amino acids between the immunoglobulin and fibronectin type-III repeats. Overexpression of the shorter Frazzled isoform rescues some but not all mutant phenotypes, suggesting that the longer isoform has some distinct functions. Mutants of *frazzled* also have defects in intersegmental nerves. These project normally towards netrin-expressing muscles, but they wander into adjacent segments and make contact with inappropriate muscles, implying that Frazzled has an additional function in target recognition.

Taken together, the three new papers[7–9] constitute a considerable step forward. But inevitably the picture may be more complicated than it might seem, and other molecules besides those cloned so far may well be involved in netrin signalling. For example, DCC is expressed on spinal motor neurons, which are not sensitive to netrin. So DCC alone is not sufficient to confer responsiveness to netrin, and there may be a requirement for an additional molecule (or molecules) that is expressed on commissural but not spinal motor axons[8]. On motor neurons, DCC could interact with a different ligand or be antagonized by unknown downstream signalling molecules.

Alternatively, the netrin receptor may be heteromeric — studies on *C. elegans* show that some dorsal migrations have a dual requirement for UNC-5 and UNC-40 (ref. 11), and the phenotype of *unc-40* null mutations is less severe than those resulting from mutations to other alleles which result in truncated versions of UNC-40 protein[7]. So the truncated forms may act as dominant negatives by inhibiting a normal binding partner of UNC-40.

Finally, because DCC is expressed at very low levels on trochlear motor neurons[8], which are repelled by netrin-1 (ref. 18), there may be other receptors for netrin. One candidate might be an UNC-5 homologue, because UNC-5 is involved in dorsally directed movements of axons in *C. elegans*.

It was Ramón y Cajal who, more than a century ago, proposed[19] that migrating axons are guided to their destinations by chemoattractants. This latest work paves the way for the identification of co-receptors for netrins, and other downstream molecules, and for a modern, molecular understanding of his idea. □

Uwe Drescher is in the Department of Physical Biology, Max-Planck-Institute for Developmental Biology, 72076 Tübingen, Germany.

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DAEDALUS

Life inside out

ALMOST all living things are made of cells densely packed together in a watery intercellular medium. The outer cell membrane may simply be an oily bilayer of phospholipid molecules, with various proteins swimming in it. An assembly of cells is rather like a dense emulsion of oil in water, but with a living interior in each droplet.

Now dense emulsions have a remarkable trick. They can invert. Agitation, or chemical additives, can flip a dense emulsion of oil in water over into a dilute one of water in oil. The densely packed oil drops merge into a continuous phase, isolating the water phase as little drops within the oil. So Daedalus wants to invert living tissue. The result would have all the cells merged into one continuous phase, with the intercellular medium dispersed within it as small droplets. Furthermore, says Daedalus, the product would still be alive and healthy. The same area of bilayer surface would separate the merged cells from their 'external' medium, despite the fact that it was now inside them. The cell phase would go on absorbing oxygen and nutrients from that medium, and dumping waste material into it. Ultimately, of course, the entrapped drops of 'external' medium would be exhausted; but for a while the tissue would not even notice its inversion.

To invert the tissue, Daedalus plans to shrink the outer monolayers of its cell membranes, encouraging them to flip from convex to concave. A sudden douche of heavy water, he argues, should do it. It will reduce the hydration energy of the outer phospholipid layer, thus dehydrating and therefore shrinking that layer. (Excessive heavy water is bad for many organisms, maybe for this very reason.) Ultrasonic agitation of the strained tissue should then invert it.

Daedalus wants to try it on cultured tissue first. The obvious experiment on an inverted tissue is to dose it with plasmids; with no cell membranes to penetrate, they will diffuse freely through the continuous multi-nucleate mass. But fast work will be needed. The droplets of intercellular medium within that mass will rapidly lose their heavy water and nutrients by outward diffusion. Soon it will be time to apply a blast of fresh heavy water and ultrasonics, and invert the tissue back again.

It will be somewhat disorganized. As the continuous mass repartitions into cells again, some will enclose two or more nuclei or other organelles, while others have none. Many will die, but the survivors will teach surprising and instructive lessons. Biology will take another step forward. David Jones