Life on the road

John G. Flanagan

As nerve axons migrate during wiring of the nervous system, molecular signposts at intermediate targets show them the way. Such a target has now been found to keep axons alive — provided they're on the right track.

To form the connections of the nervous system, axons must find their targets by setting out on a journey that may be long and complicated. How do they select the right path? What happens to those that stray off in the wrong direction? Intermediate targets are known to act as signposts along the way, sending out guidance molecules that tell the axons which way to go. On page 765 of this issue, Wang and Tessier-Lavigne[1] show that intermediate targets may have another function — they can also support the survival of migrating axons. Such a mechanism, by keeping axons alive only if they follow the right route, could ensure that the correct neural connections are formed.

The concept that nerves derive support from the targets they ultimately innervate can be traced back more than a century[2]. The best-known way in which this support — now known as a neurotrophic effect — occurs is by prevention of programmed cell death. The idea is that, during development, an excess of neurons is produced initially. Their axons then compete for a limiting amount of neurotrophic factors in the target. The result is a match in the sizes of the nerve and target, as well as elimination of mistargeted axons. A molecular basis for this phenomenon has been established through studies on nerve growth factor, the related neurotrophins, and other molecules that act as neurotrophic factors in final targets[3].

Wang and Tessier-Lavigne[1] set out to investigate whether similar principles might apply to an intermediate target — an idea they refer to as *en passant* (in passing) neurotrophic activity. As their model system they chose spinal commissural axons (those that cross from one side of the spinal cord to the other), and their intermediate target was a wedge-shaped group of cells called the spinal-cord floor plate[4] (Fig. 1a). Commissural axons are guided towards the floor plate, at least in part, by diffusible chemoattractants belonging to the netrin family. After crossing the midline, these axons turn and grow alongside the floor plate towards the brain, eventually heading off to reach their final targets.

As a first step, Wang and Tessier-Lavigne developed an *in vitro* assay. In the presence of netrin-1, rat dorsal spinal-cord explants send out commissural axons. But within days the axons degenerate and the cell bodies die. Could this degeneration be rescued by the intermediate target? The authors found that the axon degeneration and cell death were indeed dramatically rescued with floor plate-conditioned culture medium (FPCM), revealing a powerful neurotrophic activity.

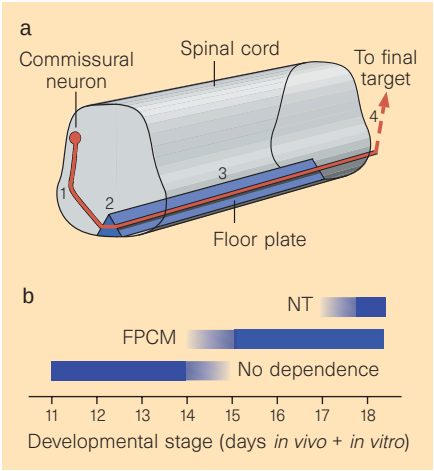

Figure 1 **Commissural neurons and their intermediate target, the floor plate.** a, **Commissural neurons in the rat dorsal spinal cord send out their axons, which soon turn (1) towards the floor plate, guided by chemoattractants. They then cross the floor plate (2) and turn again to grow alongside it (3) in the direction of the brain. Later they leave (4) to reach their final targets.** b, **Wang and Tessier-Lavigne[1] grew commissural axons from spinal-cord explants *in vitro*. They found that the explants degenerated at about the time they should normally have reached the floor plate. However, floor plate-conditioned culture medium (FPCM) contains a neurotrophic activity that can rescue them. Later, FPCM is not sufficient, but the axons can be rescued by FPCM if molecules in the neurotrophin family (NT) are also added.**

Is the activity localized to the intermediate target? The molecule responsible has not yet been identified, and, although it seems to be a polypeptide, tests of many candidates failed to identify any with appropriate activity. So, Wang and Tessier-Lavigne tested the localization by assaying slices taken from different parts of the spinal cord. They found that the activity is highly concentrated in the floor plate, with some also in more dorsal regions. These results indicate either lower production of the activity in dorsal regions, or diffusion of a signal produced in the floor plate.

If this activity is to fit the *en passant* neurotrophic model, a key issue is whether it can promote survival of cell bodies by acting at a distance on their axons. This was shown for nerve growth factor in classic experiments using the 'Campenot chamber', where axons were separated from their cell bodies by growth through a sealed barrier. Spinal commissural axons grow too slowly for this approach to be used, but Wang and Tessier-Lavigne developed an innovative and persuasive alternative. They placed a dorsal spinal-cord explant next to a floor plate explant in a collagen gel. By examining the axons and staining for dying cell bodies, they showed that, independent of the location of the cell bodies, the cells survived only if the axons had grown near the floor plate. This result is consistent with the idea that survival of commissural neurons in the embryo could depend on the distant journey of their axons.

So spatial aspects seem to fit the model — but what about time? *In vivo*, commissural axons grow out between embryonic day 11 (E11) and E13, and take about a day to reach the floor plate. Wang and Tessier-Lavigne found that, in the absence of FPCM, all of the dorsal spinal-cord explants looked healthy at the equivalent of E14. Yet, just one day later, all had begun to degenerate (Fig. 1b). This was true irrespective of whether the explants were placed in culture at E11 or E13, suggesting that a clock runs in the dorsal spinal cord *in vivo* or *in vitro*. This clock sets off a requirement for neurotrophic support just as the commissural axons are supposed to be reaching the floor plate. Axons that don't get there by the time the clock strikes presumably suffer a fate worse than Cinderella's.

Later, after the developing axons grow away from the floor plate, one might expect them to come to depend on neurotrophic activity from their final targets. Consistent with this idea, Wang and Tessier-Lavigne found that, at the equivalent of E18, axons can no longer be supported by FPCM. Instead, they need a combination of FPCM and neurotrophins (Fig. 1b). This could fit with a two-step model of development, in which the growing axons first depend on an *en passant* signal from the intermediate target, and later need an additional signal from the final target.

The *en passant* neurotrophic activity identified in these elegant studies could help solve the neural wiring problem in several

100 YEARS AGO

A bicycle ride will be none the less enjoyable if you train yourself, not merely to travel far, but to take an interest in the sights and scenes through which you pass. For the sake of example, let me remind you that no country is so rich as England in the architecture of its village churches. It is no hard matter to learn to recognise the principal peculiarities of the architectural types which prevailed from the days of the Saxons to Sir Christopher Wren. ... But as soon as the elements of English church architecture are known, an old church ceases to be merely a picturesque object. It is an historical document which you yourself can read. You do not need the aid of the sexton to tell you which is the oldest part. You can make a good guess at when that aisle was added, or that window knocked in a wall obviously older than itself. A visit to a cathedral becomes an intellectual pleasure. Weariness at the drone of the verger as he recites his oft-repeated lesson is replaced by an alert desire to know if the authorities from whom he learnt it confirm or correct the rapid conclusions as to date or history to which you yourself have come.

From *Nature* 19 October 1899.

50 YEARS AGO

In a recent investigation, I have even demonstrated, among other matters, the existence of red blood corpuscle remnants in ancient Swedish skeletons (Viking age), buried without the embalming procedures used in Egypt and elsewhere. Pictures of relatively well-preserved organic framework of bone tissue were obtained with material even from so early a period as the Upper Stone Age. Various substances have been identified in mummified tissues by means of chemical methods. ... It was now thought possible to ascertain ... whether histamine occurs in measurable amounts in mummy tissue and other ancient human remains. Soft tissue (skin from the neck) and bone (cervical vertebra) from a mummy of the Egyptian Museum in Stockholm and supposed to be about three thousand years old were ground to a fine powder. … A definite spasmogenic activity was found in the extract thus prepared. The spasm of the isolated guinea pig's small intestine elicited with the extract could be prevented with an antihistamine drug and was to a certain degree counteracted by atropine.

From *Nature* 22 October 1949.

ways. First, if axons go astray, this mechanism might help to eliminate them quickly, before they interfere with the orderly pioneering and assortment of axon tracts. Second, it could prevent axons reaching the wrong final target, where they might otherwise be incorporated in aberrant neural circuits[1]. Third, it opens the possibility of a combinatorial mechanism, where a limited number of factors derived from the intermediate or final targets could be used in different combinations to specify many distinct connections.

Wang and Tessier-Lavigne's work adds a fascinating new dimension to the increasing recognition that neurotrophic effects may go beyond the simple model of support by final targets. During development, the responsiveness of some neurons to different neurotrophins switches. Although this has not been tied unequivocally to intermediate targets, neurotrophins may contribute support at cell bodies or along pathways, while axons are still on the way to their targets[5]. There is also some analogy in later events, when motor neurons require support both from their muscle target and from the Schwann cells that wrap around their axons after reaching the target[3]. We also have several new questions. What is the precise developmental significance of the floor plate activity detected *in vitro*? Could the work have therapeutic implications in spinal-cord regeneration? What molecules are responsible for the activity? We don't have all the answers yet — but after all, life's a journey. ■

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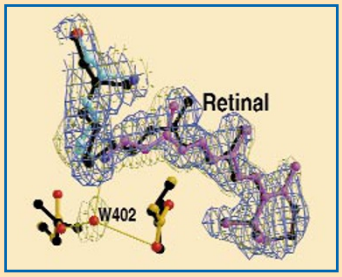
Protein crystallography

Frozen in time



R. NEUTZE

Biochemical reactions are extremely rapid, but the methods for imaging the enzymes that catalyse them can take hours. To get round this problem, structures can be determined at temperatures around 100 K, literally freezing an enzyme's movements and allowing the intermediates in its reaction cycle to be observed. This has been done for bacteriorhodopsin in studies reported by Edman *et al.* in this issue (*Nature* 401, 822–826; 1999) and Genick *et al.* (*Science* 286, 255–260; 1999).

Bacteriorhodopsin is a pump that uses light energy to drive protons across bacterial purple membranes. A cycle of structural changes is triggered when absorption of a photon of light causes isomerization of bacteriorhodopsin's bound chromophore, retinal (purple in the figure). Intermediates in this photocycle can accumulate in crystals at low temperatures (see *Nature* 392, 206–209; 1998) or in mutant proteins, as used by Genick *et al.*, but subtler techniques are needed to trap the earliest and most elusive intermediates.

Edman *et al.* maintained bacteriorhodopsin crystals in the dark, bathed in liquid nitrogen (110 K). They then drove the initial step in the protein's photocycle by illuminating the crystals with green light through an optical fibre (at a wavelength of 532 nm), and exposed them to a powerful synchrotron source to aid the rapid collection of data. The authors found that, despite the isomerization of a double bond, there is very little change in the overall shape of the retinal — in the figure, electron density after illumination (blue) is compared with that before (brown).

But there are other changes. The biggest of these is the escape of a water molecule (designated W402) from the vicinity of the retinal. This molecule previously formed part of a network of water and amino-acid residues connecting retinal to the outside of the bacterial membrane. Its loss triggers changes in this network, eventually resulting in expulsion of a proton from the bacterial cell.

One snap-shot cannot reveal the whole picture of how the bacteriorhodopsin pump works. However, by building up series of freeze-frame structures for all the intermediates in the enzyme's working cycle the mechanics of this, and other, molecular machines is being revealed.

Christopher Surridge