PERSPECTIVES: NEUROBIOLOGY

# TRK Makes the Retrograde

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One of the most interesting questions facing neurobiologists is how a growth factor binds to receptors on nerve terminals, and then transmits signals back to the cell body of the neuron located up to a meter away. This question of retrograde signaling has been intensively studied for decades, ever since the original finding that nerve growth factor (NGF) made by peripheral tissues was necessary for the survival of developing peripheral neurons. This body of work has led to a widely accepted model for the retrograde transmission of neuronal survival signals. In this model, NGF binds to TrkA receptors on axon terminals; the ligand-receptor complexes are then internalized into vesicles, which are transported down the axon to the cell body where they provide survival signals to the neuron. However, a report by MacInnis and Campenot on page 1536 of this issue (*1*) demonstrates that retrograde survival signals do not depend on the internalization and transport of NGF. This finding opens up the possibility that nature may, in fact, have developed several unique ways to solve the problem of long-distance cellular signaling.

This major conceptual advance was made possible by a unique culture system originally devised by Bob Campenot that segregates the distal axons of cultured neurons from their cell bodies and proximal axons (*1*). MacInnis and Campenot used this system, together with iodinated NGF immobilized on beads, to show that binding of NGF to TrkA receptors on axonal membranes and receptor activation were sufficient to provide a retrograde survival signal to the cell body, even in the absence of NGF internalization and transport. Thus, although many previous studies, both in vivo and in culture, have shown that NGF is retrogradely transported from nerve terminals to the neuronal cell body, and even that NGF can be retrogradely transported as a complex with its activated receptor (*2*), the new work suggests that NGF transport is not required for neuronal survival.

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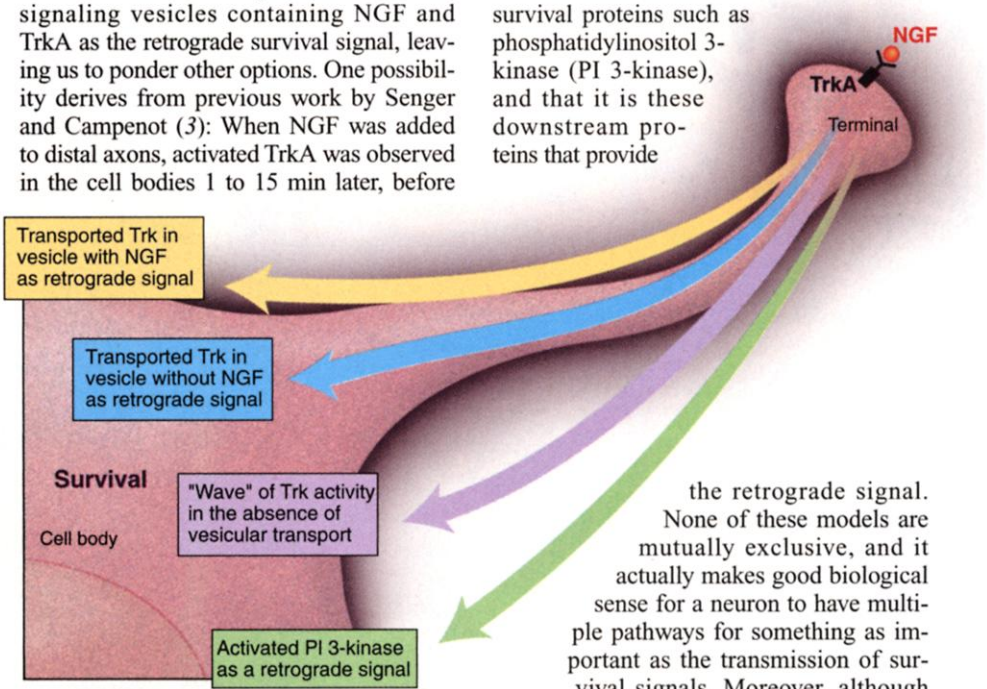
What, then, is the essential retrograde survival signal? It is clear from previous work that the activated TrkA receptor itself is a key retrograde survival signal when NGF is added to distal axons (*2*). However, the MacInnis and Campenot data rule out signaling vesicles containing NGF and TrkA as the retrograde survival signal, leaving us to ponder other options. One possibility derives from previous work by Senger and Campenot (*3*): When NGF was added to distal axons, activated TrkA was observed in the cell bodies 1 to 15 min later, before any retrograde transport of NGF. This finding led the authors to postulate that binding of NGF to TrkA receptors in nerve terminals resulted in the rapid propagation of an NGF-independent "wave" of TrkA receptor activation (see the figure). Support for this model comes from the recent finding of a ligand-independent wave of epidermal growth factor (EGF) receptor activation originating from a point source of EGF (*4*).

A second possibility is that activated TrkA, minus NGF, can be internalized into signaling vesicles and transported down the axon to the cell body, maintaining its activation state in the absence of ligand. Is there any precedent for TrkA activation in the absence of NGF? When the TrkA concentration in the neuronal membrane is increased above a certain threshold (as it might well be in a vesicle), then TrkA can dimerize and mediate survival and growth in the absence of any exogenous NGF (*5*). A third possibility is that NGF binding to TrkA on the axonal membrane activates survival proteins such as phosphatidylinositol 3-kinase (PI 3-kinase), and that it is these downstream proteins that provide the retrograde signal. None of these models are mutually exclusive, and it actually makes good biological sense for a neuron to have multiple pathways for something as important as the transmission of survival signals. Moreover, although the MacInnis and Campenot data conclusively show that NGF-TrkA vesicles are not necessary for survival, this does not rule out the possibility that such vesicles may be important for certain facets of long-distance signaling, such as phosphorylation of the CREB transcription factor in the cell body (*6*).




**Talking long-distance.** In both the developing and mature nervous system, NGF binds to TrkA receptors on axon terminals and transmits survival signals the entire length of the axon to the neuronal cell body. It has been assumed that this survival signal is transmitted along the axon by retrograde transport of NGF-TrkA ligand-receptor complexes in vesicles (yellow). However, MacInnis and Campenot (*1*) show that this survival signal does not require internalization and transport of NGF, providing support for a number of alternative models for the transmission of long-distance survival signals along axons. For example, activated TrkA receptors may be transported in vesicles in the absence of NGF (blue); a wave of TrkA receptor activation may be propagated along the axon (purple); or a signaling molecule such as PI 3-kinase may be the retrograde survival signal (green).

From the broader perspective, the MacInnis and Campenot data also speak to the issue of spatial segregation of signaling pathways within neurons, an issue brought to the forefront by the recent work of Watson *et al.* (*7*). These authors showed that addition of a mixture of neurotrophin growth factors to distal axons promoted local axonal activation of the mitogen-activated protein (MAP) kinases ERK1 and ERK2, which are also important for NGF-induced local axonal growth (*8*). However, the same axonal stimulus did not retrogradely stimulate ERK1 or ERK2 in cell bodies, but instead led to cell body activation of another MAP

kinase, ERK5. This finding implies that the signaling substrates "seen" and activated by the Trk receptors that bind neurotrophins differed according to whether signaling was local or retrograde. In contrast, the PI 3-kinase–Akt signaling pathway, which is essential for survival in response to NGF, is activated both locally and retrogradely by NGF (*2*). Interestingly, MacInnis and Campenot demonstrate that NGF immobilized on beads, which cannot be internalized, leads to activation of the PI 3-kinase–Akt pathway but not ERK1 and ERK2. This finding is similar to that seen when internalization of NGF (and presumably TrkA) is disrupted pharmacologically or by blocking the activity of dynamin, a protein required for endocytosis (*2*). Together, these findings dissociate the NGF survival signal—which does not require internalization of NGF but is dependent on PI 3-kinase—from NGF-TrkA signaling vesicles (*9*), which seem to be required for activation of ERK1 and ERK2 and may be more important for local responses such as growth (*8*).

The MacInnis and Campenot results are also relevant to how signals are transmitted in other cell types. In this regard, the requirement of receptor internalization for the activation of signaling proteins such as the ERKs has been previously noted in cells treated with EGF (*10*). The activated receptors, bound in clathrin-coated pits together with components of the endocytotic vesicle machinery, are directed to intracellular signaling proteins, culminating in the activation of the ERKs. A similar vesicle containing NGF, Trk, and Trk signaling proteins (including the ERKs) was recently characterized by Howe and colleagues (*9*). However, the MacInnis and Campenot work implies (i) that NGF can induce cell survival signals without the formation of signaling vesicles containing NGF, TrkA, and the ERKs, and (ii) that a second type of signaling pathway, potentially an activated Trk wave or a distinct type of vesicle, transmits PI 3-kinase–induced survival signals. Future studies determining whether NGF-beads can indeed stimulate the internalization of Trk, and whether the activities of different survival proteins are induced by NGF or NGF-beads, will help to elucidate these different potential pathways for survival signal transmission.

Whatever the nature of the activated TrkA retrograde signal, be it a new type of vesicle or a wave (or both), it is clearly different from the signal generated by local TrkA activation (see the figure). The TrkA retrograde signal can activate PI 3-kinase, but not ERK1 and ERK2. It is activated initially by NGF, but then is propagated and/or maintained in an NGF-independent fashion. Although these findings are surprising in terms of current biases, they are perhaps not as surprising when considered in terms of the biological necessity of signal transduction in a cell with the morphological complexity of a neuron. If you needed a receptor to maintain its activity while transported over long distances, then wouldn't it make sense to design it so that it could maintain its activation status in the absence of ligand? If you wanted one receptor to promote axonal growth locally, and then to promote cell survival at a distance, then might you not create multiple signal carriers specialized for these different functions? The MacInnis and Campenot report is not only important as a key addition to our understanding of how growth factors transduce long-distance signals, but it also provides us with a window onto possible new signaling pathways used by all cell types.

**References and Notes**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PERSPECTIVES: PALEOCLIMATE

# Cycles, Cycles Everywhere

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At the recent American Geophysical Union meeting in San Francisco, the 25th anniversary of one of the great papers in paleoclimatology was celebrated (*1*). The paper, entitled "Variations in the Earth's orbit: Pacemaker of the Ice Ages," presented important new evidence supporting the orbital theory of glaciation (*2*).

Enhanced online at www.sciencemag.org/cgi/content/full/295/5559/1473

Orbital theory goes back over a century but is most closely associated with Milankovitch (*3*), who calculated the effects of gravitational perturbations on the seasonal cycle of Earth's insolation (the radiation incident at the top of the atmosphere). Insolation varies on several time scales, including ~20,000 years (termed precession), ~40,000 years (obliquity or tilt of Earth's axis), and ~100,000 and ~400,000 years (eccentricity of Earth's orbit around the Sun). Together with the geographer Wladimir Köppen, Milankovitch hypothesized that glaciations occurred when Northern Hemisphere summer insolation was lowest. However, incomplete land records and poor chronology prevented them from fully testing their hypothesis.

In their 1976 paper, Hays *et al.* reported new evidence for the Milankovitch imprint from a deep-sea core. Advances in the chronology of deep-sea cores and the high resolution of their data allowed them to detect the precession, obliquity, and ~100,000-year eccentricity orbital periods in marine records. Although the precession and obliquity responses were linearly related to orbital forcing, there was a much larger nonlinear response to the relatively weak eccentricity forcing. The initial reaction to the paper was very positive, but some concerns were raised about the possible circularity of tuning the time scale of the core to optimize the fit to orbital forcing. This skepticism faded as evidence for a widespread imprint of orbital cycles in the geologic record mounted (*4*).

In the 25 years since the publication of (*2*), the importance of Milankovitch cycles has penetrated many areas of paleoclimatology. For example, John Kutzbach (University of Wisconsin) summarized evidence that orbital variations are now recognized to exert a strong influence on tropical climates, especially the monsoon system. Jean Jouzel (Laboratoire des Sciences du Climat et de l'Environnement, Gif sur Yvette) highlighted the importance of ice cores, which record the orbital imprint in the ice system and have provided exciting insights into the effect of trace gas changes (such as carbon dioxide and methane) on ice volume.

Virtually all credible models of ice volume change in the Quaternary [which began 1.8 million years ago (Ma)] require carbon dioxide changes to reproduce the observed record. Richard Peltier (University of Toronto) suggested that this link is so important that we may not see future ice volume increases until the present anthropogenic perturbation has been neutralized by the natural system (a process that is likely to take more than 10,000 years). According to Peltier, the magnitude of the anthropogenic perturbation may mark the

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