Developmental neurobiology

Trophic factor theory matures

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THE Nobel prize in Physiology and Medicine was awarded last September to Stanley Cohen and Rita Levi-Montalcini, recognizing the importance of their work, begun in the 1950s, on the identification and purification of nerve growth factor (NGF), a protein essential for the survival of several neuronal cell types in the peripheral nervous system. By extrapolation, NGF is thought by many neurobiologists to be a representative example of a hypothetical class of agents that mediate an obligatory trophic dependence of neurons for the targets they innervate. As first suggested by Levi-Montalcini and Viktor Hamburger, neuronal survival may depend on successful competition for target-derived trophic factor that is in limited supply. Over the past few years the trophic hypothesis has been strengthened by the demonstration that the targets of NGF-sensitive neurons do indeed synthesize this protein[1,2] and by the equally important finding that NGF-sensitive neurons have specific receptors for NGF[3–5]. The details of the trophic dependence, however, including the exact role and mechanism of NGF action, remain obscure. On page 353 of this issue[6], Davies *et al.* contribute a noteworthy study that focuses on the details of trophic factor and trophic factor receptor expression. Davies *et al.* address fundamental questions regarding the 'when' and the 'where' of NGF action, and their answers are in many ways surprising.

Differentiation

The differentiation of neurons occurs in phases of growth (proliferation, axon outgrowth and synapse formation) and regression (cell death and synapse elimination). Before the work of Davies *et al.* the potential scope of NGF action ranged throughout nearly the entire life of neurons. With the exception of neuronal proliferation, which seems to be independent of it, NGF has been implicated in virtually all other phases of neuronal differentiation. For example, NGF puffed out of a pipette[7] or injected intracerebrally *in vivo*[8] can redirect the orientation of growing axons towards the source of the agent. Hence, during axon outgrowth it was not unreasonable to think that NGF may be a chemotactic agent that guides growth cones up a concentration gradient towards their targets. Also at issue is the site of NGF action: non-target tissues such as Schwann cells appear to be NGF-immunoreactive[9,10]. Identifying the site of expression is clearly important to the understanding of when and where neurons first come into contact with NGF.

Davies *et al.*[6] examined NGF and NGF-receptor expression in early embryos of mice at a stage when ingrowing sensory fibres of the trigeminal ganglion first reach the maxillary process and developing whisker pad. Asking when NGF messenger RNA and NGF could first be detected, they find, surprisingly, that the onset of transcription and subsequent translation does not preceed but is actually coincident with the arrival of the first sensory axons. That detectable levels of the messenger RNA or NGF itself are not present before the arrival of the first axons strongly suggests that NGF action begins only after target innervation; that messenger RNA levels are also temporally correlated allows for the unexpected possibility that axons elicit the synthesis of NGF in their targets. These data provide strong evidence that NGF has little if any role in guiding growth cones to their appropriate targets.

But what of neuronal receptivity to NGF? Equally important in defining the time period of NGF action is to determine when neurons first express NGF receptors on their surfaces. Using an ^{125}I-labelled NGF probe, Davies *et al.* report that the receptors are not detectable on sensory neurons until after they arrive at their target. This finding is consistent with the observation in tissue culture that developing neurons are, for a while, not dependent on NGF. The picture is emerging that NGF begins to mediate survival after neurons have contacted their prospective targets and not before.

This interpretation critically depends on the sensitivity of the assays used to measure NGF and NGF-receptor expression. In the case of specific RNA and protein assays, the levels of sensitivity meet reasonable expectations: for example, the two-site immunoassay can detect less than a picogram of NGF (500 times more sensitive than earlier procedures)[11]. In the case of the assay for receptor levels, performed with labelled NGF and autoradiography, there is reason to suspend judgement as to the exact time of receptor expression. An immunoprecipitation assay[12] using a monoclonal antibody against NGF receptor turned out to be 50 times more sensitive than detection using directly labelled NGF.

Two final points are worth mentioning. First, Davies *et al.* demonstrate NGF messenger RNA expression in the developing whisker pad by *in situ* hydridization techniques. This simple finding establishes, by the most direct means to date, that the target is at least one source of NGF. It also counters suggestions that trophic support derives exclusively from Schwann cells[9]: Davies *et al.* detect significant concentrations of the messenger RNA within the cutaneous epithelium where there are no Schwann cells. Second, trigeminal ganglion explants from periods of development that preceed axon outgrowth nevertheless show NGF-receptor expression along growing axons at the appropriate time. Hence, despite lack of contact with the target, neurons are capable of entering the NGF-dependent phase.

Time of action

The work of Davies *et al.* merits attention because it places NGF expression and receptivity within a coherent cellular and developmental context. The results show that the time of NGF's action ensues only after target innervation. This further suggests that it is in the realm of competition between neurons and of long-term survival that NGF action must be explained. Finally, the temporal details provided suggest a more complex interpretation of neuronal development than previously thought. Before these studies, a broader view of the scope of NGF action from the period of neurogenesis to that of competition prevailed: now it appears that neurons initially have intrinsic capabilities that enable them to reach the stages of competitive interactions, or perhaps that they require yet other factors from elements of the cellular environment in which they proliferate and through which their axons grow.

It is curious why nature chose to use a protein factor — NGF — to promote the survival of a sub-population of neurons. How is NGF action mediated; what are the biochemical and physiological effects of NGF on individual neurons; what are the consequences of those effects on long-term survival; and does this sort of interaction hold for all neurons? The simple yet elegant measurements of Davies *et al.* help to refine the role of NGF, to define where and when to look for other trophic factors and help to elucidate what these factors are doing. □

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