Summary  
During development, neurons die if they do not receive neurotrophin support from the target cells they are innervating. Neurotrophins are delivered from the target to the cell bodies of the innervating neurons by interacting with specific receptors located on the nerve terminals and then together are retrogradely transported to the cell body. This process consists of a number of distinct events including endocytosis of neurotrophin and its receptor into coated vesicles; vesicle sorting followed by retrograde axonal transport to the cell body, where interaction of the activated receptor initiates a signalling cascade at the cell body that causes the survival response. It has recently been shown that the signalling molecules associated with retrograde transport differ between neuronal populations. In sympathetic but not sensory neurons, a wortmannin-sensitive molecule (phosphatidylinositol kinase) is essential for the retrograde transport of neurotrophins. In sensory but not sympathetic neurons, a rapamycin-sensitive molecule (pp70S6K) is associated with retrograde transport of neurotrophins. This is strong evidence that sympathetic and sensory neurons utilize different signalling pathways to perform the same cellular function; retrograde transport. These findings may provide clues to understanding neurological diseases, such as motor neuron disease, in which axonal transport is impaired specifically in motor neurons.

Key words: motor neuron disease, neurotrophins, NGF, retrograde axonal transport.
neurotransmitters and the growth of neurites (axons and dendrites).\textsuperscript{17–19} The low-affinity receptor (p75) is important for mediating the survival of calcitonin gene-related peptide (CGRP) and substance P containing DRG neurons\textsuperscript{20,21} and regulating the retrograde axonal transport of neurotrophins in sensory neurons.\textsuperscript{22}

**Neurotrophin-mediated neuronal survival**

The actions of neurotrophins in mediating the survival of neurons is thought to follow a simple model and involve the following steps. Neurotrophins are synthesized in the targets, and are then released following a signal from the innervating nerve. The neurotrophin binds to its corresponding receptor located on the nerve terminals of the innervating neurons. The neurotrophin-receptor complex is internalized and retrogradely transported to the cell body, where changes in gene expression associated with neuronal survival take place.\textsuperscript{23–25} However, there are additional layers of complexity at each stage of the process in delivering the neurotrophin to the cell body (Fig. 1).

**Receptor binding, activation and clustering**

It has been proposed that neurotrophins bind to their respective trk receptors, resulting in activation of the intracellular domain of the receptor. It is not clear at this stage if this may be an oversimplification because all the neurotrophins appear to have some affinity for each of the different trk receptors, and the low affinity p75 receptor can also modulate the affinity of the trk receptor for its neurotrophin.\textsuperscript{26,27} The trk receptors may then cluster before internalization into coated vesicles, as proposed for other receptors such as the epidermal growth factor receptors.\textsuperscript{28} This process may be controlled by intracellular signalling mechanisms inside the cell, such as the movement of the actin cytoskeleton which pulls together the individual receptors into clusters\textsuperscript{29,30} or alternatively by shear mechanical changes associated with the formation of the receptor clusters. The commonly held view is that trk receptors form homodimers, however, on the nerve terminal in the synaptic cleft, the individual trk receptors (trk A, B and C) may be in close proximity with one another, and it may be possible for the trk receptors to heterodimerize, but this remains to be determined. In addition, it may be possible that the individual neurotrophins may also form heterodimers.

**Internalization and endocytosis**

Nerve growth factor has been shown to induce rapid and extensive endocytosis of the trkA receptor in PC12 cells.\textsuperscript{31} The receptor remains phosphorylated during the retrograde transport process, suggesting that the receptor is able to remain activated and continue to signal at the cell body.\textsuperscript{32–35} It is unclear if a signal generated from the activation of the intracellular domain of the receptor results in the recruitment of a specific subset of molecules that then cause the endocytosis of the trk receptors into coated vesicles. There is evidence in PC12 cells that the intracellular domain of the receptor may be associated with endocytosis. Following treatment of PC12 cells with NGF, some endocytic organelles were found to contain NGF and its phosphorylated receptor, trkA, and the intracellular signalling molecule PLC-\gamma1.\textsuperscript{36} Some of the endocytic organelles were associated with clathrin and \(\alpha\)-adaptin, suggesting the NGF-trkA receptor complex is endocytosed into clathrin-coated vesicles in PC12 cells.\textsuperscript{36} However, it has been reported in nervous tissue that the majority of clathrin-coated vesicles are associated with synaptic vesicles;\textsuperscript{47} this suggests that the NGF-trkA receptor complex is endocytosed into non-clathrin-coated vesicles in neurons. The molecular machinery regulating receptor-mediated endocytosis in neurons is not well understood, with the exception of dynamin, which has been shown to be an essential molecule for regulating synaptic vesicle recycling.\textsuperscript{38}

**Vesicle sorting and targeting**

It is still unclear how different types of vesicles are differentiated from one another in the pre-synaptic nerve terminal. The majority of studies examining neuronal endocytosis have concentrated on membrane retrieval of neurotransmitter into clathrin-coated vesicles following its release from pre-synaptic nerve terminals;\textsuperscript{49} these vesicles

![Figure 1](image-url)  
**Figure 1**  Molecules regulating the retrograde axonal transport of neurotrophins.
are rapidly recycled at the nerve terminal and are sometimes retrogradely transported.40 The neurotrophin-trk receptor vesicles are retrogradely transported15 but may also be recycled. It is not known if vesicles destined for retrograde transport are sorted from synaptic vesicles in endosomal structures, as described in lymphocytes and epithelial cells.41,42 Endosomal structures have been observed in nerve terminals,43 but there is currently little evidence to support the role of endosomes in the sorting of vesicles in pre-synaptic nerve terminals. Alternatively, following endocytosis, multivesicular bodies may form in the nerve terminal and this complex may then be tugged specifically for retrograde transport.

**Transport**

A general description of axonal transport has been provided by studies examining the transport of horseradish peroxidase (HRP), ovalbumin and transferrin in cultured hippocampal neurons. It had been assumed that neurotrophin-receptor complexes follow a similar pathway. In the case of HRP, it is transported in large vesicular-like structures,44,45 in association with the minus-end-directed motor protein, cytoplasmic dynein, using the microtubular system.46–48 Experiments with antibodies against dynein have indicated that dynein would be the primary motor for retrograde transport.49 We have now shown in vivo that actin, microtubules and the motor protein, dynein, are involved in retrograde axonal transport of NGF and NT-3 in sympathetic and sensory neurons.50 This suggests that the neurotrophin-receptor complexes utilize similar machinery during axonal transport, as demonstrated for HRP in both sympathetic and sensory neurons.

**The signalling molecules regulating the transport of neurotrophins**

Different arrays of signalling molecules may participate in each of the steps outlined in the previous section. It was assumed that these molecules would be similar in all neurons. However, we have evidence that the intracellular signalling molecules associated with retrograde axonal transport of neurotrophins differ between neuronal populations. In sympathetic but not in sensory neurons, a wortmannin-sensitive isoform of PI3-kinase is involved in the retrograde axonal transport of 125I-NGF,125I-NT-3,125I-NT-4/5 and fast blue.50,52 In sensory neurons but not in sympathetic neurons, a LY294002-sensitive isoform of PI3-kinase and rapamycin-sensitive, pp70S6K, are associated with retrograde transport of 125I-βNGF.50 The differences in sensitivity to these pharmacological inhibitors suggest that neurons have different complements of signalling molecules. In support of this idea, we have also shown that sympathetic and sensory neurons differentially express PI3-kinase isoforms, because the wortmannin-insensitive isoform of PI3-kinase, p170, is expressed in sensory and not in sympathetic neurons.52 The exciting findings are that there are clear molecular differences in the signalling pathways involved in mediating a similar cellular function, retrograde axonal transport in neurons. Until these signalling pathways are understood, it will not be possible to understand how neurotrophins cause their effects in neurons.

**Implications for neurological diseases**

Neurotrophins are necessary but not sufficient for the survival of many neuronal populations both during development and throughout life. Target-derived neurotrophins interact with their receptors on a given neuron, not in isolation, but in a complex environment. There will be interactions with molecules on the outside of the neuron and the target cell, such as molecules of the extracellular matrix, with other cell surface receptors and with molecules on the inside of the neuron. If any of the motor neuron-specific signalling molecules are compromised in neurological diseases such as motor neuron disease, then it is likely that neurotrophins would not have been very successful therapeutic agents.53 This would also explain why other neuronal populations such as sympathetic and sensory neurons remain unaffected.

Motor neuron disease is a late onset, progressive deterioration of motor neurons leading to paralysis and death. Many causes have been proposed for this disease, and a genetic basis has been recognized in ~ 10–15% of cases.54,55 There are two types of amyotrophic lateral sclerosis (ALS): familial (FALS) and sporadic (SALS), and the clinical symptoms of each type cannot be differentiated. A genetic linkage has been established in which FALS was linked to chromosome 21q in some families,56 which led to the identification of 11 mutations in 13 families in the gene encoding Cu/Zn superoxide dismutase (SOD1).57 Nearly all are missense mutations which do not lead to an elimination of SOD1 enzymatic activity. The gene product is a metalloenzyme of 153 amino acids, and is expressed in virtually all cells of all organisms above bacteria and is highly conserved across species. Overexpression in mice of a SOD1 transgene with an ALS mutation causes paralysis and death. These animals become weak at ~ 3–4 months of age and rapidly lose function over 4–6 weeks.58 The two major histological features at autopsy are: in the earliest phases of the disease there is a marked vacuolar degeneration of motor neurons and later in the disease a dramatic loss of motor neurons.59 However, biochemical studies have shown that the mutant transgenic animals have an aggregate increase in total SOD activity, but the disease is not attributable to this because animals that express similar levels of wild-type human SOD1 do not become paralysed.

It has been shown that components of axonal transport are affected in animals that are overexpressing the mutant form of SOD1, which occurs prior to the onset of the pathological signs of the disease.60,61 This does not explain why motor neurons are specifically targeted because all neurons express the mutated forms of SOD1, and axonal transport in these cells is unaffected. However, it does suggest that mutated SOD1 is binding to a molecule or affecting a process associated with axonal transport that is specific to motor neurons. Our results from studies examining transport in sympathetic and sensory neurons imply that this may be the case but it remains to be investigated. If mutant forms of SOD affect a process associated with
transport that results in a small dampening of the overall response of the neurons to a particular growth factor, then summing this over time may lead to motor neuronal cell death. The transgenic animal provides the opportunity to explore these hypotheses and to determine some of the molecules that are specifically associated with transport within motor neurons. If this is the case, then understanding the mechanisms that are associated with transport in all types of neurons is important so that therapeutic agents can be given before the onset of the pathological changes, and neuronal cell death may be prevented.

References
Transport of neurotrophins


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