Molecular Neurobiology

ISSN0893-7648/93/7(3–4): 363–382/$5.00

Development of Trophic Interactions in the Vertebrate Peripheral Nervous System

Kristine S. Vogel

Molecular Embryology Section; NCI-Frederick Cancer Research and Development Center; ABL-Basic Research Program; Frederick, MD 21702-1201

Contents

Abstract

During embryogenesis, the neurons of vertebrate sympathetic and sensory ganglia become dependent on neurotrophic factors, derived from their targets, for survival and maintenance of differentiated functions. Many of these interactions are mediated by the neurotrophins NGF, BDNF, and NT3 and the receptor tyrosine kinases encoded by genes of the *trk* family. Both sympathetic and sensory neurons undergo developmental changes in their responsiveness to NGF, the first neurotrophin to be identified and characterized. Subpopulations of sensory neurons do not require NGF for survival, but respond instead to BDNF or NT3 with enhanced survival.

In addition to their classic effects on neuron survival, neurotrophins influence the differentiation and proliferation of neural crest-derived neuronal precursors. In both sympathetic and sensory systems, production of neurotrophins by target cells and expression of neurotrophin receptors by neurons are correlated temporally and spatially with innervation patterns. In vitro, embryonic sympathetic neurons require exposure to environmental cues, such as basic FGF and retinoic acid to acquire neurotrophin-responsiveness; in contrast, embryonic sensory neurons acquire neurotrophin-responsiveness on schedule in the absence of these molecules.

Index Entries: Neurotrophin; neurotrophin receptor; trophic interactions; sensory neuron; sympathetic neuron; neural crest.

Introduction

During embryogenesis, the neurons of vertebrate sympathetic and sensory ganglia become dependent on neurotrophic factors, derived from their targets, for survival and maintenance of differentiated functions (reviewed by Davies, 1987; Barde, 1989). The consequences of this trophic interaction between neurons and their targets include a match between the number of innervating neurons and target size, after superfluous neurons are eliminated during a period of apoptosis (reviewed by Cowan et al., 1984; Oppenheim, 1985; Purves, 1988), and a match between neuron modality or neurotrophic factor responsiveness and target type (reviewed by Davies, 1987, 1992). In addition to these "classic" effects on neuron survival, several neurotrophic factors affect the differentiation, proliferation, and survival of the neural crest cell precursors for sympathetic and sensory neurons. The subjects of this review are the signals and signal receptors that mediate trophic interactions between sympathetic and sensory neurons and their targets in vertebrate embryos. Specifically, I will focus on members of the NGF/BDNF family of neurotrophic factors (neurotrophins) and their cell surface receptors, including the low affinity receptor p75 and members of the *trk* family of receptor tyrosine kinases (Table 1). In the first section, I will compare and contrast the effects of neurotrophins on the survival of sympathetic and sensory neurons throughout development and discuss the possible roles of three neurotrophins in the differentiation of neural crest cells. The second section will include a discussion of temporal patterns of neurotrophin production by target tissues and receptor expression by neurons, and a comparison of the acquisition of neurotrophin-responsiveness in sympathetic and sensory systems. Before I review these aspects of trophic interactions during development, it is necessary to introduce the neurons and their precursors, and the signals and signal receptors that mediate the interactions.

Table 1
Neurotrophins and their Receptors

Neurotrophin	Neurotrophin receptor*
NGF	LNGFR, *trk*
BDNF	LNGFR, *trkB*
NT-3	LNGFR, *trk*, *trkB*, *trkC*
NT-4/5	LNGFR, *trkB*

**See* text for references.

Origins of Sensory and Sympathetic Neurons

Sensory neurons in the trunk of the vertebrate embryo, dorsal root ganglion (DRG) neurons, are derived from the neural crest, a transient population of embryonic stem cells that arise from the lateral edges of the neural plate (reviewed by Weston, 1970, 1991; LeDouarin, 1982). In the head, sensory neurons arise from two sources: cranial neural crest and epidermal placodes, which are thickenings of surface ectoderm (D'Amico-Martel and Noden, 1983; reviewed by LeDouarin et al., 1986; Vogel, 1992; Noden, 1993). Both neural crest and placodal cells undergo epithelial/mesenchymal transitions (Tosney, 1982; D'Amico-Martel and Noden, 1983), and after varying degrees

of migration localize to form ganglion primordia. The precursors of sensory neurons undergo terminal mitosis, and extend axons to their central (spinal cord, hindbrain) and peripheral (muscle spindles, cutaneous receptors, sensory receptors in the viscera) targets.

Sympathetic neurons and adrenal chromaffin cells arise from bipotential neural crest-derived precursors in the primary sympathetic chain (Anderson and Axel, 1986; Vogel and Weston, 1990a); the differentiation of these precursors is influenced by environmental conditions present in the secondary sympathetic chains and adrenal medulla (reviewed by Anderson, 1993). Sympathetic neurons are contacted by the axons of cholinergic preganglionic neurons (Rubin, 1985a,b), whose cell bodies are located in the spinal cord, and extend axons to peripheral targets (heart, blood vessels, and sweat, lacrimal, and salivary glands; Rubin, 1985a). Many of the neural crest-derived cells that enter the adrenal gland initially express neuronal markers (Anderson and Axel, 1985, 1986; Vogel and Weston, 1990a); most of these cells lose expression of these neuron-specific traits under the influence of high levels of glucocorticoids present in the adrenal gland (reviewed by Anderson, 1988). Both the experimental accessibility of sensory and sympathetic neurons and their precursors throughout embryonic development and the identification of neurotrophins and their receptors (*see* next section) make the peripheral nervous system an ideal subject for the study of cell interactions during development.

Neurotrophins and Neurotrophin Receptors

Nerve growth factor (NGF) was the first neurotrophin to be identified and characterized (Cohen, 1960; reviewed by Levi-Montalcini, 1987). Barde and colleagues (1982) purified another neurotrophic factor, brain-derived neurotrophic factor (BDNF), from pig brains. Sequence analysis of BDNF (Leibrock et al., 1989) revealed a high degree of homology with NGF. This led to a flurry of PCR mania and the identification of a third member of the neurotrophin family, neurotrophin-three (NT3; Ernfors et al., 1990; Hohn et al., 1990; Jones and Reichardt, 1990; Kaisho et al., 1990; Maisonpierre et al., 1990; Rosenthal et al., 1990). Neurotrophins four and five (NT4, NT5) have recently been identified (Berkemeier et al., 1991; Hallböök et al., 1991; Ip et al., 1992). The biochemical characteristics of neurotrophins have been reviewed recently by Ebendal (1992).

The low affinity ($K_d = 10^{-9}M$) nerve growth factor receptor, p75, binds NGF, BDNF, NT3, and NT4 (Sutter et al., 1979; Godfrey and Shooter, 1986; Ernfors et al., 1990; Rodriguez-Tébar et al., 1990; Hallböök et al., 1991; Squinto et al., 1991; Rodriguez-Tébar and Rohrer, 1992). The role of this receptor in signal transduction and neurotrophin responsiveness is controversial (reviewed by Chao, 1992). The p75 protein alone does not appear to transduce a signal that results in activation of immediate early genes (e.g., c-*fos*; reviewed by Doucet et al., 1990), internalization of neurotrophins, or enhanced neurite outgrowth (Green et al., 1986; Hempstead et al., 1989). Recently, the tyrosine kinase product of the *trk* gene (gp 140; Martin-Zanca et al., 1990) has been identified as a component of the high affinity ($K_d = 10^{-11}M$) receptor for NGF (Hempstead et al., 1991; Kaplan et al., 1991a,b; Klein et al., 1991; Meakin et al., 1992) and mediates cellular responses to this neurotrophin, including c-*fos* induction, neurite outgrowth, survival, and proliferation (Cordon-Cardo et al., 1991; Loeb et al., 1991). The receptor encoded by the *trk* gene also mediates cellular responses to NT3 and NT5 (Berkemeier et al., 1991; Cordon-Cardo et al., 1991). The related *trkB* gene encodes a receptor tyrosine kinase that mediates cellular responses to BDNF, NT3, NT4, and NT5 (Soppet et al., 1991; Squinto et al., 1991; Klein et al., 1992). A third member of the family, *trkC*, encodes a high affinity receptor specific for NT3 (Lamballe et al., 1991). The biochemical and binding characteristics of neurotrophin receptors have been reviewed by Bothwell (1991), Ross (1991), and Chao (1992). (Table 1).

Roles of Neurotrophins During Development

Neuron Survival

Both sympathetic and sensory neurons transport neurotrophins retrogradely from their targets (Hendry et al., 1974; Stöckel and Thoenen, 1975; Johnson et al., 1978; Brunso-Bechtold and Hamburger, 1979; Korsching and Thoenen, 1983a; Richardson and Riopelle, 1984; DiStephano et al., 1992), and respond to NGF with changes in neurotransmitter and enzyme expression (Hayashi et al., 1985; reviewed by Thoenen and Barde, 1980; Lindsay, 1992). Although both sympathetic and sensory neurons exhibit an initial period of neurotrophin independence, they differ in the duration of the subsequent period of neurotrophin dependence. In addition, sensory neurons may require different neurotrophins produced by their central and peripheral targets for survival (reviewed by Davies, 1987, 1992). In this section on neuron survival I will describe experiments that demonstrate changes in NGF requirements during development and that characterize the survival-promoting effects of BDNF and NT3 on sensory neurons.

The "classic" role of neurotrophins during development, to support the survival of neurons once they have innervated their targets, has been demonstrated by a variety of techniques both in vivo and in vitro. For example, in vivo, neurotrophin levels can be augmented by injection of the protein (NGF, BDNF) or diminished by introduction of antibodies that block the function of the neurotrophin (anti-NGF; reviewed by Levi-Montalcini and Angeletti, 1968; Johnson et al., 1986). In vitro, neurons isolated from embryos at precise developmental stages can be exposed to defined levels of a single neurotrophin or to combinations of neurotrophins for identified periods of time. These in vivo and in vitro studies of neurotrophin action on embryonic sympathetic and sensory neurons have led to two important conclusions. First, the requirements of sympathetic and sensory neurons for one neurotrophin, NGF, change during development. Second, different subpopulations of sympathetic and sensory neurons require different neurotrophins for survival.

NGF Requirements Change During Development

Sympathetic Neurons

Both avian and mammalian sympathetic neurons are dependent on NGF for survival throughout much of development and postnatal life. Postnatal injections of NGF reduce naturally occurring neuron death in the rodent sympathetic superior cervical ganglion (SCG; Hendry and Campbell, 1976). Similarly, sympathetic chain size and neuron numbers are increased in chicken embryos that receive injections of NGF (Oppenheim et al., 1982; Hofer and Barde, 1988). When rodents are exposed to anti-NGF late in embryogenesis or during the neonatal period, up to 99% of the neurons in the SCG and paravertebral sympathetic chains die (Levi-Montalcini and Booker, 1960; Klingman and Klingman, 1967; Johnson et al., 1980; reviewed by Levi-Montalcini and Angeletti, 1968). Anti-NGF treatment causes increased neuron death in the adult mouse (Angeletti et al., 1971) and guinea pig (Rich et al., 1984) SCG, and a decrease in neuron cell body size in the adult rat SCG (Otten et al., 1979). The results of in vitro studies with sympathetic neurons isolated from late embryonic and neonatal rodents (Coughlin et al., 1977, 1978; Coughlin and Collins, 1985; Chun and Patterson, 1977) and from avian embryos (Levi-Montalcini and Angeletti, 1963; Partlow and Larrabee, 1971; Greene, 1977a; Leah and Kidson, 1983; Edgar et al., 1981; Ernsberger et al., 1989) are also consistent with a major role for NGF in sympathetic neuron survival during development and throughout postnatal life.

However, sympathetic neurons isolated from young rodent (Coughlin et al., 1977, 1978; Coughlin and Collins, 1985) and avian (Leah and Kidson, 1983; Ernsberger et al., 1989) embryos can survive and extend neurites in vitro in the absence of NGF. Thus, a large proportion of SCG neurons isolated from E13 and E14 mice survive and extend neurites without NGF (Coughlin and Collins, 1985). Similarly, Klingman (1966) and Kessler and Black (1980) found that exposure of

mouse embryos to anti-NGF early in development (E7–E13) had only a slight negative effect on SCG neurons. By E16 most SCG neurons have become dependent on NGF for survival (Coughlin and Collins, 1985) and they retain this dependence throughout postnatal life. Klingman and Klingman (1967) have suggested that populations of sympathetic neurons that innervate different targets (e.g., spleen, heart, submaxillary glands) may differ in their periods of susceptibility to NGF deprivation. Sympathetic neuroblasts isolated from E7 and E8 chicks can survive and proliferate in the absence of NGF (Leah and Kidson, 1983; Ernsberger et al., 1989), especially when grown on a laminin substratum (Ernsberger et al., 1989). By E11, 70% of avian sympathetic neurons require NGF for survival (Ernsberger et al., 1989), and the survival-promoting effects of NGF are greatest for E12 sympathetic neurons (Edgar et al., 1981). Thus, both avian and mammalian sympathetic neurons undergo a transition from NGF-independence early in development to NGF-dependence later in development. In rodents, this NGF-dependence persists through the neonatal period and into adulthood.

Sensory Neurons

Like sympathetic neurons, avian and mammalian sensory neurons also require NGF for survival during development. In the chick, NGF injections can rescue many DRG neurons that would die during normal embryonic development (Levi-Montalcini and Hamburger, 1951; Hamburger et al., 1981; Hofer and Barde, 1988) or as a result of removing peripheral targets (Hamburger and Yip, 1984). Exposure of quail embryos (Rohrer et al., 1988) or zebrafish larvae (Weis, 1968) to anti-NGF causes a reduction in the numbers of DRG neurons. In vitro, NGF supports the survival of neural crest-derived DRG, dorsomedial trigeminal, and jugular sensory neurons isolated from E6 and E9 chicks (Davies and Lindsay, 1984, 1985) and promotes neurite outgrowth from explants of E10 and E12 mouse trigeminal ganglia (Davies and Lumsden, 1983, 1984).

The results of both in vivo and in vitro manipulations of NGF levels indicate that avian and mammalian sensory neurons have more limited periods of NGF-dependence during development than do sympathetic neurons. For example, although exposure to anti-NGF *in utero* causes an 80% decrease in the numbers of neurons in the sensory DRG, trigeminal, and jugular ganglia in rats (Johnson et al., 1980) and guinea pigs (Johnson et al., 1980; Pearson et al., 1983), animals exposed to anti-NGF during the postnatal period only show no decrease in the number of DRG neurons (Johnson et al., 1980). Like sympathetic neurons, sensory neurons isolated from early embryos can survive in the absence of NGF. Thus, the initial neurite outgrowth (Ludueña, 1973) and survival (Wright et al., 1992) of DRG neurons isolated from E3.5 and E4.5 chicks is independent of NGF, as is the survival and neurite outgrowth of trigeminal neurons isolated from E9 mice (Davies and Lumsden, 1984). By E10–E12, NGF supports the survival of 40% of chick DRG neurons; however, by E16 NGF no longer supports the survival of these neurons above control levels (Greene, 1977b; Barde et al., 1980). This loss of NGF-responsiveness coincides with a decrease in the number of NGF binding sites on the DRG neurons (Herrup and Shooter, 1975). Similarly, mouse trigeminal neurons undergo developmental changes in responsiveness to NGF, as indicated by neurite outgrowth assays (Davies and Lumsden, 1984). Trigeminal neurons isolated from E9 mice prior to axon outgrowth are refractory to NGF, whereas neurons isolated during the period of target innervation and cell death extend neurites in response to NGF. Thus, after an initial period of NGF-independent survival, some sensory neurons pass through a phase of NGF-dependence and subsequently become refractory to the survival-promoting effects of NGF, or perhaps require additional factors for survival.

Subpopulations of Neurons Require Neurotrophins Other than NGF

Several observations on the survival requirements of embryonic neurons led to the discovery and characterization of other members of the NGF family of neurotrophins. First, as described above, the requirements of sympathetic and sen-

sory neurons for NGF change during development. Second, NGF fails to support the survival of all sympathetic and sensory neurons, even during periods of maximum responsiveness. Third, many sympathetic and sensory neurons that are not supported by NGF respond with increased survival to conditioned medium factors and extracts of central and peripheral targets (reviewed by Davies, 1987; Barde, 1989). Although some sympathetic neurons clearly respond to factors other than NGF with increased survival, the responses of sensory neurons to the identified neurotrophins BDNF and NT3 have been characterized in detail.

Sympathetic Neurons

In vivo, a proportion (46%) of SCG neurons in the mouse embryo survive exposure to anti-NGF between E12 and E17 (Klingman, 1966). In vitro, a subpopulation of E16 mouse sympathetic neurons survives in the presence of conditioned medium factors and anti-NGF (Coughlin and Collins, 1985). Similarly, a subpopulation of embryonic chick sympathetic neurons can survive in the presence of heart-conditioned medium and anti-NGF (Edgar et al., 1981; Rohrer et al., 1983). Sympathetic neurons supported by heart-conditioned medium alone differ from those neurons supported by NGF in their expression of neurotransmitter synthesis enzymes (Edgar et al., 1981) and norepinephrine uptake (Rohrer et al., 1983). NT3 supports the survival of 30% of E10 chick sympathetic neurons (Rosenthal et al., 1990) and NT3 mRNA is present in some targets of sympathetic neurons (Ernfors et al., 1990; Hohn et al., 1990; Jones and Reichardt, 1990; Kaisho et al., 1990; Maisonpierre et al., 1990; Rosenthal et al., 1990); however, the conditioned medium factors that support the survival of NGF-independent sympathetic neurons have not been definitively identified.

Sensory Neurons

Subpopulations of embryonic DRG sensory neurons are refractory to the negative effects of anti-NGF in vivo (Johnson et al., 1980; Pearson et al., 1983) and to the survival-promoting effects of NGF in vitro (Barde et al., 1980). Moreover, by these same operational criteria, some sensory ganglia (e.g., nodose) contain no NGF-dependent neurons at any stage examined (Pearson et al., 1983; Lindsay and Rohrer, 1985; but *see* Hedlund and Ebendal, 1978; Katz et al., 1990). Subpopulations of embryonic sensory neurons that do not respond to NGF can be maintained in vitro by conditioned medium factors produced by target cells and extracts of central or peripheral target tissues. For example, spinal cord or brain extracts support the survival of subpopulations of DRG neurons isolated from E8–E16 chicks (Lindsay and Tarbit, 1979; Barde et al., 1980; Lindsay and Peters, 1984). In combination with NGF, the survival-promoting effects of these components are more than additive (Barde et al., 1980), indicating that some neurons may require more than one factor for survival. The novel survival-promoting activity was purified from pig brains as brain-derived neurotrophic factor (BDNF; Barde et al., 1982), a protein related to NGF (Leibrock et al., 1989). In vitro, BDNF promotes the survival of at least some neurons in every type of embryonic sensory ganglion examined (Lindsay et al., 1985; Davies et al., 1986; reviewed by Davies, 1987, 1992). In vivo, many neurons in the developing chick nodose ganglion are rescued by daily injections of BDNF between E3 and E7 (Hofer and Barde, 1988); none of the neurons in this ganglion responds to NGF with increased survival in vivo (Hofer and Barde, 1988) or in vitro (Lindsay and Rohrer, 1985). Other populations of placode-derived neurons that are refractory to NGF (e.g., vestibular, petrosal, ventrolateral trigeminal; Davies and Lumsden, 1983; Davies and Lindsay, 1985) respond to BDNF with enhanced survival in vitro (Lindsay et al., 1985; Davies et al., 1986). BDNF injections also rescue a percentage of the neural crest-derived DRG neurons in chick embryos (Hofer and Barde, 1988); in vitro, the survival-promoting effects of BDNF on E8 chick DRG neurons are additive to those of NGF (Leibrock et al., 1989).

Novel survival-promoting activities have also been identified in the peripheral targets of sensory neurons. Thus, extracts of liver and heart promote survival and neurite outgrowth of nodose neurons isolated from E5–E16 chicks (Lindsay and Rohrer, 1985). Similarly, skeletal muscle extract supports the survival and neurite

outgrowth of 70% of neurons in the chick trigeminal mesencephalic nucleus (TMN), a population of neural crest-derived proprioceptive sensory neurons that do not respond to NGF (Davies, 1986; Davies et al., 1986b). Cutaneous sensory neurons in the trigeminal ganglion, which require NGF for survival (Davies and Lindsay, 1985), do not respond to skeletal muscle or liver extracts (Davies and Lindsay, 1984; Davies, 1986). BDNF also promotes the survival of 70–80% of TMN neurons in vitro; at concentrations below saturation, the effects of BDNF and skeletal muscle extract are additive (Davies et al., 1986b). Davies and colleagues have proposed that the survival of embryonic sensory neurons is dependent on a neurotrophin(s) from the appropriate peripheral target and a different neurotrophin from the central target (Davies et al., 1986; reviewed by Davies, 1987). Although NT3 protein has not been purified from peripheral target tissues, recombinant NT3 produced by COS cells supports the survival of embryonic chick nodose and TMN neurons in vitro (Ernfors et al., 1990; Hohn et al., 1990; Maisonpierre et al., 1990; Rosenthal et al., 1990; Jones and Reichardt, 1990). Moreover, NT3 mRNA is present in the peripheral targets of these neurons (liver, heart, muscle; Ernfors et al., 1990; Hohn et al., 1990; Jones and Reichardt, 1990; Kaisho et al., 1990; Maisonpierre et al., 1990; Rosenthal et al., 1990). Thus, NT3 is likely to represent at least part of the survival-promoting activity present in extracts of these tissues. The survival-promoting effects of neurotrophins on embryonic sympathetic and sensory neurons are summarized in Table 2.

Effects on Development of Neuronal Precursors

In addition to their survival-promoting effects on sensory and sympathetic neurons, neurotrophins act earlier in development to influence the proliferation, survival, and differentiation of the neural crest-derived precursors of these neurons.

NGF

As described earlier, the survival of embryonic sympathetic neurons is initially independent of NGF (Leah and Kidson, 1983; Coughlin and Collins, 1985; Ernsberger et al., 1989). However, NGF has well-characterized effects on the proliferation and differentiation of adrenal chromaffin cells and bipotential sympathoadrenal precursors. NGF is a mitogen for adrenal chromaffin cells isolated from young rats (Lillien and Claude, 1985), and promotes neuronal differentiation of adrenal chromaffin cells isolated from newborn and early postnatal mammals (Unsicker et al., 1978; Aloe and Levi-Montalcini, 1978; Naujoks et al., 1982; Doupe et al., 1985; Anderson and Axel, 1985, 1986). However, NGF is not required earlier in development for *initial* neuronal differentiation in the sympathoadrenal lineage. Thus, about 50% of the sympathoadrenal precursors that populate the embryonic rat adrenal glands initially express neuronal markers in the absence of NGF (Anderson and Axel, 1985, 1986); the expression of these markers is extinguished by glucocorticoids present at high levels in the adrenal gland (Anderson and Axel, 1986; reviewed by Anderson, 1993).

Neuronal precursors isolated from E6 quail DRG can survive and differentiate as neurons on a laminin substratum in the absence of NGF (Ernsberger and Rohrer, 1986). Moreover, NGF does not increase the number of neurons that differentiate in cultures of quail neural crest cells (Kalcheim and Gendreau, 1988; Vogel and Weston, 1990b). Thus, for both sensory neuron and sympathoadrenal precursors, NGF is unlikely to have a role in initial neuronal differentiation.

BDNF

In vivo, BDNF temporarily rescues the neural crest-derived precursors of the DRG after the ganglia anlage are separated from the neural tube by a silastic implant (Kalcheim et al., 1987). In vitro, BDNF increases the number of neurons that arise in cultures of trunk neural crest and somites isolated from E3 quail (Kalcheim and Gendreau, 1988). Similarly, BDNF causes an increase in the number of sensory neuron precursor cells, recognized by the monoclonal antibody SSEA-1, that arise in clonal cultures of quail neural crest cells (Sieber-Blum, 1991). Sieber-Blum (1991) has proposed that BDNF instructs pluripotent neural crest cells to differentiate as sensory

Table 2
Survival Response of Neurons to Neurotrophins*

	SYMP	DRG	VL-TRIG	DM-TRIG	TMN	GEN	VEST	PET	JUG	NOD
NGF	+	+	+	+	−	−	−	−	+	−
BDNF	−	+	+	+	+	+	+	+	+	+
NT3	±	+	ND	ND	+	ND	+	ND	ND	+

*Abbreviations: SYMP, sympathetic ganglion neurons; DRG, dorsal root ganglion neurons; VL-TRIG, ventrolateral trigeminal ganglion neurons (nerve V); DM-TRIG, dorsomedial trigeminal ganglion neurons; TMN-trigeminal mesencephalic nucleus neurons; GEN, geniculate ganglion neurons (nerve VII); VEST, vestibuloacoustic ganglion neurons (nerve VIII); PET, petrosal neurons (nerve IX); JUG, superior/jugular ganglion neurons (nerves IX/X); NOD, nodose ganglion neurons (nerve X).

neurons. However, BDNF does not appear to be required for the expression of neuronal markers by undifferentiated neuronal precursors isolated from E6 quail DRG (Ernsberger and Rohrer, 1986); these precursors may have been exposed to BDNF in vivo earlier in development. In cultures of younger (E4.5) chick DRG, BDNF enhances the maturation of small, neurofilament$^+$ (68 kDa; NF$^+$) cells into neurons with large cell bodies and long neurites (Wright et al., 1992). Thus, in addition to the "classic" effects of BDNF on DRG neuron survival, this neurotrophin acts earlier in development to enhance the survival and influence the differentiation of the NF$^-$ neural crest cell precursors of DRG neurons.

NT3

Recently, Kalcheim and colleagues (1992) have demonstrated that NT3 acts as a mitogen for neural crest cells isolated from E2–E3 quail. Somite cells enhance the proliferative response of neural crest cells to NT3 (Kalcheim et al., 1992). In cultures of E4.5 chick DRG, NT3 enhances the morphological maturation of small NF$^+$ cells and causes an increase in the total number of NF$^+$ cells (Wright et al., 1992). Although this increase is not caused by an effect on the survival of existing neurons, it is not known whether NT3 promotes the proliferation, differentiation, or survival of neuronal precursors (Wright et al., 1992).

Coordination of Trophic Interactions

During development, production of neurotrophins by target cells and expression of neurotrophin receptors by responding neurons must be coordinated both spatially and temporally. In the mammalian embryo, two systems have been well-characterized with respect to the timing of target innervation, temporal and spatial aspects of neurotrophin production by target cells, and development of neurotrophin responsiveness and receptor expression by neurons. Thus, in this section I will compare sympathetic innervation of the heart and sensory innervation of the facial primordia. I will then contrast the mechanisms by which sympathetic and sensory neurons acquire neurotrophin dependence.

Expression of Neurotrophins and Neurotrophin Receptors

In situ and Northern blot analyses of neurotrophin mRNAs (NGF, BDNF, NT3) and immunological assays of neurotrophin protein (NGF) provide information on when and where neurotrophins are synthesized and in what quantities. The presence of neurotrophin receptors can be determined using immunocytochemical methods, radiolabeled neurotrophin binding studies, and *in situ* and Northern blot analyses of

receptor mRNAs. In addition, the development of responsiveness to neurotrophins by embryonic neurons can be assayed by measuring survival, biochemical maturation, and neurite outgrowth in vitro.

Sympathetic Neurons and Targets

In the rat embryo, SCG neurons begin to extend axons as early as E12 and may innervate peripheral targets, such as the lacrimal gland and carotid artery smooth muscle, by E15 (Rubin, 1985a). Using a sensitive two-site immunoassay (Korsching and Thoenen, 1987), Korsching and Thoenen (1988) first detected NGF protein in the heart ventricle, a target of superior cervical and stellate ganglion sympathetic neurons, on E12 in the mouse. NGF levels in the ventricle increased to a maximum by E14; at birth, these levels had decreased by 30%. Comparison of these data with the time course of sympathetic innervation in the rat embryo (Rubin, 1985a) led Korsching and Thoenen (1988) to conclude that the onset of NGF synthesis in sympathetic target organs coincides with the onset of innervation by these neurons. Clegg and colleagues (1989) analyzed NGF mRNA levels in rat heart ventricles throughout late embryonic and postnatal development. They concluded that the onset of and increases in NGF synthesis in the heart coincide with the differentiation of innervating sympathetic neurons and the cessation of neuron death, as well as with the arrival of sympathetic axons in the target field. In adult animals, the levels of NGF protein (Korsching and Thoenen, 1983b) and mRNA (Heumann et al., 1984; Shelton and Reichardt, 1984) in target tissues correlate with the density of sympathetic innervation.

A proportion of SCG sympathetic neurons can respond to NGF with increased survival and neurite outgrowth as early as E14 in the mouse (Coughlin and Collins, 1985) and E15 in the rat (Lahtinen et al., 1986). This corresponds to the initial phases of innervation, since some of the SCG neurons have contacted their peripheral targets by E15 in the rat (Rubin, 1985a). The mRNAs for p75 and *trk* NGF receptors are detectable in mouse sympathetic neurons on E14.5 and E15.5, respectively (Schecterson and Bothwell, 1992). Thus, during development of the mammalian sympathetic nervous system, initial production of NGF by the target organs coincides with the onset of NGF responsiveness in the neurons and the arrival of their axons in the target field.

Sensory Neurons and Targets

The availability of sensitive assays for NGF and its receptors allowed Davies and colleagues to characterize and quantify in detail the temporal and spatial patterns of expression of these molecules during the development of the mouse trigeminal system (reviewed by Davies, 1988). The cutaneous sensory neurons of the trigeminal ganglion begin to extend peripheral axons on E9.5 and first contact their peripheral targets, the epithelia of the maxillary and mandibular processes, by E10.5–E11 (Davies and Lumsden, 1984). With the two-site immunoassay (Korsching and Thoenen, 1987), NGF protein can first be detected in the maxillary process at E11; NGF mRNA is present in very small amounts 12 h earlier, at E10.5 (Davies et al., 1987). Although NGF mRNA is present in both the epithelium and the mesenchyme of the maxillary process, the more densely innervated epithelium contains a higher concentration of NGF message (Davies et al., 1987; Bandtlow et al., 1987). As in the sympathetic nervous system (Heumann et al., 1984; Shelton and Reichardt, 1984), the levels of NGF mRNA in the different target fields of trigeminal neurons (maxillary, mandibular, ophthalmic) are correlated with innervation density (Harper and Davies, 1990).

Trigeminal neurons first begin to respond to NGF with increased neurite outgrowth on E10 (Davies and Lumsden, 1984), and a proportion of neurons in E10 ganglia can bind [^{125}I]NGF with high affinity in vitro. By E14, almost 100% of trigeminal neurons can be labeled with [^{125}I]NGF (Davies et al., 1987a). A detailed Northern blot analysis revealed that trigeminal neurons express low levels of p75 mRNA prior to target contact and that the levels of p75 message per neuron increase fivefold throughout the period of target field innervation (E10.5–E13; Wyatt et al., 1990). *Trk* transcripts can be detected in the trigeminal ganglion as early as E11.5, and virtually all

trigeminal neurons express *trk* mRNA by E15 (Martin-Zanca et al., 1990; Schecterson and Bothwell, 1992). As in the case of sympathetic innervation of the heart, production of NGF by the maxillary process and onset of NGF responsiveness in trigeminal neurons occur at the same time in development, and these events coincide with the arrival of trigeminal axons in the target field.

Acquisition of Neurotrophin Responsiveness

Because of the experimental accessibility of sympathetic and sensory neuron precursors, the acquisition of neurotrophin responsiveness and its relationship to neurotrophin receptor expression can be examined very early in development, using cells isolated prior to target contact.

Sympathetic Neurons

In the mammalian sympathoadrenal lineage, acquisition of NGF-responsiveness depends on signals produced by other cells. Thus, Stemple et al. (1988) found that basic fibroblast growth factor (bFGF) induces neuronal differentiation and NGF-dependence in neonatal rat chromaffin cells. Birren and Anderson (1990) isolated a *v-myc*-immortalized cell line (MAH cells) from E14.5 rat sympathoadrenal precursors to study this issue in detail. E14.5 rat sympathoadrenal precursors can survive and proliferate in the absence of NGF (Anderson and Axel, 1986). MAH cells are unresponsive to NGF and express neither p75 nor *trk* NGF receptors. Basic FGF induces expression of neuronal markers and p75 in MAH cells, and allows a very small proportion of them to respond to NGF with increased survival (Birren and Anderson, 1990). When MAH cells are depolarized with 40 m*M* KCl, they are induced to express *trk* but not p75 mRNAs; in the presence of NGF, these cells extend neurites and 20–25% survive for 5 d in culture (Birren et al., 1992). Thus, the *trk* NGF receptor is sufficient to mediate NGF responsiveness in MAH cells (Birren et al., 1992).

Avian sympathetic neurons also depend on environmental cues to acquire NGF-responsiveness. Sympathetic neuroblasts isolated from E7 chicks survive and proliferate in the absence of NGF, but do not acquire NGF-responsiveness with time in culture (Leah and Kidson, 1983; Ernsberger et al., 1989). Retinoic acid, which increases the number of low- and high-affinity NGF receptors in a neuroblastoma cell line (Haskell et al., 1987), induces expression of high-affinity NGF receptors and NGF-responsiveness in E7 sympathetic neurons (Rodriguez-Tébar and Rohrer, 1991). In contrast, NGF-independent SCG neurons isolated from E14 mice develop NGF-dependence in vitro in the absence of target interaction (Coughlin and Collins, 1985). However, these neurons may represent a later stage in the development of neurotrophin dependence than do E7 chick sympathetic neurons.

Sensory Neurons

Unlike sympathetic neurons, sensory neuron precursors and young sensory neurons isolated prior to target contact acquire neurotrophin responsiveness in the absence of environmental cues. Thus, neuronal precursors isolated from E6 quail DRG do not depend on NGF or BDNF for initial survival and neurite outgrowth; however, these cells respond to NGF and BDNF with increased survival after several days in vitro (Ernsberger and Rohrer, 1988). Similarly, chick nodose neurons isolated prior to target contact (E3.5) survive for several days in the absence of neurotrophins (Vogel and Davies, 1991; Fig. 1). After 72 h in vitro, E3.5 nodose neurons begin to respond to BDNF with increased survival, regardless of whether they have been cultured in the presence or absence of this neurotrophin. Acquisition of BDNF-dependence in chick nodose neurons and other populations of placode-derived cranial sensory neurons is correlated with the time-course of hindbrain innervation. Thus, neurons whose axons reach the hindbrain first (e.g., vestibular) survive for a short time in vitro before responding to BDNF, whereas neurons whose axons reach the hindbrain later (e.g., nodose) survive longer before responding to BDNF (Fig. 2; Vogel and Davies, 1991). Other types of sensory neurons isolated prior to target contact also acquire neurotrophin responsiveness in vitro. For example,

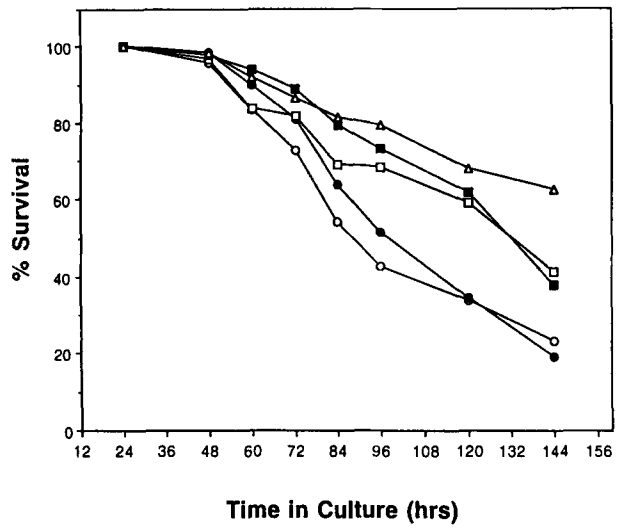

Fig. 1. Nodose ganglion sensory neurons isolated prior to target innervation survive for several days in the absence of neurotrophins. E3.5 chick nodose ganglia were dissociated and plated at very low density in F14 medium containing 10% heat-inactivated horse serum and 5% heat-inactivated fetal calf serum. Twenty-four hours after plating cohorts of neurons were identified using a grid scored on the bottom of each dish; the survival of these cohorts of neurons was monitored over a period of 6 d (Vogel and Davies, 1991). After 72 h in vitro, the survival of neurons in control cultures (no neurotrophins, open circles; and mock-transfected COS cell supernatant, solid circles) was not significantly different from the survival of neurons in cultures supplemented with BDNF (open squares), NT3-transfected COS cell supernatant (solid squares), or BDNF + NT3 (open triangles). After 84 h in vitro, the survival of neurons in cultures supplemented with NT3 (solid squares) is increased compared to controls (open circles, solid circles); BDNF does not increase survival over control levels until 96 h in vitro.

trigeminal neurons isolated from E9 mice acquire NGF-responsiveness on schedule, in the absence of targets and constituents of axon pathways (Davies and Lumsden, 1984; Davies et al., 1987).

Future Directions

The identification of new neurotrophins and receptors and the availability of sensitive probes for these molecules provide endless opportuni-

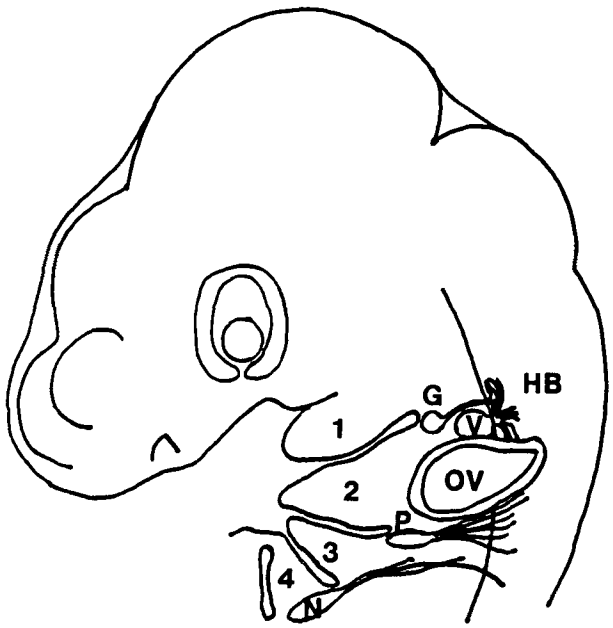

Fig. 2. Cranal sensory ganglia in an E3.S chick. The central projections are based on camera lucida drawings of dil-labeled neurons. Note that nodose axons have the greatest distance to grow to reach the hindbrain; vestibular neurons are closest, and geniculate and petrosal axons have intermediate distances to grow to reach the hindbrain. The peripheral projections are not included in this drawing. V, vestibular ganglion; G, geniculate ganglion; P, petrosal ganglion; N, nodose ganglion; HB, hindbrain; OV, otic vesicle; 1,2,3, and 4, branchial arches 1,2,3,4. From Vogel and Davies, 1991.

ties for researchers to examine the development of trophic interactions in the vertebrate peripheral nervous system. Some issues raised by the results discussed in this review are given below.

Roles of Neurotrophins During Development

Development of the Neural Crest and Placodal Precursors of the PNS

Although both BDNF and NT3 increase the number of neurons in cultures of neural crest cells and young dorsal root ganglia (Kalcheim and Gendreau, 1988; Sieber-Blum, 1991; Wright et al., 1992), it is not clear whether these neurotrophins act to enhance the survival, proliferation, or dif-

ferentiation of neuronal precursors. Clonal analyses (Sieber-Blum, 1991) and time-lapse observations of individual cells will be required to distinguish between these possibilities. Neural crest cell subpopulations that respond to different neurotrophins could be identified with probes specific for *trk* receptors.

Functional Identities of the Subpopulations of Sympathetic and Sensory Neurons Responding to Different Neurotrophins

Both in vivo and in vitro studies have revealed a correlation between function or modality and neurotrophin responsiveness in sensory neurons. Thus, anti-NGF administered to rat fetuses *in utero* causes a 90% decrease in the number of small, unmyelinated DRG nerve fibers, whereas large myelinated fibers are unaffected (Goedert et al., 1984). The small-diameter neurons lost following anti-NGF treatment normally project to laminae I and II of the dorsal horn of the spinal cord, and correspond to nociceptive and thermoreceptive neurons (Ruit et al., 1992). Recently, Carroll et al. (1992) have demonstrated that NGF deprivation *in utero* causes a selective loss of DRG neurons that express *trk* mRNA; neurons that express *trkB* or *trkC* are unaffected. Treatment with anti-NGF postnatally eliminates the population of sural nerve A-delta fibers, which represent cutaneous high-threshold mechanoreceptors that transmit nociceptive stimuli (Ritter et al., 1991).

Whereas cutaneous sensory neurons require NGF for survival during development, proprioceptive sensory neurons, which innervate muscle spindles, do not respond to this factor (Davies et al., 1986b, 1987b). BDNF and NT3 support the survival of the proprioceptive neurons of the embryonic chick trigeminal mesencephalic nucleus in vitro (Davies et al., 1986b; Hohn et al., 1990). Innervation patterns (Ruit et al., 1992), neurotrophin receptor expression (Carroll et al., 1992), neurotransmitter phenotype (Lawson, 1992), and cell surface markers (Marusich et al., 1986) can be used to characterize further the subpopulations of sensory and sympathetic neurons that respond to different neurotrophins.

Combinatorial Effects on Sensory Neurons of Neurotrophins from Central and Peripheral Targets

Davies and colleagues (1986a) have proposed that sensory neurons may require different neurotrophins from their central and peripheral targets to survive in vivo. Seventy to eighty percent of E10 chick TMN proprioceptive neurons survive in the presence of saturating levels of BDNF in vitro; a similar proportion of TMN neurons survive in the presence of saturating levels of skeletal muscle extract. All surviving neurons respond to both factors, since at saturating levels, a combination of BDNF and skeletal muscle extract does not promote survival of additional neurons. At concentrations below saturation, the survival-promoting effects of BDNF and skeletal muscle extracts are additive (Davies, 1986; Davies et al., 1986a). Clearly, to test the above hypothesis, one must be able to determine the concentrations of neurotrophins in the target tissues that are actually available to neurons. In addition, neurotrophin "switching" experiments (Acheson et al., 1987; Ernsberger and Rohrer, 1988) can be performed in vitro, to determine which neurotrophins support the survival of identified neurons and at what concentration they are effective.

Coordination of Trophic Interactions

Neurotrophin Production by Target Cells Throughout Development and First Availability Neurons and Their Precursors

The development of a sensitive two-site immunoassay to detect NGF protein (Korsching and Thoenen, 1987) allowed Davies and colleagues (1987a) to correlate neurotrophin production by target maxillary process cells with innervation by trigeminal neurons in the mouse embryo. Clearly, similar assays specific for other neurotrophins would be invaluable. *In situ* (Bandtlow et al., 1987; Schecterson and Bothwell, 1992) and Northern blot (Clegg et al., 1989; Harper and Davies, 1990) analyses reveal the

presence and allow quantification of neurotrophin mRNAs in target tissues during development. However, the question of when neurotrophins are available to responding neurons and neuronal precursors remains difficult to address. Neurotrophins may be present in forms that neurons cannot use or recognize, and their function may depend on the presence of specific extracellular matrix molecules (Kalcheim et al., 1987).

Neurotrophin Receptor Expression by Neurons and Their Precursors Throughout Development and Neuron Response to and Dependence on Neurotrophins

In situ studies (Klein et al., 1989; Martin-Zanca et al., 1990; Schecterson and Bothwell, 1992) have identified populations of neurons and neuronal precursors that express *trk* mRNAs in mammalian embryos. Antibodies specific for *trk* proteins would allow determination of temporal and spatial patterns of receptor expression during embryogenesis. Populations of neuronal precursors that differentiate in vitro and of neurons isolated prior to target contact (Ernsberger and Rohrer, 1988; Vogel and Weston, 1990b; Sieber-Blum, 1991; Vogel and Davies, 1991) can be used to determine the earliest periods of neurotrophin-responsiveness.

Control of Neurotrophin Production Onset by Target Cells and Receptor Expression by Neurons: Spatial and Temporal Relation to Innervation Patterns

Synthesis of NGF in the peripheral targets of sympathetic and sensory neurons (Shelton and Reichardt, 1986; Rohrer et al., 1988; Clegg et al., 1989) is not dependent on innervation. Moreover, initial differences in innervation density in the mouse trigeminal system do not result from regional differences in NGF synthesis by target epithelia. Thus, the differences in NGF synthesis in ophthalmic, maxillary, and mandibular target areas may serve only to *maintain* previously established differences in innervation density (Davies and Lumsden, 1984; Harper and Davies, 1990). Similarly, the development of neurotrophin-responsiveness in sensory neurons occurs in the absence of target interaction (Davies and Lumsden, 1984; Ernsberger and Rohrer, 1988; Vogel and Davies, 1991). Although fluorescent dyes (e.g., diI; Honig and Hume, 1986) allow analysis of temporal and spatial patterns of target innervation by neurons, the cell interactions and regulatory molecules that may coordinate receptor expression and neurotrophin production remain unknown.

Cellular Mechanisms Underlying Acquisition of Neurotrophin Dependence in Neurons: Relationship Between Neurotrophin Receptor Expression and Neurotrophin Dependence

Larmet et al. (1992) have proposed that electrical activity may play a role in regulating the survival of embryonic chick nodose neurons during the early phases of target field innervation. Thus, depolarizing levels of K^+, acting through L-type calcium channels, first enhance the survival of E3.5 chick nodose neurons in vitro at about the time they become BDNF-dependent and innervate the hindbrain (Vogel and Davies, 1991; Larmet et al., 1992). Depolarizing levels of K^+ induce *trk* mRNA expression and NGF-responsiveness in an immortalized sympathoadrenal progenitor cell line (Birren et al., 1992). Neurotrophins themselves can also influence receptor expression. Thus, NGF induces an increase in *trk* mRNA expression in adult rat cholinergic neurons (Holtzman et al., 1992). The mechanisms by which neurotrophins and other signals affect receptor levels are not known.

Perturbations of p75 and *trk* expression may allow analysis of the relationship between receptor expression and neurotrophin dependence. For example, targeted mutation of the p75 gene in vivo (Lee et al., 1992) or addition of antisense oligonucleotides against the p75 message to cultured neurons (Wright et al., 1992) eliminate or decrease p75 levels, resulting in deficits in sensory innervation, and delayed maturation of DRG neurons, respectively. Recently, Hempstead et al. (1992) have demonstrated that overexpression of *trk* mRNA in a pheochromocytoma (adrenal

chromaffin cell) cell line accelerates NGF-induced differentiation.

Advances in molecular, genetic, biochemical, anatomical, and cell culture techniques have been exploited to enhance our understanding of the development of trophic interactions in the vertebrate peripheral nervous system. Whatever approaches are used, it will always be essential to relate observations on neurotrophin and receptor expression to the temporal and spatial patterns of cell interactions in the developing embryo.

Acknowledgments

Thanks to Kathleen Morrison-Graham and Paul Henion for constructive criticisms of the manuscript and to David Raible for help with the graph.

References

Acheson A., Barde Y.-A., and Thoenen H. (1987) High K^+-mediated survival of spinal sensory neurons depends on developmental stage. *Exp. Cell. Res.* **170,** 56–63.

Aloe L. and Levi-Montalcini R. (1979) Nerve growth factor-induced transformation of immature chromaffin cells in vivo into sympathetic neurons: effect of antiserum to nerve growth factor. *Proc. Natl. Acad. Sci. USA* **76,** 1246–1250.

Anderson D. J. (1993) Cell fate determination in the peripheral nervous system: the sympathoadrenal progenitor. *J. Neurobiol.* **24,** 185–198.

Anderson D. J. (1988) Cell fate and gene expression in the developing neural crest, in *Neural Development and Regeneration, NATO ASI Series,* vol. 22 (Gorio A., ed.), Springer-Verlag, Berlin, pp. 188–198.

Anderson D. J. and Axel R. (1985) Molecular probes for the development and plasticity of neural crest derivatives. *Cell* **42,** 649–662.

Anderson D. J. and Axel R. (1986) A bipotential neuroendocrine precursor whose choice of cell fate is determined by NGF and glucocorticoids. *Cell* **47,** 1079–1090.

Angeletti P. U., Levi-Montalcini R., and Caramia F. (1971) Analysis of the effects of the antiserum to the nerve growth factor in adult mice. *Brain Res.* **27,** 343–355.

Bandtlow C. E., Heumann R., Schwab M. E., and Thoenen H. (1987) Cellular localization of nerve growth factor synthesis by *in situ* hybridization. *EMBO J.* **6,** 891–899.

Barde, Y.-A. (1989) Trophic factors and neuronal survival. *Neuron* **2,** 1525–1534.

Barde Y.-A., Edgar D., and Thoenen H. (1982) Purification of a new neurotrophic factor from mammalian brain. *EMBO J.* **1,** 549–553.

Barde Y.-A., Edgar D., and Thoenen H. (1980) Sensory neurons in culture: changing requirements for survival factors during embryonic development. *Proc. Natl. Acad. Sci. USA* **77,** 1199–1203.

Berkemeier L. R., Winslow J. W., Kaplan D. R., Nikolics K., Goeddel D. V., and Rosenthal A. (1991) Neurotrophin-5: a novel neurotrophic factor that activates *trk* and *trkB*. *Neuron* **7,** 857–866.

Birren, S. J. and Anderson, D. J. (1990) A v-*myc*-immortalized sympathoadrenal progenitor cell line in which neuronal differentiation is initiated by FGF but not NGF. *Neuron* **4,** 189–201.

Birren S. J., Verdi J. M., and Anderson D. J. (1992) Membrane depolarization induces $p140^{trk}$ and NGF responsiveness, but not $p75^{LNGFR}$, in MAH cells. *Science* **257,** 395–397.

Bothwell M. (1991) Keeping track of neurotrophin receptors. *Cell* **65,** 915–918.

Brunso-Bechtold J. and Hamburger H. (1979) Retrograde transport of nerve growth factor in chicken embryo. *Proc. Natl. Acad. Sci. USA* **76,** 1494–1496.

Carroll S. L., Silos-Santiago I., Frese S. E., Ruit K. G., Milbrandt J., and Snider W. D. (1992) Dorsal root ganglion neurons expressing *trk* are selectively sensitive to NGF deprivation *in utero*. *Neuron* **9,** 779–788.

Chao M. V. (1992) Neurotrophin receptors: a window into neuronal differentiation. *Neuron* **9,** 583–593.

Chun L. L. Y. and Patterson P. H. (1977) Role of nerve growth factor in the development of rat sympathetic neurons *in vitro*. 1. Survival, growth, and differentiation of catecholamine production. *J. Cell Biol.* **75,** 694–704.

Clegg D. O., Large T. H., Bodary S. C., and Reichardt L. F. (1989) Regulation of nerve growth factor mRNA levels in developing rat heart ventricle is not altered by sympathectomy. *Dev. Biol.* **134,** 30–37.

Cohen S. (1960) Purification of a nerve growth-promoting protein from the mouse salivary gland and its neuro-cytotoxic antiserum. *Proc. Natl. Acad. Sci. USA* **46,** 302–311.

Cordon-Cardo C., Tapley P., Jing S., Nanduri V., O'Rourke E., Lamballe F., Kovary K., Klein R., Jones K. R., Reichardt L. F., and Barbacid M. (1991) The *trk* tyrosine protein kinase mediates the mitogenic properties of nerve growth factor and neurotrophin-3. *Cell* **66,** 173–183.

Coughlin M. D., Boyer D. M., and Black I. B. (1977) Embryologic development of a mouse sympathetic ganglion *in vivo* and *in vitro*. *Proc. Natl. Acad. Sci. USA* **74,** 3438–3442.

Coughlin M. D., Dibner M. D., Boyer D. M., and Black I. B. (1978) Factors regulating development of an embryonic mouse sympathetic ganglion. *Dev. Biol.* **66,** 513–528.

Coughlin M. D. and Collins M. B. (1985) Nerve growth factor-independent development of embryonic mouse sympathetic neurons in dissociated cell culture. *Dev. Biol.* **110,** 392–401.

Cowan W. M., Fawcett J. W., O'Leary D. D. M., and Stanfield B. B. (1984) Regressive events in neurogenesis. *Science* **225,** 1258–1265.

D'Amico-Martel A., and Noden D. M. (1983) Contributions of placodal and neural crest cells to avian periphel ganglia. *Am. J. Anat.* **166,** 445–468.

Davies A. M. (1986) The survival and growth of embryonic proprioceptive neurons is promoted by a factor present in skeletal muscle. *Dev. Biol.* **115,** 56–67.

Davies A. M. (1987) Molecular and cellular aspects of patterning sensory neurone connections in the vertebrate nervous system. *Development* **101,** 185–208.

Davies A. M. (1988) The trigeminal system: an advantageous experimental model for studying neuronal development. *Development* **103 (Suppl.),** 175–183.

Davies A. M. (1992) Cell death and the trophic requirements of developing sensory neurons, in *Sensory Neurons: Diversity, Development, and Plasticity* (Scott S. A., ed.), Oxford University Press, New York, NY, pp. 194–214.

Davies A. and Lumsden A. (1983) Influence of nerve growth factor on developing dorso-medial and ventro lateral neurons of chick and mouse trigeminal ganglia. *Int. J. Devel. Neurosci.* **1,** 171–177.

Davies A. and Lumsden A. (1984) Relation of target encounter and neuronal death to nerve growth factor responsiveness in the developing mouse trigeminal ganglion. *J. Comp. Neurol.* **223,** 124–137.

Davies A. M. and Lindsay R. M. (1984) Neural crest-derived spinal and cranial sensory neurones are equally sensitive to NGF but differ in their response to tissue extracts. *Dev. Brain Res.* **14,** 121–127.

Davies A. M. and Lindsay R. M. (1985) The cranial sensory ganglia in culture: differences in the response of placode-derived and neural crest-derived neurons to nerve growth factor. *Dev. Biol.* **111,** 62–72.

Davies A. M., Thoenen H., and Barde Y.-A. (1986a) The response of chick sensory neurons to brain-derived neurotrophic factor. *J. Neurosci.* **6,** 1897–1904.

Davies A. M., Thoenen H., and Barde Y.-A. (1986b) Different factors from the central nervous system and periphery regulate the survival of sensory neurons. *Nature* **319,** 497–499.

Davies A. M., Bandtlow C., Heumann R., Korsching S., Rohrer H., and Thoenen H. (1987a) Timing and site of nerve growth factor synthesis in developing skin in relation to innervation and expression of the receptor. *Nature* **326,** 353–358.

Davies A. M., Lumsden A. G. S., and Rohrer H. (1987b) Neural crest-derived proprioceptive neurons express nerve growth factor-receptors but are not supported by nerve growth factor in culture. *Neuroscience* **1,** 37–46.

DiStephano P. S., Friedman B., Radziejewski C., Alexander C., Boland P., Schick C. M., Lindsay R., and Weigand S. J. (1992) The neurotrophins BDNF, NT-3, and NGF display distinct patterns of retrograde axonal transport in peripheral and central neurons. *Neuron* **8,** 983–993.

Doupe A. J., Landis S. C., and Patterson P. H. (1985) Environmental influences in the development of neural crest derivatives: glucocorticoids, growth factors, and chromaffin cell plasticity. *J. Neurosci.* **5,** 2119–2142.

Ebendal T. (1992) Function and evolution in the NGF family and its receptors. *J. Neurosci. Res.* **32,** 461–470.

Edgar D., Barde Y.-A., and Thoenen H. (1981) Subpopulations of cultured chick sympathetic neurones differ in their requirements for survival factors. *Nature* **289,** 294–295.

Ernsberger U. and Rohrer H. (1988) Neuronal precursor cells in chick dorsal root ganglia: differentiation and survival *in vitro*. *Dev. Biol.* **126,** 420–432.

Ernsberger U., Edgar D., and Rohrer H. (1989) The survival of early chick sympathetic neurons in vitro is dependent on a suitable substrate but independent of NGF. *Dev. Biol.* **135,** 250–262.

Ernfors P., Ibañez C. F., Ebendal T., Olson L., and Persson H. (1990) Molecular cloning and neurotrophic activities of a protein with similarities to nerve growth factor: developmental and topographical expression in the brain. *Proc. Natl. Acad. Sci. USA* **87,** 5454–5458.

Godfrey E. W. and Shooter E. M. (1986) Nerve growth factor receptors on chick embryo sympathetic ganglion cells: binding characteristics and development. *J. Neurosci.* **6,** 2543–2550.

Goedert M., Otten U., Hunt S. P., Bond A., Chapman D., Schlumpf M., and Lichtensteiger W. (1984) Biochemical and anatomical effects of antibodies against nerve growth factor on developing rat sensory ganglia. *Proc. Natl. Acad. Sci. USA* **81,** 1580–1584.

Green S. H., Rydel R. E., Connolly J. L., and Greene L. A. (1986) PC12 cell mutants that possess low-but not high-affinity nerve growth factor receptors neither respond to nor internalize nerve growth factor. *J. Cell Biol.* **102,** 830–843.

Greene L. A. (1977a) Quantitative studies on the nerve growth factor (NGF) requirement of neurons I. Sympathetic neurons. *Dev. Biol.* **58,** 96–105.

Greene L. A. (1977b) Quantitative in vitro studies on the nerve growth factor (NGF) requirement of neurons II. Sensory neurons. *Dev. Biol.* **58,** 106–113.

Hallböök F., Ibañez C. F., and Persson H. (1991) Evolutionary studies of the nerve growth factor family reveal a novel member abundantly expressed in *Xenopus* ovary. *Neuron* **6,** 845–858.

Hamburger V., Brunso-Bechtold J. K., and Yip J. W. (1981) Neuronal death in the spinal ganglia of the chick embryo and its reduction by nerve growth factor. *J. Neurosci.* **1,** 60–71.

Hamburger V. and Yip, J. W. (1984) Reduction of experimentally induced neuronal death in spinal ganglia of the chick embryo by nerve growth factor. *J. Neurosci.* **4,** 767–774.

Harper S. and Davies A. M. (1990) NGF mRNA expression in developing cutaneous epithelium related to innervation density. *Development* **110,** 515–519.

Haskell B. E., Stach R. E., Werrbach-Perez K., and Perez-Polo J. R. (1987) Effect of retinoic acid on nerve growth factor receptors. *Cell Tiss. Res.* **247,** 67–73.

Hayashi M., Edgar D., and Thoenen H. (1985) Nerve growth factor changes the relative levels of neuropeptides in developing sensory and sympathetic ganglia of the chick embryo. *Dev. Biol.* **108,** 49–55.

Hempstead B. L., Patil N., Olson K., and Chao M. V. (1988) Molecular analysis of the nerve growth factor receptor. *Cold Spring Harbor Symp. Quant. Biol.* **53,** 477–485.

Hempstead B. L., Martin-Zanca D., Kaplan D. R., Parada L. F., and Chao M. V. (1991) High-affinity NGF binding requires coexpression of the *trk* proto-oncogene and the low-affinity NGF receptor. *Nature* **350,** 678–683.

Hempstead B. L., Rabin S. J., Kaplan L., Reis S., Parada L. F., and Kaplan D. R. (1992) Overexpression of the *trk* tyrosine kinase rapidly accelerates nerve growth factor-induced differentiation. *Neuron* **9,** 883–896.

Hendry I. A., Stöckel K., Thoenen H., and Iversen L. L. (1974) The retrograde axonal transport of nerve growth factor. *Brain Res.* **68,** 103–121.

Hendry I. A. and Campbell J. (1976) Morphometric analysis of rat superior cervical ganglion after axotomy and nerve growth factor treatment. *J. Neurocytol.* **5,** 351–360.

Herrup K. and Shooter E. M. (1975) Properties of the B-nerve growth factor receptor in development. *J. Cell Biol.* **67,** 118–125.

Heumann R., Korsching S., Scott J., and Thoenen H. (1984) Relationship between levels of nerve growth factor (NGF) and its messenger RNA in sympathetic ganglia and peripheral target tissues. *EMBO J.* **3,** 3183–3189.

Hofer M. M. and Barde Y.-A. (1988) Brain-derived neurotrophic factor prevents neuronal death *in vivo*. *Nature* **331,** 261–262.

Hohn A., Leibrock J., Bailey K., and Barde Y.-A. (1990) Identification and characterization of a novel member of the nerve growth factor/brain-derived neurotrophic factor family. *Nature* **344,** 339–341.

Holtzman D. M., Li Y., Parada L. F., Kinsman S., Chen C.-K., Valletta J. S., Zhou J., Long J. B., and Mobley W. C. (1992) p140^{trk} mRNA marks NGF-responsive forebrain neurons: evidence that *trk* gene expression is induced by NGF. *Neuron* **9,** 465–478.

Honig M. G. and Hume R. I. (1986) Fluorescent carbacyanine dyes allow living neurons of identified origin to be studied in long-term cultures. *J. Cell Biol.* **103,** 171–197.

Ip, N. Y., Ibáñez C. F., Nye S. H., McClain J., Jones P. F., Gies D. R., Belluscio L., LeBeau M. M., Espinosa III R., Squinto S. P., Persson H., and Yancopolous G. D. (1992) Mammalian neurotrophin-4: structure, chromosomal location, tissue distribution, and receptor specificity. *Proc. Natl. Acad. Sci. USA* **89,** 3060–3064.

Johnson E. M., Andres R. Y., and Bradshaw R. A. (1978) Characterization of the retrograde transport of nerve growth factor (NGF) using high specific activity [^{125}I]NGF. *Brain Res.* **150,** 319–331.

Johnson E. M., Gorin P. D., Brandeis L. D., and Pearson J. (1980) Dorsal root ganglion neurons are destroyed by exposure *in utero* to maternal antibody to nerve growth factor. *Science* **210,** 916–918.

Johnson E. M., Rich K. M., and Yip H. K. (1986) The role of NGF in sensory neurons *in vivo*. *Trends Neurosci.* **9,** 33–37.

Jones K. R. and Reichardt L. F. (1990) Molecular cloning of the human gene that is a member of the nerve grow factor family. *Proc. Natl. Acad Sci. USA* **87,** 8060–8064.

Kaisho Y., Yashimura K., and Nakahama K. (1990) Cloning and expression of a cDNA encoding a novel human neurotrophic factor. *FEBS Lett.* **266,** 187–191.

Kalcheim C., Barde Y.-A., Thoenen H., and LeDouarin N. M. (1987) *In vivo* effect of brain-derived neurotrophic factor on the survival of developing dorsal root ganglion cells. *EMBO J.* **6,** 2871–2873.

Kalcheim C. and Gendreau M. (1988) Brain-derived neurotrophic factor stimulates survival and neuronal differentiation in cultured avian neural crest. *Dev. Brain Res.* **41,** 79–86.

Kalcheim C., Carmeli C., and Rosenthal A. (1992) Neurotrophin-3 is a mitogen for cultured neural crest cells. *Proc. Natl. Acad Sci. USA* **89,** 1661–1665.

Kaplan D. R., Hempstead B. L., Martin-Zanca D., Chao M. V., and Parada L. F. (1991a) The *trk* proto-oncogene product: a signal transducing receptor for nerve growth factor. *Science* **252,** 554–558.

Kaplan D. R., Martin-Zanca D., and Parada L. F. (1991b) Tyrosine phosphorylation and tyrosine kinase activity of the *trk* proto-oncogene product induced by NGF. *Nature* **350,** 158–160.

Katz D. M., Erb M., Lillis R., and Neet K. (1990) Trophic regulation of nodose ganglion cell development. *Exp. Neurol.* **110,** 1–10.

Kessler J. A. and Black I. B. (1980) The effects of nerve growth factor (NGF) and antiserum to NGF on the development of embryonic sympathetic neurons *in vivo*. *Brain Res.* **189,** 157–168.

Klein R., Parada L. F., Coulier F., and Barbacid M. (1989) *trkB*, a novel tyrosine protein kinase receptor expressed during mouse neural development. *EMBO J.* **8,** 3701–3709.

Klein R., Martin-Zanca D., Barbacid M., and Parada L. F. (1990) Expression of the tyrosine kinase receptor gene *trkB* is confined to the murine embryonic and adult nervous system. *Development* **109,** 845–850.

Klein R., Nanduri V., Jing S., Lamballe F., Tapley P., Bryant S., Cordon-Cardo C., Jones K. R., Reichardt L. F., and Barbacid M. (1991) The *trkB* tyrosine protein kinase is a receptor for brain-derived neurotrophic factor and neurotrophin-3. *Cell* **66,** 395–403.

Klein R., Lamballe F., Bryant S., and Barbacid M. (1992) The *trkB* tyrosine protein kinase is a receptor for neurotrophin-4. *Neuron* **8,** 947–956.

Klingman G. I. (1966) *In utero* sympathectomy of mice. *Int. J. Neuropharmacol.* **5,** 163–170.

Klingman G. I. and Klingman J. D. (1967) Catecholamines in peripheral tissues of mice and cell counts of sympathetic ganglia after the prenatal and postnatal administration of the nerve growth factor antiserum. *Int. J. Neuropharmacol.* **6,** 501–508.

Korsching S. and Thoenen H. (1983a) Quantitative demonstration of the retrograde axonal transport of endogenous nerve growth factor. *Neurosci. Lett.* **39,** 1–4.

Korsching S. and Thoenen H. (1983b) Nerve growth factor in sympathetic ganglia and corresponding target organs of the rat: correlation with density of sympathetic innervation. *Proc. Natl. Acad. Sci. USA* **80,** 3513–3516.

Korsching S. and Thoenen H. (1987) Two-site enzyme immunoassay for nerve growth factor, in *Methods in Enzymology*, vol. 147, Academic, Orlando, FL, pp. 167–185.

Korsching S. and Thoenen H. (1988) Developmental changes of nerve growth factor levels in sympathetic ganglia and their target organs. *Dev. Biol.* **126,** 40–46.

Lahtinen T., Soinila S., and Eranko O. (1986) Age-dependent stimulation by atrium explants or nerve growth factor of nerve fibre outgrowth from cocultured embryonic rat sympathetic ganglia. *Dev. Brain Res.* **27,** 51–57.

Lamballe F., Klein R., and Barbacid M. (1991) *trkC*, a new member of the *trk* family of tyrosine protein kinases, is a receptor for neurotrophin-3. *Cell* **66,** 967–979.

Larmet Y., Dolphin A. C., and Davies A. M. (1992) Intracellular calcium regulates the survival of early sensory neurons before they become dependent on neurotrophic factors. *Neuron* **9,** 563–574.

Lawson S. N. (1992) Morphological and biochemical cell types of sensory neurons, in *Sensory Neurons: Diversity, Development, and Plasticity*, Oxford University Press, New York, NY, pp. 27–59.

Leah J. and Kidson C. (1983) Survival of chick embryo sympathetic neurons in cell culture. *Int. J. Dev. Neurosci.* **1,** 403–409.

LeDouarin N. M. (1982) *The Neural Crest.* Cambridge University Press, Cambridge, UK.

LeDouarin N. M., Fontaine-Pérus J., and Couly G. (1986) Cephalic ectodermal placodes and neurogenesis. *Trends Neurosci.* **9,** 175–180.

Lee K.-F., Li E., Huber J., Landis S. C., Sharpe A. H., Chao M. V., and Jaenisch R. (1992) Targeted mutation of the gene encoding the low affinity NGF receptor p75 leads to deficits in the peripheral sensory nervous system. *Cell* **69,** 737–749.

Leibrock J., Lottspeich F., Hohn A., Hofer M., Hengerer B., Masiakowski P., Thoenen H., and Barde Y.-A. (1989) Molecular cloning and expression of brain-derived neurotrophic factor. *Nature* **341,** 149–152.

Levi-Montalcini R. (1987) The nerve growth factor: thirty-five years later. *EMBO J.* **6,** 1145–1154.

Levi-Montalcini R. and Hamburger V. (1951) Selective growth-stimulating effects of mouse sarcoma on the sensory and sympathetic nervous system of the chick embryo. *J. Exp. Zool.* **116,** 321–362.

Levi-Montalcini R. and Booker B. (1960) Destruction of the sympathetic ganglia in mammals by an antiserum to a nerve-growth protein. *Proc. Natl. Acad. Sci. USA* **46,** 384–391.

Levi-Montalcini R. and Angeletti P. U. (1963) Essential role of the nerve growth factor in the survival and maintenance of dissociated sensory and sympathetic embryonic nerve cells *in vitro*. *Dev. Biol.* **7,** 653–659.

Levi-Montalcini R. and Angeletti P. U. (1968) Nerve growth factor. *Physiol. Rev.* **48,** 534–569.

Lillien L. E. and Claude P. (1985) Nerve growth factor is a mitogen for cultured chromaffin cells. *Nature* **317,** 632–634.

Lindsay R. M. (1992) The role of neurotrophic factors in the functional maintenance of mature sensory neurons, in *Sensory Neurons: Diversity, Development, and Plasticity* (Scott S. A., ed.), Oxford University Press, New York, NY, pp. 404–420.

Lindsay R. M. and Tarbit J. (1979) Developmentally regulated induction of neurite outgrowth from immature chick sensory neurons (DRG) by homogenates of avian or mammalian heart, liver, and brain. *Neurosci. Lett.* **12,** 195–200.

Lindsay R. M. and Peters C. (1984) Spinal cord contains neurotrophic activity for spinal nerve sensory neurons. Late developmental appearance of a survival factor distinct from nerve growth factor. *Neuroscience* **12,** 45–51.

Lindsay R. M. and Rohrer H. (1985) Placodal sensory neurons in culture: nodose ganglion neurons are unresponsive to NGF, lack NGF receptors but are supported by a liver-derived neurotrophic factor. *Dev. Biol.* **112,** 30–48.

Lindsay R. M., Thoenen H., and Barde Y.-A. (1985) Placode-and neural crest-derived sensory neurons are responsive at early developmental stages to brain-derived neurotrophic factor. *Dev. Biol.* **112,** 319–328.

Loeb D. M., Maragos J., Martin-Zanca D., Chao M., Parada L. F., and Greene L. A. (1991) The *trk* proto-oncogene rescues NGF responsiveness in mutant NGF-nonresponsive PC12 cell lines. *Cell* **66,** 961–966.

Ludueña M. A. (1973) Nerve cell differentiation *in vitro*. *Dev. Biol.* **33,** 268–284.

Maisonpierre P. C., Belluscio L., Squinto S., Ip N. Y., Furth M. E., Lindsay R. M., and Yancopoulos G. D. (1990) Neurotrophin-3: a neurotrophic factor related to NGF and BDNF. *Science* **247,** 1446–1451.

Martin-Zanca D., Barbacid M., and Parada L. F. (1990) Expression of the *trk* proto-oncogene is restricted to the sensory cranial and spinal ganglia of neural crest origin in mouse development. *Genes Dev.* **4,** 683–694.

Marusich M. F., Pourmehr K. P., and Weston J. A. (1986) A monoclonal antibody (SNl) identifies a subpopulation of avian sensory neurons whose distribution is correlated with axial level. *Dev. Biol.* **118,** 494–504.

Meakin S. O., Suter U., Drinkwater C. C., Welcher A. A., and Shooter E. M. (1992) The rat *trk* proto-oncogene exhibits properties characteristic of the slow NGF receptor. *Proc. Natl. Acad. Sci. USA* **89,** 2374–2378.

Naujoks K. W., Korsching S., Rohrer H., and Thoenen H. (1982) Nerve growth factor-mediated induction of tyrosine hydroxylase and of neurite outgrowth in cultures of bovine adrenal cells: dependence on developmental stage. *Dev. Biol.* **92,** 365–379.

Noden D. M. (1993) Spatial integration among cells forming the cranial peripheral nervous system. *J. Neurobiol.* **24,** 248–261.

Oppenheim R. W. (1985) Naturally occurring cell death during neural development. *Trends Neurosci.* **8,** 487–493.

Oppenheim R. W., Maderdrut J. L., and Wells D. J. (1982) Cell death of motoneurons in the chick embryo spinal cord. VI. Reduction of naturally occurring cell death in the thoracolumbar column of Terni by nerve growth factor. *J. Comp. Neurol.* **210,** 174–189.

Otten U., Goedert M., Schwab M., and Thibault J. (1979) Immunization of adult rats against 2.5S NGF: effects on the peripheral sympathetic nervous system. *Brain Res.* **176,** 79–90.

Partlow L. M. and Larrabee M. G. (1971) Effects of a nerve growth factor, embryonic age and metabolic inhibitors on synthesis of ribonucleic acid and protein in embryonic sensory ganglia. *J. Neurochem.* **18,** 2101–2118.

Pearson J., Johnson E. M., and Brandeis L. (1983) Effects of antibodies to nerve growth factor on intrauterine development of derivatives of cranial neural crest and placode in the guinea pig. *Dev. Biol.* **96,** 32–36.

Purves D. (1988) *Body and Brain. A Trophic Theory of Neuronal Connections.* Harvard University Press, Cambridge, MA.

Rich K. M., Yip H. K., Osborne P. A., Schmidt R. E., and Johnson E. M. (1984) Role of nerve growth factor in the adult dorsal root ganglia neuron and its response to injury. *J. Comp. Neurol.* **230,** 110–118.

Richardson P. M. and Riopelle R. J. (1984) Uptake of nerve growth factor along peripheral and spinal axons of primary sensory neurons. *J. Neuroscience* **4,** 1683–1689.

Ritter A. M., Lewin G. R., Krema N. E., and Mendell L. M. (1991) Requirement for nerve growth factor in the development of myelinated nociceptors *in vivo*. *Nature* **350,** 500–502.

Rodriguez-Tébar A., Dechant G., and Barde Y.-A. (1990) Binding of brain-derived neurotrophic factor to the nerve growth factor receptor. *Neuron* **4,** 487–492.

Rodriguez-Tébar A. and Rohrer H. (1991) Retinoic acid induces NGF-dependent survival response and high-affinity NGF receptors in immature chick sympathetic neurons. *Development* **112,** 813–820.

Rodriguez-Tébar A., Dechant G., Götz R., and Barde Y.-A. (1992) Binding of neurotrophin-3 to its neuronal receptors and interactions with nerve growth factor and brain-derived neurotrophic factor. *EMBO J.* **11,** 917–922.

Rohrer H. and Barde Y.-A. (1982) Presence and disappearance of nerve growth factor receptors on sensory neurons in culture. *Dev. Biol.* **89,** 309–315.

Rohrer H., Thoenen H., and Edgar D. (1983) Presence of nerve growth factor receptors and catecholamine uptake in subpopulations of chick sympathetic neurons: correlation with survival factor requirements in culture. *Dev. Biol.* **99,** 34–40.

Rohrer H., Hofer M., Hellweg R., Korsching S., Stehle A. D., Saadat S., and Thoenen H. (1988a) Antibodies against mouse nerve growth factor interfere in vivo with the development of avian sensory and sympathetic neurones. *Development* **103,** 545–552.

Rohrer H., Heumann R., and Thoenen H. (1988b) The synthesis of nerve growth factor (NGF) in developing skin is independent of innervation. *Dev. Biol.* **128,** 240–244.

Rosenthal A., Goeddel D. V., Nguyen T., Lewis M., Shih A., Laramee G. R., Nikolics K., and Winslow J. W. (1990) Primary structure and biological activity of a novel human neurotrophic factor. *Neuron* **4,** 767–773.

Ross A. H. (1991) Identification of tyrosine kinase *trk* as a nerve growth factor receptor. *Cell Regul.* **2,** 685–690.

Rubin E. (1985a) Development of the rat superior cervical ganglion: ganglion cell maturation. *J. Neurosci.* **5,** 673–684.

Rubin E. (1985b) Development of the rat superior cervical ganglion: ingrowth of preganglionic axons. *J. Neurosci.* **5,** 685–696.

Ruit K. G., Elliott J. L., Osborne P. A., Yan Q., and Snider W. D. (1992) Selective dependence of mammalian dorsal root ganglion neurons on nerve growth factor during embryonic development. *Neuron* **8,** 573–587.

Schecterson L. C. and Bothwell M. (1992) Novel roles for neurotrophins are suggested by BDNF and NT3 mRNA expression in developing neurons. *Neuron* **9,** 449–463.

Shelton D. L. and Reichardt L. F. (1984) Expression of the B-nerve growth factor gene correlates with the density of sympathetic innervation in effector organs. *Proc. Natl. Acad. Sci. USA* **81,** 7951–7955.

Shelton D. L. and Reichardt L. F. (1986) Studies on the regulation of B nerve growth factor gene expression in iris: the level of mRNA encoding nerve growth factor is increased in iris in explant cultures *in vitro*, but not in irises deprived of sensory or sympathetic innervation *in vivo*. *J. Cell Biol.* **102,** 1940–1948.

Sieber-Blum M. (1991) Role of the neurotrophic factors BDNF and NGF in the commitment of pluripotent neural crest cells. *Neuron* **6,** 949–955.

Soppet D., Escandon E., Maragos J., Middlemas D. S., Reid S. W., Blair J., Burton L. E., Stanton B. R., Kaplan D. R., Hunter T., Nikolics K., and Parada L. F. (1991) The neurotrophic factors brain-derived neurotrophic factor and neurotrophin-3 are ligands for the *trkB* tyrosine kinase receptor. *Cell* **65,** 895–903.

Squinto S. P., Stitt T. N., Aldrich T. H., Davis S., Bianco S. M., Radziejewski C., Glass D. J., Masiakowski P., Furth M. E., Valenzuela D. M., DiStephano P. S., and Yancopolous G. D. (1991) *trkB* encodes a functional receptor for brain-derived neurotrophic factor and neurotrophin-3 but not nerve growth factor. *Cell* **65,** 885–893.

Stemple D. L., Mahanthappa N. K., and Anderson D. J. (1988) Basic FGF induces neuronal differ-

entiation, cell division, and NGF dependence in chromaffin cells: a sequence of events in sympathetic development. *Neuron* **1,** 517–525.

Stöckel K. and Thoenen H. (1975) Retrograde axonal transport of nerve growth factor: specificity and biological importance. *Brain Res.* **85,** 337–341.

Sutter A., Riopelle R. J., Harris-Warrick R. M., and Shooter E. M. (1979) Nerve growth factor receptors: characterization of two distinct binding sites on chick embryo sensory ganglia cells. *J. Biol. Chem.* **254,** 5972–5982.

Thoenen H. and Barde Y.-A. (1980) Physiology of nerve growth factor. *Physiol. Rev.* **60,** 1284–1335.

Tosney K. W. (1982) The segregation and early migration of cranial neural crest cells in the avian embryo. *Dev. Biol.* **89,** 13–24.

Unsicker K., Krisch B., Otten U., and Thoenen H. (1978) Nerve growth factor-induced fiber outgrowth from isolated rat adrenal chromaffin cells: impairment by glucocorticoids. *Proc. Natl. Acad. Sci. USA* **75,** 3498–3502.

Vogel K. S. and Weston J. A. (1990a) The sympathoadrenal lineage in avian embryos I. Adrenal chromaffin cells lose neuronal traits during embryogenesis. *Dev. Biol.* **139,** 1–12.

Vogel K. S. and Weston J. A. (1990b) The sympathoadrenal lineage in avian embryos II. Effects of glucocorticoids on cultured neural crest cells. *Dev. Biol.* **139,** 13–23.

Vogel K. S. and Davies A. M. (1991) The duration of neurotrophic factor independence in early sensory neurons is matched to the time course of target field innervation. *Neuron* **7,** 819–830.

Vogel K. S. (1992) Origins and early development of vertebrate cranial sensory neurons, in *Sensory Neurons: Diversity, Development, and Plasticity* (Scott S. A., ed.), Oxford University Press, New York, NY, pp. 171–193.

Weis J. S. (1968) Analysis of the development of the nervous system of the zebrafish, *Brachydanio rerio* II. The effect of nerve growth factor and its antiserum on the nervous system of the zebrafish. *J. Embryol. Exp. Morphol.* **19,** 121–135.

Weskamp G. and Reichardt L. F. (1991) Evidence that biological activity of NGF is mediated through a novel subclass of high affinity receptors. *Neuron* **6,** 649–663.

Weston J. A. (1970) The migration and differentiation of neural crest cells. *Adv. Morphogen.* **8,** 41–114.

Weston, J. A. (1991) The sequential segregation and fate of developmentally-restricted intermediate cell populations in the neural crest lineage. *Curr. Top. Dev. Biol.* **25,** 133–153.

Wright E. M., Vogel K. S., and Davies A. M. (1992) Neurotrophic factors promote the maturation of developing sensory neurons before they become dependent on these factors for survival. *Neuron* **9,** 139–150.

Wyatt S., Shooter E. M., and Davies A. M. (1990) Expression of the NGF receptor gene in sensory neurons and their cutaneous targets prior to and during innervation. *Neuron* **2,** 421–427.