Molecules That Make Axons Grow

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Abstract

The study of neurite growth in tissue culture has been a productive way to identify substances that may control the behavior of axons in vivo. Molecules that promote the outgrowth of neurites include nerve growth factor, laminin, fibronectin, and a protease inhibitor derived from glia. Evidence that these molecules may influence axon growth and guidance in vivo is discussed. The effects these molecules have at the cellular level are compared, in an attempt to identify common mechanisms of action. Several less well-characterized molecules that influence the behavior of neurites are also discussed.

Index Entries: Axon; neurite; tissue culture; nerve growth factor; laminin; fibronectin; protease nexin; extracellular matrix.

Introduction

The job of axons is to connect neurons with one another and with the outside world. In the nervous systems of higher animals, connections of enormous complexity somehow assemble themselves during development. This accomplishment can be credited largely to populations of growing axons, whoso precisely orchestrated behaviors are executed with high reliability and, typically, in very brief periods of time (days to weeks for many animals). How do axons manage this feat?

After a century of experimentation and debate, this question remains a central motivation for much of the work at the forefront of developmental neurobiology. Attempts to reduce the complexity of the question have been frequent. For example, it has been pointed out that axons could conceivably be quite sloppy—or even random—about where and when they grow, provided that those axons that do reach an appropriate target "recognize" it and stop growing, whereas those axons that do not find a target eventually degenerate and disappear. That axonal regression can be an important force in determining the mature pattern of connectivity has, in fact, been documented in several systems (reviewed by Cowan et al., 1984). However, the idea that such phenomena explain most of the specificity observed in neural connections has not held up well. Instead, a large and steadily increasing number of examples have been found of axons that seem to "know" exactly what path to follow to reach, unfailingly, an appropriate target. These examples come from vertebrates and invertebrates, from studies of central as well as peripheral neurons, and from sensory as well as motor systems (e.g., Constantine-Paton, 1979; Katz et al., 1980; Lance-Jones and Landmesser, 1981; Bentley and Caudy, 1983; Raper et al., 1984; Tosney and Landmesser, 1984; Stirling and Summerbell, 1985; Blair et al., 1985; Harris, 1986; Kuwada, 1986).

Because axonal pathfinding seems to be so important in determining nervous system form and function, there has been considerable interest in the molecular mechanisms that govern axon growth and guidance. Largely, although not exclusively, the success of research in this area has depended on the fact that axon growth can be studied in vitro by observing the behavior of neurites.* The discussion that follows is intended to review what is known about molecules that specifically influence the growth and guidance of neurites. It is hoped that the concerted study of such molecules may give rise to an understanding of the sorts of molecular events that control axon behavior in vivo. It should be stressed, however, that the control of axon growth and guidance in vivo need not depend on "specific" molecules in the axonal environment. Electric fields, mechanical contraints, adhesion, and the intrinsic morphogenetic properties of individual classes of neurons may all influence axon behavior (Letourneau, 1975; Constantine-Paton, 1979; Johnston and Wessells, 1980; Bray, 1982; Solomon, 1984; Patel et al., 1985; Trinkaus, 1985; Letourneau, 1985; Bray et al., 1987). Nevertheless, specific molecular interactions are appealing because they could convey a nearly limitless number of unique instructions to a great number of neuronal populations.

Axon Behavior

Before considering how the molecular environment of axons might control their growth, it is necessary to review the behaviors that growing axons exhibit: Axon growth must begin

*The term "axon" implies morphological and functional specializations that distinguish axons from dendrites. In tissue culture studies it may involve considerable work to determine whether a particular neuronal process is an axon or a dendrite. Usually, this is not done, and the noncommittal term "neurite" is applied instead.

(initiation), it must proceed at some rate (elongation), it must stop at some point (termination), it may proceed in one direction in favor of another (navigation), and it may involve the formation of axonal branches (branching). Of these behaviors, elongation and navigation have been the easiest to manipulate and quantify in vitro, and most of the experiments discussed below concern these two.

What cellular mechanisms are responsible for the behaviors axons exhibit? Clearly important is the activity of growth cones, the motile organelles at the leading edge of growing axons. Among the observations most relevant to this discussion are that growth cone shape is very dynamic, that transient extention and retraction of filopodia (microspikes) enables growth cones to sample the environment in a wide radius, and that factors such as mechanical tension, ion fluxes, and second messenger systems may play roles in determining how a growth cone responds to an environmental signal (Yamada et al., 1971; Johnston and Wessells, 1980; Gundersen and Barrett, 1980; Landis, 1983; Letourneau, 1985; Freeman et al., 1985; Cohan et al., 1986). The ability of growth cones to sample the environment in many directions makes growth cones ideally suited to play a role in axon navigation. Observations of growth cone behavior in vitro and in vivo are consistent with this notion (e.g., Bray and Chapman, 1985; Tsui et al., 1985; Hammarback and Letourneau, 1986; Bentley and Toroian-Raymond, 1986; Bovolenta and Mason, 1987). Growth cones probably also play a role in influencing axon elongation. Nevertheless, elongation can occur in the absence of growth cone motility (Marsh and Letourneau, 1984).

Because axon growth is dependent on the delivery of new membranes, cytoplasm, and organelles into the axon, axonal transport is another cellular mechanism that could play a role in influencing axon behavior. On some level, axonal transport is, of course, essential for axonal elongation, but not enough is known about the molecular bases of the different modes of axonal transport to decide whether environmental influences that modulate axonal elongation might act via effects on axonal transport.

Finally, it is impossible to ignore the importance of the genetic and protein-synthetic machinery housed in the neuronal soma. Indeed, there are particular proteins that are synthesized and transported to growing axons that are apparently not made by neurons with nongrowing axons (Skene, 1984). However, environmental signals are often received by growing axons at distances far from the cell body. The time that is required for axonal transport of newly synthesized materials places limitations on how active a role the cell body can play in navigation or in short-term modulation of elongation.

In Vitro Assays

In vivo experiments have been crucial in demonstrating the importance of axonal growth and guidance in neural development. Some in vivo experiments have even identified cells or structures that seem to bear guidance cues (e.g., Bentley and Caudy, 1983; Raper et al., 1984; Kuwada, 1986). Determining the molecular nature of such cues, however, has been extremely difficult to do in vivo. As a result, tissue culture assays of neurite growth have played an important part in the approach to this question. In vitro assays have advantages in terms of ease, rapidity, and the ability to control for many more variables than would be possible in vivo. On the other hand, in vitro assays can be difficult to interpret, particularly since cells do not always behave in vitro as they would in vivo.

As a result, before any substance or molecule that influences neurite elongation or navigation in vitro can be considered a candidate for an in vivo role, it must be investigated thor-

oughly to determine whether it is likely to have similar effects in vivo, or whether its actions in vitro are merely an accident of the experimental design. This kind of inquiry requires posing certain questions. *"Is the substance actually affecting the neurons in the culture, or is its action mediated indirectly by nonneurons?"* Sometimes, nonneurons can be removed or depleted from the cultures to check this. *"Is the substance influencing neurite growth per se, or is it actually affecting some general parameter such as cell viability or metabolic rate, that then indirectly affects neurite growth?"* To resolve this question requires dissociating the substance's effects on neurite growth from any other effects it may have, or else gathering sufficient evidence that the substance really has no significant effect on parameters such as viability and metabolism. This has been addressed for only a few molecules (these will be discussed below). It should be pointed out, however, that even a molecule with only indirect effects on neurite growth could still play an important role in regulating axon growth in vivo. Gathering evidence to support such a role, however, would likely be an unusually difficult task. *"Does the substance occur in vivo at a time and place appropriate for a role in influencing axon growth or guidance?"* This can sometimes be determined directly. *"Does the substance interact with a specific neuronal receptor?"* It is not necessary for a substance to interact with a specific receptor to be a candidate for in vivo relevance. However, the less specific its molecular interaction, the greater the likelihood that a large number of other molecules could substitute for the substance. Under these circumstances it would be difficult to have confidence in the biological importance of any one such molecule.

In the following pages, the characteristics of several molecules that influence neurite growth are described. Particular attention is focused on four molecules: nerve growth factor, laminin, fibronectin, and a glial-derived protease inhibitor (Table 1). These are molecules for which the above questions have at least been addressed, if not always answered. In the final section, substances whose effects on neurons are less well characterized (Table 2) will also be discussed. Because the aim of this review is to compare what is known about a variety of molecules that are not usually considered together, the discussion focuses narrowly on those points most useful for such a comparison. As a result, many interesting and important studies are, regrettably, not mentioned.

Molecules That Promote Neurite Growth

Nerve Growth Factor

Of all the substances that influence neurite growth, Nerve Growth Factor (NGF) is by far the best characterized. NGF* was first identified as a substance released by certain mouse sarcomas that, when transplanted into chick embryos, caused sensory and sympathetic axons to grow toward and ramify throughout the tumor (Bueker, 1948; Levi-Montalcini 1952, 1987). The subsequent demonstration that these sarcoma cells stimulate neurite outgrowth by embryonic avian and mammalian ganglia in vitro made the purification of NGF possible (Levi-Montalcini et al., 1954). The fortuitous existence of extremely rich sources of NGF (e.g., male mouse salivary gland) has enabled many groups, over more than thirty years, to characterize in detail the structure and

*Abbreviations used: NGF, nerve growth factor; CNS, central nervous system; PNS, peripheral nervous system; ECM, extracellular matrix; CM, conditioned medium; NOPF, neurite outgrowth-promoting factor; GdNPF, glia-derived neurite promoting factor; cyclic AMP, adenosine 3':5'-cyclic monophosphate; BDNF, brain-derived neurotrophic factor.

Table 1

Molecules That Promote Neurite Growth

	Type of molecule	M_r	Active form	Can control neurite navigation	Relevant distribution in vivo	Other effects
Nerve growth factor	Polypeptide growth factor	26,000[a]	Soluble[b]	Yes	Yes	Trophic
Laminin	ECM glycoprotein	850,000	Substratum bound	Yes	Yes	Potentiates NGF action
Fibronectin	ECM glycoprotein	440,000	Substratum bound	[c]	Yes	[d]
GdNPF	Serine protease inhibitor	43,000	[d]	[e]	Yes	Inhibits neuron migration

[a]The M_r of the beta-NGF dimer;
[b]Appears also to be active when substratum-bound (Gundersen, 1985);
[c]One negative result has been reported (Gundersen, 1987);
[d]Not determined;
[e]Not determined for GdNPF, but work with other protease inhibitors (Hawkins and Seeds, 1986b) suggests that the answer will be yes.

physiological actions of this molecule. These characteristics have already been presented in several reviews (e.g., Levi-Montalcini, 1976, 1987; Bradshaw, 1978; Thoenen and Barde, 1980; Yankner and Shooter, 1982). Briefly, beta-NGF, the active component of the NGF complex purified from salivary gland, is a dimer of a 13,000 M_r polypeptide that is derived by proteolytic processing from a larger precursor. It acts, in vivo and in vitro, on a restricted set of neurons, including sympathetic and sensory neurons of the peripheral nervous system (PNS). Recent findings suggest it may act on certain central nervous system (CNS) neurons as well (Korsching, 1986). It binds to a high affinity receptor on the plasma membrane of responsive cells, enabling it to be effective at sub-nanomolar concentrations.

Although NGF is generally assayed by its ability to stimulate neurite growth, it has wide-ranging effects. Most importantly, NGF is a "trophic agent," i.e., it has a positive effect on the health and viability of responsive neurons. For sympathetic and sensory neurons, this has been demonstrated in vivo as well as in vitro (Chun and Patterson, 1977; Greene, 1977; reviewed by Yankner and Shooter, 1982). Indeed, at appropriate times during development, these classes of neurons appear to be de-

Table 2

Other Molecules That Influence Neurite Growth

	Effect on neurite elongation	Other effects	References
Trophic factors and other polypeptide growth hormones			
Brain-derived neurotrophic factor	⇑	Trophic	Barde et al.,1982; Lindsay et al., 1985; Johnson et al., 1986
Basic fibroblast growth factor	⇑	Trophic, heparin-binding	Morrison et al., 1986; Walicke et al., 1986; Schubert et al., 1987; Rydel and Greene, 1987
Acidic fibroblast growth factor	⇑	Heparin-binding, heparin-stimulated	Walicke and Cowan, 1986; Rydel and Greene, 1987
Retinal-derived growth factor[a]	⇑	Trophic (?), heparin-binding.	Wagner and D'Amore, 1986
Neurotransmitters			
Serotonin	⇓	Synaptic transmitter	McCobb and Kater, 1986; Haydon et al., 1987
Dopamine	⇓	Synaptic transmitter	Lankford et al., 1986
Gangliosides	⇑,⇓	Varied	Roisen et al., 1981; Ferrari et al., 1983; Leskawa and Hogan, 1985; Nakajima et al., 1986
Other substances			
S-100-beta	⇑	Calcium-binding	Kligman and Marshak, 1985
Substance released by target of trigeminal neurons	⇑	Chemotactic for axons	Lumsden and Davies, 1986
Component(s) of non-neuronal	⇑		Fallon, 1985a, b; Tomaselli, Reichardt, and Bixby, 1986; Bixby et al., 1987
Component of CNS myelin and cultured oligodendrocytes	⇓		Schwab and Caroni, 1986
Components of neurites	⇑,⇓		Kapfhammer et al., 1986, Kapfhammer and Raper, 1987; Chang et al., 1987

[a]May be identical to acidic fibroblast growth factor.

pendent on NGF for their survival. It is known that NGF is synthesized by tissues innervated by these classes of neurons, and that target-derived NGF is retrogradely transported from axon terminals to cell bodies (Hendry et al., 1975; Thoenen and Barde, 1980; Korsching and Thoenen, 1983a,b; Shelton and Reichardt, 1984; Bandtlow et al., 1987). Retrograde transport, either of NGF itself, or of some intracellular signal produced in response to NGF, appears to be necessary for NGF's action at the cell body (reviewed by Oppenheim, 1981; Cowan et al., 1984). For these reasons, it has been proposed that a major role of NGF in vivo is to modulate the level of cell death in sympathetic and sensory ganglia, matching each ganglion's neuronal number to the size of its target field. The results of chronic administration of NGF or anti-NGF antibodies to embryos and immature animals are consistent with this view (e.g., Gorin and Johnson, 1979; Kessler and Black, 1980; Hamburger et al., 1981).

Because of its trophic actions, the effect of NGF on neurite growth cannot be assumed to be direct. It could be argued that explanted ganglia extend neurites in the presence of NGF simply because the neurons in the ganglia survive in the presence of NGF, and that surviving neurons tend to extend neurites, while moribund or dead neurons do not. Two classes of experiments argue against this interpretation.

First, when a source of NGF is applied locally to an embryo or immature animal (e.g., transplantation of a mouse sarcoma, or localized injections of NGF), one observes not only a generalized hypertrophy of responsive ganglia (trophic effect), but often the directed outgrowth of axons *toward* the NGF source. For example, intracerebral injections of NGF can induce sympathetic axons to enter dorsal roots and travel up the spinal cord to the vicintiy of the injection site (Levi-Montalcini, 1976).

Second, several observations in tissue culture demonstrate that NGF can exert local effects on individual neurites and growth cones. For example, Campenot (1977) demonstrated that growth and maintenance of sympathetic neurites requires that NGF be present *in the localized environment* of the neurites: NGF presented to cell bodies, or to other neurites of the same group of cells, is insufficient. These observations establish a direct effect of NGF on neurite extension. Gunderson and Barrett (1979, 1980) also obtained evidence for a direct effect of NGF on neurite navigation by observing a chemotactic response of growth cones towards an NGF-filled micropipet. Other studies have further documented rapid effects of NGF on growth cone morphology and behavior (Griffin and Letourneau, 1980; Greene, 1984; Connolly et al., 1985).

Although it seems clear that NGF can influence neurite growth, it is particularly difficult to determine the importance of this role in vivo, since no suitable agent exists to block NGF's effects on axons while preserving its trophic effects. Although it has long been suggested that NGF secretion by target tissues acts to guide embyonic axons to them, recent evidence in one system suggests that neither the synthesis of NGF by target tissues nor the expression of NGF receptors by neurons begins until after axons are in the vicinity of their targets (Davies et al., 1987). Perhaps NGF is more involved in controlling the degree of ramification of axons within their target fields. This interpretation would fit well with the observation by Shelton and Reichardt (1984) that NGF mRNA levels in sympathetic target tissues were closely correlated with norepinephrine content (a measure of sympathetic innervation density).

Laminin

Laminin is a glycoprotein of the extracellular matrix (ECM). It is a major component of basement membranes (basal laminae), a specialized form of ECM, and in adult animals appears to be restricted to these structures (reviewed by

Timpl et al., 1982; Timpl et al., 1983; Kleinman et al., 1985). Laminin is an unusually large molecule consisting of an A chain (~400,000 M_r) and two B chains (B1 and B2, each ~210,000 M_r) disulfide-linked to each other (Timpl et al., 1982; Barlow et al., 1984; Sasaki et al., 1987). These polypeptides are physically arranged so as to give laminin a characteristic cross-shaped structure when viewed under the electron microscope (Engel et al., 1981).

Although it has been less than ten years since laminin was first identified (Timpl et al., 1979; Chung et al., 1979), a number of physiologic activities have been suggested for the molecule. Laminin binds to several other components of the ECM, including type IV collagen (Bachinger et al., 1982), proteoglycans (Del Rosso et al., 1981), entactin (Carlin et al., 1981), as well as to itself (Charonis et al., 1986). These observations suggest that laminin may play a structural role in directing the assembly or maintaining the integrity of the ECM (Kleinman et al., 1984). In addition, laminin binds to components on the surface of cells. One of these is a high affinity (~2 nM K_d) receptor on the surface of epithelial, muscle, and certain other cells (Malinoff and Wicha, 1983; Rao et al., 1983; Lesot et al., 1983). Observations in vitro confirm that laminin is an attachment factor for these types of cells (Terranova et al., 1983; Wewer et al., 1986). This role is consistent with the fact that epithelia and myocytes are found adjacent to basement membranes in vivo.

Laminin also interacts with other cell types, by way of interactions of lower affinity. These interactions may be important in cell attachment, migration, or other behaviors (Kleinman et al., 1984, 1985). These effects may be mediated by interactions between laminin and cell surface proteoglycans (Hassell et al., 1986), glycolipids (Roberts et al., 1985), or members of a newly described class of polypeptide receptors that have been named "integrins" (Hynes, 1987). More will be said about these receptors in the following sections.

The first report that laminin promotes neurite growth appeared only five years ago (Baron-van Evercooren et al., 1982). However, the effects of laminin on neurite behavior had come under scrutiny several years earlier, as a result of several groups' interest in tissue culture-derived factors that promoted neurite growth. The earliest of these observations came from Collins (1978), who, with other investigators (Helfand et al., 1978), had begun to look at heart cell-conditioned medium (cell culture medium in which heart cells had been cultured for several days) as a possible source of a trophic molecule that would affect parasympathetic neurons the way NGF affects sympathetic and sensory neurons. Heart cells were chosen because the heart is parasympathetically innervated in vivo. As hoped, the conditioned medium (CM) supported both the survival and neurite growth of parasympathetic neurons. Surprisingly, these effects appeared to be caused by two distinct factors, one with a purely trophic (survival-enhancing) action, and one that stimulated neurite growth. The two factors were distinguishable because incubation of the CM over a series of polyornithine-coated tissue culture dishes depleted the latter, but not the former. Indeed, if such dishes were then washed, it could be shown that the neurite outgrowth-promoting activity became associated with the culture dish itself: Neurons cultured on these dishes responded with rapid and very extensive neurite outgrowth. The trophic activity did not associate with the dish (Collins, 1978).

These observations suggested that the neurite outgrowth-promoting factor (NOPF) in the CM might actually act from the substratum, rather than in solution. Collins and Garret (1980) tested this by preparing a dish with a sharp border separating CM-treated and untreated areas of the substratum. Neurons cultured on these dishes extended profuse neurites over the treated area exactly to, but rarely beyond, the border, indicating that neurites could respond to a substratum-bound

pattern of the NOPF. Using more precise patterns of substratum treatment, Collins and Lee (1984) provided compelling visual evidence that this factor can control neurite navigation as well as stimulate neurite elongation (Fig. 1).

The production of a NOPF by cells that are normally innervated in vivo seemed nicely consistent with a role for target tissues in controlling axon growth and guidance. Subsequently, however, Adler et al. (1981), Coughlin et al. (1981), Lander et al. (1982), and others (e.g., Calof and Reichardt, 1984) showed that CM taken from a wide variety of cultured cells —indeed from almost any type of cell that grows atttached to a culture dish—contained a substratum-binding neurite outgrowth-promoting activity indistinguishable from that in heart cell CM. These investigators also showed that a large number of different types of neurons—including sensory, sympathetic, spinal motor, and retinal neurons—responded to substrata treated with these CM-derived NOPFs in exactly the same way that parasympathetic neurons did, by rapidly extending neurites.

It was suspected early on in the characterization of these NOPFs that they consisted of some component of ECM, and preparations of ECM produced in vitro indeed contained a similar activity (Collins, 1980; Lander et al., 1982). Nevertheless, the identification of laminin as the active component of CM-derived NOPFs was hampered by a variety of complications, including the following.

1. The NOPFs in CMs occur as complexes or aggregates of two or more ECM molecules. One of these is identifiable as laminin on the basis of biochemical and immunochemical properties, and it has been found to be part of the NOPF in every CM tested (Lander et al., 1985a; Davis et al., 1985a; Calof and Reichardt, 1985). A second component is a heparan sulfate proteoglycan, and it is believed to associate with laminin by way of laminin's heparin-binding domain. There is direct evidence that a proteoglycan is associated with the NOPF in some CMs; for other CMs indirect evidence exists (Lander et al., 1985a,b; Davis et al., 1985a; Steele and Hoffman, 1986). A third ECM molecule, entactin, has been found associated with the NOPFs in some but not all CMs (Lander et al., 1985a,b). Of these molecules, the evidence indicates that the ability to promote neurite growth rests primarily, if not entirely, with laminin. Purified laminin (from several sources) can fully substitute for the factor isolated from CM, while purified proteoglycans and entactin are ineffective (Manthorpe et al., 1983; Lander et al., 1985a,b; Rogers et al., 1983; Wewer et al., 1983; Davis et al., 1985a,b). In addition, when most of the proteoglycan and entactin were removed from an isolated NOPF, no loss of activity was observed (Lander et al., 1985b). Nevertheless, the association of a proteoglycan with the NOPFs in CMs does confer a unique property on them: highly efficient adsorption to polycationic (polysine- or polyornithine-coated) substrata (Lander et al., 1985b).*
2. When the laminin-containing NOPFs in CM are compared with laminin purified from the Engelbreth-Holm-Swarm sarcoma (the source of the laminin with which most biochemical and immunochemical studies have been done), a few differences stand out. Most strikingly, antisera prepared against sarcoma-derived laminin block the neurite outgrowth-promoting activity of that laminin, but not the activity of the NOPFs in most CMs (Lander et al., 1983, 1985a; Manthorpe et al., 1983). Conversely, antisera directed against NOPFs block the activity of the NOPFs, but frequently do not block the activity of sarcoma-derived laminin (Edgar et al., 1984). The explana-

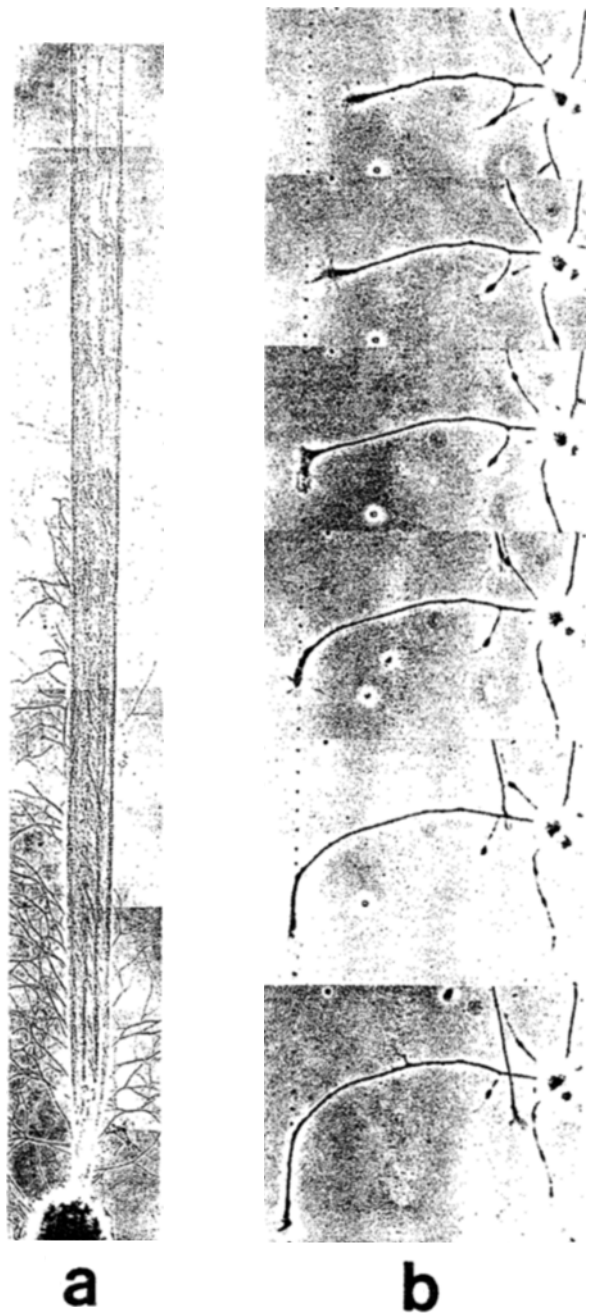

Fig. 1. Restriction of ciliary ganglion cell neurite outgrowth to a pathway of substratum-adsorbed material from heart cell conditioned medium. A. Channeling of neurite growth from an explant 72 h after plating. B. The turning response of a growth cone at the edge of the pathway. The series of photomicrographs condense 2 min of elapsed time (both photographs reproduced from Collins and Lee, 1984; © Society for Neuroscience, 1984). The active substratum-binding component of heart cell and other conditioned media appears to be laminin (*see* text) and data similar to these have also been obtained using purified laminin (e.g., Gundersen, 1987).

tion for these findings is still not clear. It may be that the association of laminin with proteoglycans in CMs induces conformational changes or creates new epitopes that give it distinct immunochemical properties. Consistent with this idea, a monoclonal antibody that blocks the activity of the NOPF in certain CMs appears to recognize an eptiope that only exists (or becomes accessible) when laminin and a heparan sulfate proteoglycan are present together (Chiu et al., 1986). Alternatively, the laminin in CMs and sarcoma-derived laminin may represent different but related polypeptides. It is known that fibronectin, another ECM glycoprotein, exists in multiple isoforms (Hynes, 1985). There is even some indirect evidence to suggest that this may be the case for laminin as well (Wan et al., 1984; Wewer et al., 1987).

3. There has been some controversy over the polypeptide composition of the laminin in CMs, with some authors suggesting that CM-derived laminin lacks all, or part of, the ~400,000 M_r A-chain (Palm and Furcht, 1983; Cornbrooks et al., 1983; Davis et al., 1985a; Coughlin et al., 1986). Because of this, some have suggested that the molecule in CM be called by some name other than laminin (e.g., "neuronectin" [Coughlin et al., 1986]). With regard to this issue, certain points are relevant: Because the A-chain of laminin is very sensitive to proteolysis (Ott et al., 1982), and because it is not possible to adequately control for proteolytic degradation that occurs during the conditioning of medium, the absence or truncation of A-chains in CM-derived laminin does not necessarily imply the existence of a biosynthetically different species of molecule. Indeed, such an interpretation is challenged by electrophoretic analysis of one NOPF that was performed under nonreducing conditions (so that polypeptides that had become partially degraded might still remain intact owing to intrachain disulfide bonds). In this case (Lander et al., 1985b), a consistently normal molecular weight was observed for the laminin in the NOPF, even with samples which, when analyzed under reducing conditions, showed little or no A-chain present.

*If nothing else, this is of historical significance. In most CMs laminin is of very low abundance compared to other proteins (particularly if the CM contains serum). Calculations suggest that, were all proteins in CM able to adsorb equally well to a substratum, the substratum would become saturated long before laminin molecules had attached in sufficient quantity to be able to detectably promote neurite growth. Proteoglycans, however, bind very strongly to polycations, presumably because proteoglycans bear polyanionic glycosaminoglycan chains. Experiments in which the proteoglycan component of a NOPF is first digested with heparitinase (to remove its glycosaminoglycan) confirm that laminin's association with a proteoglycan is what enables it to adsorb in preference to other proteins. In short, were the laminin in CM not associated with a proteoglycan, its neurite outgrowth-promoting effects would probably not have initially been noticed by Collins (1978), Adler (1981), Coughlin (1981), Lander (1982), and others.

Before considering what role laminin may play in vivo, it is useful to compare laminin's actions with those of NGF.

1. Both molecules promote neurite outgrowth and exert local effects on individual neurites, enabling both to influence neuronal navigation.
2. NGF possesses trophic activities, whereas laminin does not [although laminin does appear to potentiate the effects of suboptimal levels of trophic factors, including

NGF (Edgar and Thoenen, 1982; Edgar et al., 1984)].

3. Laminin exerts its actions on all types of PNS neurons and many types of CNS neurons (e.g. spinal notoneurons, retinal ganglion cells, hippocampal neurons, hypothalamic neurons, and others). NGF's actions in the PNS are restricted to sympathetic and sensory neurons; in the CNS it seems likely to act on certain cholinergic populations (Korsching, 1986).
4. Neurons that respond to laminin respond to it in a substratum-bound form. Indeed, neurons *only* extend neurites in response to laminin that is substratum-bound, a fact that can be illustrated by culturing neurons in the presence of soluble laminin, but on a substratum to which laminin cannot adsorb, e.g., serum-saturated polylysine-coated tissue culture plastic (Lander, unpublished observations). In contrast, neurons clearly respond to soluble NGF, although, under some conditions, substratum-bound NGF can also promote and guide neurite growth (Gundersen, 1985).
5. The neurite outgrowth response to laminin usually begins considerably sooner and proceeds at a more rapid pace than the response to NGF (Lander et al., 1982). For some neurons, the combination of NGF and substratum-bound laminin is, at early times in culture, no more effective than laminin alone; eventually, however, NGF-dependent neurons die on laminin, unless NGF is also present (Lander et al., 1982).

These observations portray laminin as a potent but rather general stimulator of neurite growth. Roles it might play in directing axon growth in vivo can, at present, only be surmised from laminin's distribution. Laminin's presence in basement membranes suggests that it may interact with those axons that grow along basement membranes. These include peripheral axons contacting skin and muscle (Roberts and Patton, 1985; Sanes et al., 1986), very early central axons contacting the basement membrane that surrounds the forming CNS (Easter et al., 1984; Roberts and Patton, 1985), and regenerating peripheral axons within their endoneurial sheaths (a form of basement membrane) (Cornbrooks et al., 1983; Palm and Furcht, 1983; Bignami et al., 1984). What about axons that do not contact basement membranes? These too may interact with laminin in vivo, since, during embyogenesis, laminin also appears in the interstitial ECM within and around forming dorsal root and trigeminal sensory ganglia, along the pathways taken by motor axons as they leave the spinal cord and brainstem (Fig. 2), and in other locations (Rogers et al., 1986; Krotoski et al., 1986; Riggott and Moody, 1987). Laminin has also been reported in the parenchyma of the embryonic CNS (Liesi 1985a; Madsen et al., 1986; Cohen et al., 1987), but not in the adult (Alitalo et al., 1982; Smallheiser et al., 1984). One group of authors has obtained evidence that laminin-immunoreactivity faintly and transiently marks a pathway connecting the chick retina to its target, the optic tectum (Cohen et al., 1987). A similar observation has been made in rat optic nerve (McLoon et al., 1986). An additional level of developmental control appears to operate in this system: Retinal ganglion cells are only transiently responsive to the neurite outgrowth-promoting effects of laminin (Cohen et al., 1986; Hall et al., 1986). As one might expect, their period of responsiveness occurs while they are sending axons to the tectum, which corresponds to the time that laminin is expressed along that pathway. These and other results (e.g., Liesei et al., 1985b) have exciting implications; however, immunohistochemcial observations must be interpreted cautiously. Most of these studies used polyclonal antisera directed against mouse laminin to stain tissues of chick, frog, and other species.

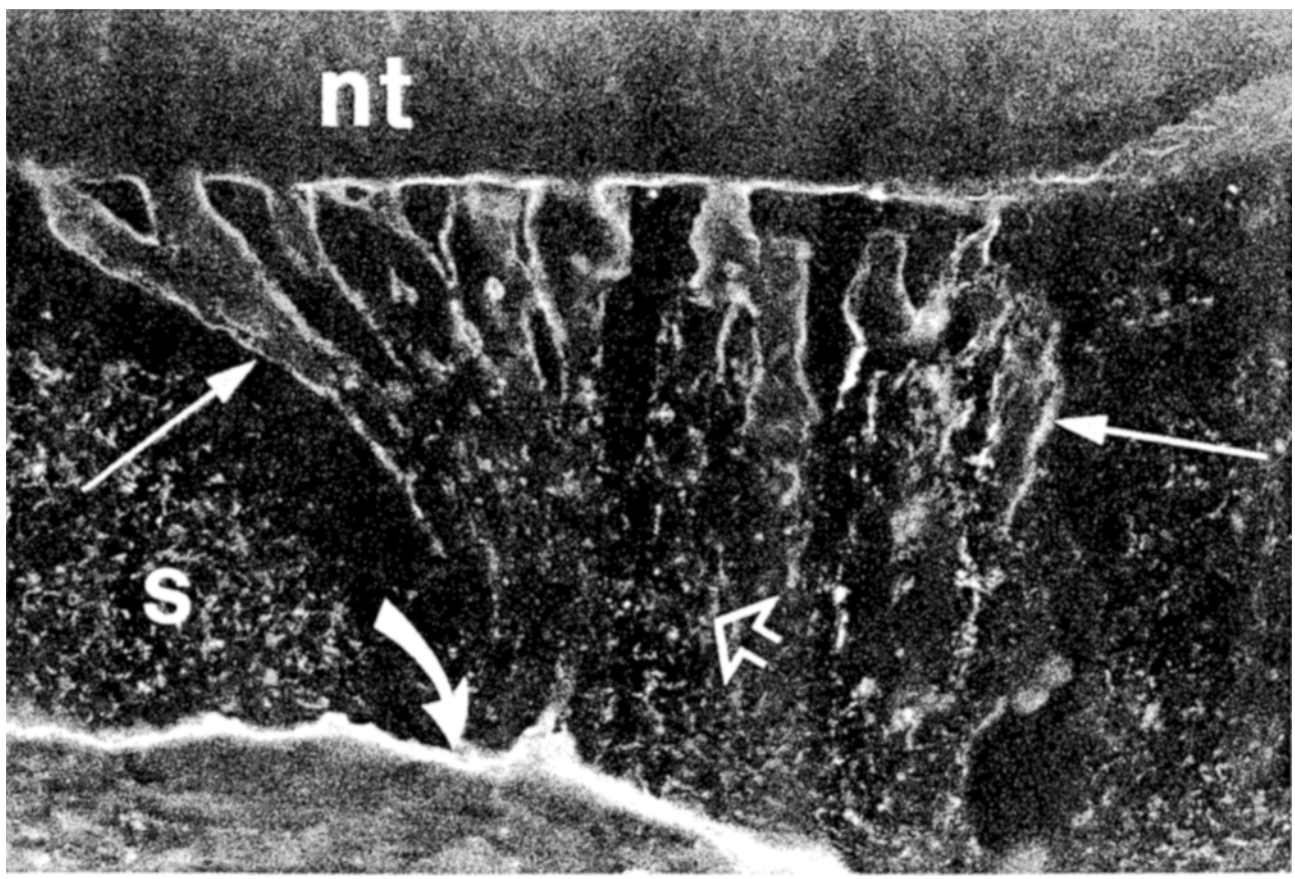

Fig. 2. Laminin immunoreactivity demonstrated in a horizontal section through the ventral root of a stage 17–18 chick embryo. Ventral rootlets (axon bundles) can be seen extending from the neural tube (nt). Immunoreactivity is found in basement membranes surrounding the maturing rootlets (arrows) as well as in a punctate meshwork (arrowhead) in the forming ventral roots, sclerotome cells; curved arrow, edge of dermomyotome. Reproduced from Rogers et al., 1986 © Academic Press, 1986.

In order to have confidence in the sometimes faint staining obtained under these conditions, it will be important to show that laminin is the only molecule these antisera recognize in these species. Showing that mouse laminin will absorb out staining is helpful in these cases, but not entirely satisfactory.

If laminin does play a role in axon growth and guidance in vivo, it should be possible, in principle, to use antibodies to disturb that role. In practice, however, this may be difficult. The abundant laminin in basement membranes may "soak up" antibodies applied in vivo. Furthermore, antibodies that block all of laminin's functions may be lethal to an embryo, for reasons unrelated to the nervous system. Finally, it will be necessary to know what kind of anti-laminin antibodies would be appropriate to use in vivo, the kind that blocks the neurite outgrowth-promoting activity of mouse sarcoma-derived laminin, or the kind that blocks the activity of the laminin in CM-derived NOPFs. Recent studies of neurite outgrowth on tissue slices (Sandrock and Matthew, 1985, 1987) and of regeneration in the iris (W. D. Matthew, personal communication) suggest that the latter kind should be used.

Fibronectin

Like laminin, fibronectin is a component of the ECM, although fibronectin is widely distributed in interstitial matrices as well as in basement membranes (reviewed by Yamada 1983; Furcht, 1983; Hynes, 1985). It is also a component of blood plasma. Fibronectin contains two disulfide-linked polypeptides of ~220,000 M_r. Both chains are encoded by the same gene, but a number of variations in RNA splicing and post-translational modifications give rise to a variety of related subunit isoforms (Hynes, 1985; Kornblihtt et al., 1985; Sekiguchi et al., 1985). The significance of the different isoforms is just beginning to be elucidated (Humphries et al., 1986; Jones et al., 1986; Schwarzbauer et al., 1987).

A number of physiological functions have been proposed for fibronectin, including the mediation of attachment by a variety of cell types, control of cell migration, opsonization of antigens, a role in blood clotting, and a structural role in matrix assembly (Yamada, 1983; Furcht, 1983; Hynes, 1985). These functions presumably depend on fibronectin's ability to bind to interstitial collagens, heparin and heparan sulfate, bacteria, fibrin, and at least one type of cell surface polypeptide receptor. In addition, fibronectin can polymerize into fibrils.

There exist several reports that fibronectin promotes the growth of neurites (Akers et al., 1981; Baron-van Evercooren et al., 1982; Rogers et al., 1983; Manthorpe et al., 1983; Gundersen, 1987). In each case, fibronectin was presented to neurons in a substratum-bound form. Under these conditions, appropriate types of neurons responded with rapid and extensive neurite outgrowth, much as they would have on a substratum treated with laminin. However, two features distinguish neurite growth promoted by fibronectin from that promoted by laminin.

First, the neuronal response to fibronectin is, in a number of respects, consistently weaker than the response to laminin. For example, both the rate of neurite elongation, and the proportion of neurons that respond is less on fibronectin (Baron-van Evercooren et al., 1982; Rogers et al., 1983; Manthorpe et al., 1983; Gundersen, 1987). In addition, the precise guidance of neurites that can be observed along patterns of substratum-bound laminin has not been observed with similar patterns of fibronectin (Gundersen, 1987). Thus, evidence for direct control of neurite navigation by fibronectin is lacking.

Second, fewer types of neurons respond to fibronectin than to laminin. For example, chick embryo autonomic and sensory ganglion neurons respond to fibronectin, but neonatal rat sympathetic neurons apparently do not (Rogers et al., 1983; Manthorpe et al., 1983; Lander

et al., 1983). Fetal human sensory neurons appear to respond to fibronectin, but this has only been assayed under conditions in which significant neurite outgrowth occurred even without fibronectin; in these cases the response observed was a 20–50% enhancement of neurite growth by fibronectin (Baron-van Evercooren et al., 1982). In the CNS, no type of neuron has yet been found to extend neurites in response to fibronectin, with the exception of embryonic d-7 chick retinal cells (Akers et al., 1981). A number of studies of d-8 retinal cells have, however, failed to detect the effect (Rogers et al., 1983; Adler et al., 1985), although one study of d-6 retinal cells reported a small effect when very high concentrations of fibronectin were used (Hall et al., 1987). A recent study on the behavior of Zebrafish neurons suggests that, for some neurons, fibronectin may actually inhibit neurite outgrowth (Frost and Westerfield, 1986). Some of these negative results may be considered tentative, since, in some cases, questions remain about methods for preparing fibronectin-treated substrata [e.g., the pH at which to apply the fibronectin (Akers et al., 1981), or whether to apply it to a substratum containing polyornithine (cf. Manthorpe et al., 1983)].

It is difficult to speculate on whether fibronectin is involved in axon growth and guidance in vivo. The distribution of fibronectin, in embryos and adults, has been reported (Furcht, 1983; Sanes, 1986; Krotoski et al., 1986), and it is widely distributed in interstitial matrices outside the CNS. Although this patttern is not particularly illuminating, it is possible that individual fibronectin isoforms are distributed in more interesting ways. If so, that might be physiologically significant, since functional differences among fibronectin isoforms have been observed (e.g., Humphries et al., 1986). Fibronectin has not beeen detected within the parenchyma of the adult CNS, however, as with laminin, recent reports suggest that localized regions of fibronectin expression in the CNS exist transiently during embryonic development (Pearlman et al., 1986; Chun et al., 1986).

A Glia-Derived Protease Inhibitor

In an attempt to identify agents released by glial cells that regulate axon growth, Monard et al. (1973) cultured mouse neuroblastoma cells in the presence of medium that had been conditioned by rat glioma cells. They discovered that these conditions induced the neuroblastoma cells to extend neurites. A number of drugs and nonspecific treatments (cyclic nucleotides, serum starvation, bromodeoxyuridine) also induce neurite outgrowth by neuroblastoma cells, but these agents produce drastic changes in many aspects of cell behavior, including halting cell growth. In contrast, glioma-CM had no discernable effect on cell growth, nor did it alter cyclic AMP levels in the neuroblastoma cells.

Recently, the factor in glioma CM responsible for inducing neurite formation has been purified (Guenther et al., 1985), and its sequence deduced from a cDNA (Gloor et al., 1986). It is a single polypeptide of 43000 M_r and it promotes neurite growth at subnanomolar concentrations. It is a potent inhibitor of a number of serine proteases including thrombin, trypsin, and urokinase-type plasminogen activator (u-PA). The factor, tentatively named Glia-derived Neurite Promoting Factor (GdNPF) forms a sodium dodecyl sulfate-resistant complex with u-PA. In addition, it binds tightly to heparin. All of its biochemical properties, including molecular weight, are shared with a protease inhibitor known as protease nexin-1 (Knauer et al., 1983; Scott et al., 1985), that has been purified from fibroblasts and other cell types. The 28 amino-terminal amino acids of GdNPF and protease nexin-1 are identical, but overall differences in amino acid composition have led Gloor et al. (1986) to suggest that GdNPF and protease nexin-1 may

turn out to be related, but not identical, proteins.

The fact that GdNPF inhibits protease activity raises the possibility that other protease inhibitors might also promote neurite outgrowth. Consistent with this, Monard et al. (1983; Monard 1985a) have identified two other protease inhibitors that equally stimulate neurite outgrowth at similarly low concentrations. One is the leech thrombin inhibitor hirudin, and the other is the synthetic tripeptide D-Phe-Pro-Arg-CH_2Cl. Various other inhibitors were not found to be effective at low concentrations. Interestingly, GdNPF has other effects on neural cells (effects on neuronal migration and glial proliferation) that possibly do not involve its protease-inhibitory activity (Lindner et al., 1986).

When considering how strong a candidate GdNPF is for a molecule involved in axon-growth in vivo, a number of questions arise.

Does GdNPF Promote Neurite Growth from Primary Cultures of Neurons? Neuroblastoma cells are derived from a neural tumor and may exhibit abnormal responses to physiologic agents. Although the effects of GdNPF on primary neurons have apparently not been studied, Hawkins and Seeds (1986a,b) have examined the effects of several protease inhibitors on dorsal root ganglion cell neurite outgrowth. Unfortunately, these investigators did not test hirudin or D-Phe-Pro-Arg-CH_2Cl, the inhbitors Monard (1985) found most clearly mimicked GdNPF. Nevertheless, their results did demonstrate a significant stimulation of sensory neuron neurite outgrowth by several inhibitors of serine proteases.

Is the Stimulation of Neurite Growth a Direct or Indirect Action of GdNPF? The observation that GdNPF does not influence the viability, growth rate, or cyclic AMP content of neuroblastoma cells suggests that neurite outgrowth induced by GdNPF is not the indirect result of global changes in cell metabolism. Compelling evidence, however, such as could be provided by demonstrating a *local* effect of GdNPF on neurites, is still lacking. Nevertheless, consideration of probable mechanisms for its effects (discussed below) makes a local action for GdNPF seem reasonably likely.

Is GdNPF Appropriately Expressed in Vivo? Tissue culture studies suggest that GdNPF can be synthesized by brain glia (Schuerch-Rathgeb and Monard, 1978). Using a cDNA probe, Gloor et al. (1986) have indeed detected GdNPF gene expression in the developing rat brain. However, the regional and cellular localization of GdNPF and mRNA and its protein product have yet to be determined.

These unresolved questions make it difficult at present to propose specific functions for GdNPF in vivo. However, the existence of a purification scheme for GdNPF, antibodies directed against the protein, and a specific cDNA probe, should make it possible to answer some of these questions in the near future.

Cellular Mechanisms Involved in the Promotion of Neurite Growth

As the foregoing sections illustrate, neurite growth can be stimulated by very different kinds of molecules. NGF is a polypeptide growth factor (Jones and Bradshaw, 1984); laminin and fibronectin are multifunctional glycoproteins immobilized in the extracellular matrix; GdNPF is one of many biologically occurring serine protease inhibitors (Carrell and Travis, 1985; Knauer et al., 1983). By considering the mechanism of action of these molecules, it may be possible to (a) gain insights into how axons grow, and (b) make general predictions about the types of molecules that might influence axon growth and guidance in vivo.

The mechanism of NGF's actions has been under investigation by several groups (reviewed by Yankner and Shooter, 1982; Greene,

1984; Greene et al., 1985). Much work has taken advantage of PC12 pheochromacytoma cells, which respond to NGF by ceasing division, acquiring characteristics of sympathetic neurons, and extending neurites (Green and Tischler, 1982). Although many details remain to be elucidated, some general conclusions can be reached: The binding of NGF to a responsive cell triggers a variety of intracellular changes, some occurring in minutes, others taking hours to days. Some of these changes involve the activation of transcription of particular genes, whereas others are independent of transcription and translation altogether. It is likely that at least the initial events triggered by NGF are mediated through the networks of protein modification (e.g., phosphorylation) and second messengers (e.g., cyclic nucleotides, phosphoinositides) that appear to control an enormous variety of behaviors in all types of cells.

It is possible to make a few guesses about which of NGF's intracellular actions are involved in promoting neurite growth. The transcriptional induction of cytoskeletal and other proteins (Greene, 1984; Drubin et al., 1985; Greenberg et al., 1985) seems likely to be of importance. However, rapid, transcription/translation-independent effects must be involved to explain NGF's rapid effects on growth cone morphology and navigation (Gundersen and Barrett, 1979; Griffin and Letourneau 1980; Greene, 1984; Connolly et al., 1985). NGF's local actions on neurite elongation and maintenance (Campenot, 1977) are also more likely to depend on direct effects on neurites than on genomic effects. Such actions may depend, in part, on protein methylation (Seeley et al., 1984; Acheson et al., 1986), and could involve the modulation of protein kinase activities (Blenis and Erikson, 1986) or of the phosphorylation state of cytoskeletal elements (Black et al., 1986; Greene et al., 1986), although it is too early to make definitive statements. On theoretical grounds, however, one might expect any intracellular changes that affect cytoskeletal polymerizetion or contractility, membrane insertion or recycling, or transmembrane ion fluxes to have at least the potential to influence growth cone motility, stability, or orientation, or the rate of incorporation of new material into growing neurites. This view leads to two predictions: (a) that other physiological agents that produce intracellular signals in neurons might also influence neurite growth, and (b) that pharmacological agents that act on specific intracellular messenger systems might be useful in dissecting the cellular basis of neurite growth. The first of these predictions has been borne out by studies of polypeptide growth factors other then NGF. FGF, for example, mimics many of NGF's actions on PC12 cells, whereas EGF mimics only some (Togari et al., 1985; Greenberg et al., 1985; Blenis and Erikson, 1986; Schubert et al., 1987; Rydel and Greene, 1987). Neurotransmitters such as serotonin and dopamine can also have dramatic effects on neurite growth (discussed below). The second prediction has also been borne out by the usefulness of agents such as cyclic nucleotide analogs, activators of adenylate cyclase, inhibitors of methylation, activators of protein kinase C, and elevated potassium in manipulating neurite growth by primary neurons as well as PC12 cells (Gundersen and Barrett, 1980; Seeley et al., 1984; Rydel and Greene, 1986; Campenot 1986; Greene et al., 1986; Hsu and Chen, 1986).

The mechanisms by which laminin and fibronectin promote neurite outgrowth have not been as well studied as the mechanism of NGF action. Since laminin and fibronectin each have a number of different binding specificities, one approach has been to determine which binding domains are and are not important for effects on neurite growth. Edgar et al. (1984) obtained evidence that, of all the rabbit antibodies that could be generated against mouse laminin or fragments thereof, only antibodies recognizing a single ~50,000 M_r domain inhib-

ited laminin's neurite outgrowth-promoting activity, and this inhibition was total. The results were less clear cut, however, when fragments of laminin were themselves tested for neurite outgrowth-promoting activity. The 50,000 M_r domain had no activity, although a much larger (~280,000 M_r) fragment containing it did. The activity of the larger fragment was not as great as that of intact laminin, and surprisingly, an additional large and completely unrelated fragment also detectably stimulated neurite growth (Edgar et al., 1984). The only other functions that have been previously associated with the 50,000 M_r domain are heparin binding (Ott et al., 1982) and laminin self-association (Charonis et al., 1986). This suggests that laminin's neurite outgrowth-promoting activity may involve interactions with cell-surface heparan sulfate proteoglycans, but this view has been challenged by recent reports that a partially degraded preparation of human laminin lacks the ability to bind heparin, yet retains the ability to promote neurite outgrowth (Engvall et al., 1986).

The results of studies on fibronectin fragments are also complicated: Rogers et al. (1985, 1987) have reported that sets of fragments from two non-overlapping regions of fibronectin can promote neurite outgrowth. One set of fragments contains the major "cell binding domain" of fibronectin, now known to interact with a cell surface receptor of the integrin family (see below), and these fragments promote neurite outgrowth in much the same way as intact fibronectin (Carbonetto et al., 1983; Rogers et al. 1985). A second set of fragments includes fibronectin's carboxy-terminal heparin-binding domain. Suprisingly, these heparin-binding fragments promote neurite outgrowth not only from fibronectin-responsive neurons, but also from neurons that do not respond to intact fibronectin! Recent observations suggest that these fragments contain not only a heparin-binding domain, but also a domain that recognizes a novel cell-surface receptor (Humphries et al., 1986).

These studies on laminin- and fibronectin-derived fragments have yielded results that suggest a surprising level of complexity in the mechanisms by which these molecules influence the growth of neurites. In contrast, attempts to define neuronal cell surface receptors involved in this process have yielded a simpler picture. These studies have made use of two antibodies, both of which interfere with the attachment of fibroblasts, myoblasts, and other cells to fibronectin (Greve and Gottlieb, 1982; Horwitz et al., 1984). When included in neuronal cultures, these antibodies completely block neurite outgrowth promoted by fibronectin as well as by laminin (Bozyczko and Horwitz, 1986; Tomaselli, Reichardt, and Bixby, 1986; Hall et al., 1987). It is now apparent that both antibodies recognize several of the members of a set of structurally related receptors (Leptin, 1986; Ruoslahti and Pierschbacher, 1986; Hynes, 1987). The members of this receptor superfamily, termed "integrins" by one author (Hynes, 1987), include receptors for many ECM molecules, as well as for molecules involved in platelet and immune function. Biochemical tests have documented direct, although weak, binding of laminin and fibronectin to solubilized receptors of this group (Horwitz et al., 1985).

Knowing of receptors that laminin and fibronectin may bind to on the neuronal surface is only the first step in explaining the mechanism by which these molecules influence neurite outgrowth. One hypothesis that has been proposed by nearly all investigators in the field is that laminin and fibronectin increase neurite-substratum adhesion. This hypothesis draws historical support from a number of studies demonstrating the necessity of adequate neurite–substratum adhesion for effective neurite growth. Perhaps the most influential of these studies (Letourneau, 1975) dem-

onstrates that neurons will navigate a complex pattern of a substratum-bound adhesive molecule so as to never leave an area of high adhesivity. This sort of guidance by a substratum-bound pattern is exactly the sort of phenomenon that has been observed with laminin (as discussed above and shown in Fig. 1).

Despite these similarities, there are now good reasons to reject the adhesion hypothesis. The most telling observations were obtained by Gundersen (1987), who actualy measured growth cone-substratum adhesion by sensory neurites on substrata coated with type IV collagen, laminin, fibronectin, polylysine, or various combinations of these. Not only was there no clear positive correlation between adhesion and the extent of neurite outgrowth, but the application of laminin to a region of a collagen substrum actually *decreased* the region's adhesitivity, even though neurites elongated considerably faster on the laminin-treated area and, once on it, refused to cross back onto untreated collagen. Other investigators looking at neuronal cell body attachment to laminin, fibronectin, and collagen have also failed to see a correlation between attachment efficiency and the neurite outgrowth-promoting activity of the substratum (Adler et al., 1985; Hall et al., 1987). These intriguing observations do not really contradict earlier studies on adhesion (e.g., Letourneau, 1975). Instead, it appears that neurites do prefer an adequately adhesive substratum to a poorly adhesive one, but once within the range of adequate adhesion, they will grow faster on, and remain restricted to, an area of substratum containing a specific neurite outgrowth-promoting molecule.

If the actions of laminin and fibronectin cannot be explained by an effect on adhesion, what hypotheses remain? Again, the observations of Gundersen (1987) are pertinent: Individual growth cones were observed as they straddled a border between laminin-on-collagen and untreated collagen. If these cultures were cooled to room temperature, those cellular protrusions of growth cones (e.g., lamellipodia, filopodia) that had extended onto the laminin-treated zone failed to adhere and were completely absorbed into the body of the growth cone. This observation fits with the lower level of growth cone adhesion that had been measured on laminin. However, when the same cultures were left at 37°C and observed by time lapse cinematography, it became clear that cellular protrusions extending onto the laminin-treated area remained stably extended four times longer, on average, than protrusions on the untreated collagen.

These observations indicate that laminin can stabilize growth cone protrusions by some mechanism other than increased growth cone adhesion. This action is particularly significant, because an agent that locally affects the balance between extension and retraction of growth cone protrusions should be expected to profoundly affect neurite elongation and navigation. Whether this accounts for all of laminin's (or fibronectin's) actions on neurites is unclear. Also uncertain is the means by which growth cone stabilization occurs, although there is some evidence for a direct effect on the cytoskeleton: The cell surface receptor for laminin appears to interact on the cytoplasmic side of the plasma membrane with talin, an actin-binding protein (Horwitz et al., 1986). Possibly, substratum-bound laminin stabilizes growth cone protrusions by physically immobilizing actin filaments with respect to the substratum. This hypothesis could explain why laminin in solution has no effect on neurite outgrowth. Alternatively, cellular responses mediated by phosphorylation and/or second messengers could be involved. Indeed, laminin has certain global effects on cells [synergistic interaction with NGF's trophic activity (Edgar et al., 1984), induction of enzymes (Acheson et al., 1986)] that seem more likely to be mediated by these latter types of systems. Significantly, these actions of laminin seem to be triggered by the same domains that are in-

volved in promoting neurite outgrowth (Edgar et al., 1984; Acheson et al., 1986). To approach this question, it might be worthwhile to examine whether laminin triggers some of the same transcription independent effects in neurons that NGF does. Such a line of inquiry could be conducted with PC12 cells, since, under appropriate conditions, they will respond to the neurite outgrowth-promoting effects of laminin (Tomaselli, Damsky, and Reichardt, 1986).

Examining the actions of NGF, laminin, and fibronectin has so far focused attention on cellular events that occur within growth cones. The actions of GdNPF direct attention toward events occurring in the growth cone's external environment, where a connection between extracellular proteolysis and axon growth has been suggested by several observations. For example, motile cells release measurably more extracellular proteolytic activity than stationary cells (e.g., Ossowski et al., 1974), and this finding has been extended to migrating neurons (Krystosek and Seeds, 1981a). That growth cones, being motile organelles, also release high levels of proteases appears to be true both for neuroblastoma cells and primary neurons (Krystosek and Seeds, 1981b; Pittman, 1985).

The correlation between motility and protease release has been attributed to the need for motile cells and growth cones to digest the ECM around them in order to move. Such an hypothesis fails, however, to explain why neurite outgrowth is stimulated, not retarded, by GdNPF and other protease inhibitors. It is possible to build more complex models in which the balance between proteolysis and protease inhibition is critical, but such efforts seem premature. Neurons release and express on their surfaces a variety of different proteases that most likely have very different substrate specificities (Pittman, 1985; Monard, 1985a,b). Only by knowing more about these enzymes and their in vivo substrates will it be possible to understand their function in axon growth. For example, the pharmacology of action of GdNPF, hirudin, and D-Phe-Pro-Arg-CH_2Cl on neurite outgrowh suggests that their effects do not result from the inhibition of plasminogen activators (the class of proteases that has been most frequently mea-sured around cells and growth cones in vitro), but rather from the inhibition of a particular, unidentified protease with a thrombin-like specificity (Monard, 1985a,b). Binding and crosslinking studies may offer a means to identify this molecule (Monard, 1985a,b).

The similarity (perhaps identity) of GdNPF with protease nexin-1 brings to mind molecular characteristics that may be of functional importance. For instance, GdNPF and protease nexin-1 bind heparin, which allows them to associate with heparan sulfate proteoglycans, and thereby become selectively retained on cell surfaces or in the ECM (Knauer et al., 1983; Guenther et al., 1985). Moreover, protease nexin-1 that is bound to heparin (or to a cell surface) appears to be a more effective protease inhibitor than the free molecule (Knauer et al., 1983; Scott et al., 1985; Farrell and Cunningham, 1986). All of this suggests that proteases and protease inhibitors can be widely distributed in the axonal environment, yet still be put to use in very local ways.

Other Molecules That Influence Neurite Growth

A number of other molecules have been described that have effects on neurite growth. In most of these cases it is not known whether they act directly on neurite growth, or indirectly by influencing neuronal viability or overall metabolism.

Some of these molecules are putative trophic factors. One, the "brain-derived neurotrophic factor" (BDNF) is, like NGF, a small (M_r 12300) cationic polypeptide. It has been purified from pig brain and shown to support the survival in vitro of chick sensory neurons and rat retinal

ganglion cells (Barde et al., 1982; Lindsay et al., 1985; Johnson et al., 1986). Although most of what has beeen reported about this molecule concerns its survival-promoting effects, there is evidence that it specifically promotes the growth of neurites: When nodose ganglion cells from embryonic d-12 chick are cultured on a laminin-treated substratum, the neurons survive equally well in the presence or absence of BDNF, but only if BDNF is present will they extend neurites (Lindsay et al., 1985). To understand the basis of this effect, it will be necessary to determine how direct BDNF's stimulation of neurite growth is. For example, BDNF might act by inducing nodose ganglion neurons to synthesize molecules that are required for them to respond to the substratum-bound laminin. Alternatively, BDNF may, like NGF, exert a direct and local influence on neu-rite behavior. This latter possibility could be tested, using the methods Campenot (1977) devised to study NGF's action.

Two related polypeptide growth factors, the acidic and basic fibroblast growth factors (Thomas and Gimenez-Gallego, 1986) and a molecule related or identical to one of them (Wagner and D'Amore, 1986) are also under investigation as putative neurotrophic agents (Morrison, 1987). Basic fibroblast growth factor has been reported to promote survival andneurite outgrowth by neurons from the hippocampus and the cerebral cortex (Morrison et al., 1986; Walicke et al., 1986). Acidic fibroblast growth factor may also have these actions (Walicke and Cowan, 1986). In PC12 cells, basic and acidic fibroblast growth factor have both been found to mimic nearly all of the effects of NGF (Togari et al., 1985; Schubert et al., 1987; Rydel and Greene, 1987). Although the fibro-blast growth factors have traditionally been categorized as broad-spectrum mitogens, their particular abundance in the brain (Lobb and Fett, 1984) has always hinted at a role in neural development.

New ECM molecules that have a neurite outgrowth-promoting activity similar to that of laminin and fibronectin have yet to be described, but antibody-blocking studies suggest their existence (Sandrock and Matthew, 1986). One possibility is that neurites themselves introduce neurite outgrowth-promoting molecules into the ECM. Intriguingly, antibodies against the cell-surface receptor subfamily involved in the neuronal response to laminin and fibronectin have been found to block neurite outgrowth even on a "bare" polylysine substratum (Bozyczko and Horwitz, 1986). Since these antibodies do not simply interfere with neurite outgrowth under all circumstances (see below), this result suggests they are blocking neuronal responses to something neurons themselves may have put onto the substratum. Indeed, evidence exists that neurites release molecules that associate with the substratum (Adler and Varon, 1981; Gundersen and Barrett, 1984). It is unlikely that such a substance is laminin or fibronectin, since these proteins have not been identified among the molecules synthesized by neurons.

Progress is also being made on the identification of a substance on the surface of glial and other cells that promotes neurite outgrowth (Fallon, 1985a,b). Recent observations (Tomaselli, Reichardt, and Bixby, 1986; Bixby et al., 1987) suggest that the active factor is neither laminin nor fibronectin, but a detergent extractable component of the cell surface. Strong candidates for this component include molecules believed to mediate the adhesion of neurons to nonneuronal cells (e.g., *N*-CAM [Edelman, 1985], L1/Ng-CAM [Fassner et al., 1984; Grumet et al., 1984], and *N*-cadherin [Hatta et al., 1985]). Though the adhesive properties of these molecules suggest obvious ways in which they might influence the navigation of neurites (Rutishauser, 1985), it has only recently been suggested that they might stimulate neurite elongation as well (Chang et

al., 1987). Whatever the nature of the cell-surface component responsible for promoting neurite outgrowth, it is interesting that it appears not to interact with the same class of neuronal cell-surface receptors that laminin and fibronectin recognize (Tomaselli et al., 1986).

Other molecules reported to promote neurite outgrowth in various systems include S-100-beta, a small (~6500 M_r) calcium binding protein extractable from brain (Kligman and Marshak, 1985). In vitro studies suggest that target tissues of the mouse trigeminal ganglion may release a factor that is a potent chemoattractant for trigeminal neurites. Unlike NGF, this factor appears to be produced by cells at a very early stage in development, before axons have reached their target (Lumsden and Davies, 1986). A number of studies (e.g., Roisen et al., 1981; Ferrari et al., 1983; Leskawa and Hogan, 1985; Nakajima et al., 1986) have documented stimulatory effects of certain gangliosides on neurite outgrowth. Interpretation of these studies is clouded by the fact that gangliosides added to cells in culture end up in numerous locations, both adsorbed to cell surface proteins, and intercalated into the cell membrane (Wiegandt et al., 1982). Nonetheless, it is intriguing that a particular rare ganglioside has been found to be distributed in vivo in a dorsoventral gradient across the rat retina (Constantine-Paton et al., 1986). Such a distribution is suggestive of a molecule that plays a role in establishing the topographic projection of retinal axons to the optic tectum.

In addition to substances that stimulate neurite growth, there are a number of inhibitors of neurite growth that may be physiologically relevant. Schwab and Caroni (1986) have reported that a protein constituent of CNS myelin and cultured oligodendrocytes is a potent inhibitor of neurite growth. It is known that CNS tracts are a much poorer environment for axonal regeneration than PNS nerves (Aguayo et al., 1981); a growth-inhibitory component of CNS myelin could explain why. Inhibitory interactions may also occur between growing axons. When the growth cones of one class of neurons encounter the neurites of another class, the growth cones have been observed to "collapse" and retract (Kapfhammer et al., 1986; Kapfhammer and Raper, 1987). In the mollusc *Helisoma*, the neurotransmitter serotonin has been found to inhibit neurite elongation by particular cells by a direct effect on growth cones (Haydon et al., 1987), and this effect can be antagonized by acetylcholine (McKobb and Kater, 1986). The neurotransmitter dopamine has also been reported to inhibit growth cone motility and neurite extension by embryonic chick retinal neurons (Lankford et al., 1986). The fact that growth cones themselves can release transmitters (Hume et al., 1983; Young and Poo, 1983) raises the possibility that multiple cooperative and competitive interactions occur among growing axons in vivo.

Summary

In vitro studies have led to the identification of a number of molecules that influence the growth of neurites. Several of these are good candidates for molecules that direct the growth and guidance of axons in vivo. Moreover, consideration of the mechanism of action of these molecules suggests categories within which new neurite growth-influencing molecules may be sought. These include:

1. *Polypeptide "growth hormones,"* such as NGF, other neurotrophic factors, and the fibroblast growth factors. These molecules bind to high-affinity receptors, enabling them to act at very low concentrations. Studies on NGF suggest that they may have wide-ranging effects, including a direct effect on intracellular events within the growth cone.

2. *Substratum-bound molecules*, including molecules of the ECM, such as laminin and fibronectin, as well as molecules expressed on cell surfaces. Studies with laminin and fibronectin suggest that these molecules can exert their effects through relatively low-affinity interactions. The immobility of these molecules in the environment suggests they may act by immobilizing (and stabilizing) components of the growth cone, not necessarily by increasing growth cone–substratum adhesion, but more likely by influencing intracellular events within the growth cone, possibly involving the cytoskeleton.
3. *Molecules that affect proteolysis in the axonal environment.* Studies with GdNPF suggest that these molecules may act not by modulating proteolysis indiscriminately, but rather by influencing one or more specific neuron-associated proteases, the functions of which are unknown. One possibility is that these proteases degrade neurite outgrowth-promoting substances present in the environment or produced by neurons themselves. Other binding activities of protease inhibitors (e.g., heparin-binding) may be useful in precisely localizing these molecules in vivo.

No doubt further investigations of neurite outgrowth will add categories to the list. What is significant is the framework that is beginning to emerge, within which the *kinds* of molecular events that control axon growth and guidance may be understood. Which events are most critical? Which ones are the targets of genetic lesions and developmental abnormalities? Which ones could be manipulated to facilitate regeneration? We are still a long way from knowing the answers to these questions. One thing, however, seems clear: Axon growth and guidance is a robust cellular behavior sensitive to influences at the level of the genome, the cytoplasm, the cytoskeleton, the cell surface, and the pericellular environment. Given what we have learned in the last several years, it would not be surprising to find that every one of these influences comes into play in the control of normal nervous system development.

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References

Acheson A., Edgar D., Timpl R., and Thoenen H. (1986) Laminin increases both levels and activity of tyrosine hydroxylase in calf adrenal chromaffin cells. *J. Cell Biol.* **102**, 151–159.

Acheson A., Vogl W., Huttner W. B., and Thoenen H. (1986) Methyltransferase inhibitors block NGF-regulated survival and protein phosphorylation in sympathetic neurons. *EMBO J.* 5, 2799–2803.

Adler R., Jerdan J., and Hewitt A. T. (1985) Responses of cultured neural retinal cells to substratum-bound laminin and other extracellular matrix molecules. *Devel. Biol.* **112**, 100–114.

Adler R., Manthorpe M., Skaper S., and Varon S. (1981) Polyornitihine-attached neurite-promoting factors (PNPFs). Culture sources and responsive neurons. *Brain Res.* **206**, 129–144.

Adler R. and Varon S. (1981) Neuritic guidance by polyornithine-attached materials of ganglionic origin. *Devel. Biol.* **81**, 1–11.

Aguayo A. J., David S., and Bray G. M. (1981) Influences of the glial environment on the elongation of axons after injury: transplantation studies in adult rodents. *J. Exp. Biol.* **95**, 231–240.

Akers R. M., Mosher D.F., and Lilien J. E. (1981) Promotion of retinal neurite outgrowth by substratum-bound fibronectin. *Devel. Biol.* **86**, 179–188.

Alitalo K., Kurkinen M., Virtanen I., Mellstrom K., and Vaheri A. (1982) Deposition of basement

membrane proteins in attachment and neurite formation of cultured murine C-1300 neuroblastoma cells. *J. Cell Biochem.* 18, 25–35.

Bächinger H. P., Fessler L. E., and Fessler J. H. (1982) Mouse procollagen IV. Characterization and supramolecular association. *J. Biol. Chem.* **257**, 9796–9803.

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

Barlow D. P., Green N. M., Kurkinen M., and Hogan B. L. M. (1984) Sequencing of laminin B chain DNAs reveals C-terminal regions of coiled-coil alpha-helix. *EMBO J.* **3**, 2355–2362.

Baron-van Evercooren A., Kleinman H. D., Ohno S., Marangos P., Schwartz J. P., and Dubois-Dalcq M. E. (1982) Nerve growth factor, laminin and fibronectin promote nerve growth in human fetal sensory ganglia cultures. *J. Neurosci. Res.* **8**, 179–183.

Bentley D., and Caudy M. (1983) Pioneer axons lose directed growth after selective killing of guidepost cells. *Nature* **304**, 62–65.

Bentley D., and Toroian-Raymond A. (1986) Disoriented pathfinding by pioneer neurone growth cones deprived of filopodia by cytochalasin treatment. *Nature* **323**, 712–715.

Bignami A., Chi N. H., and Dahl D. (1984) Laminin in rat sciatic nerve undergoing Wallerian degeneration. Immunofluorescence study with laminin and neurofilament antisera. *J. Neuropathol. Expl. Neurol.* **43**, 94–103.

Bixby J. L., Pratt R. S., Lilien J., and Reichardt L. F. (1987) Neurite outgrowth on muscle cell surfaces involves extracellular matrix receptors as well as Ca^{2+}-dependent and independent cell adhesion molecules. *Proc.Natl.Acad.Sci.USA* **84**, 2555–2559.

Black M. M., Aletta J. M., and Greene L. A. (1986) Regulation of microtubule composition and stability during nerve growth factor-promoted neurite outgrowth *J. Cell Biol.* **103**, 545–557.

Blair S. S., Murray M. A., and Palka J. (1985) Axon guidance in cultured epithelia fragments of *Drosophila* wing. *Nature* **315**, 406–409.

Blenis J., and Erikson R. L. (1986) Regulation of protein kinase activities in PC12 pheochromocytoma cells. *EMBO J.* **5**, 3441–3447.

Bovolenta P., and Mason C. (1987) Growth cone morphology varies with position in the developing mouse visual pathway from retina to first targets. *J. Neurosci.* 7, 1447–1460.

Bozyczko D., and Horwitz A. F. (1986) The participation of a putative cell surface receptor for laminin and fibronectin in peripheral neurite extension *J. Neurosci.* 6, 1241–1251.

Bray D. (1982) Filopodial contraction and growth cone guidance, *Cell Behavior*, Bellairs R., Curtis A., and Dunn G., eds., Cambridge Univ. Press, Cambridge England, pp. 299–317.

Bray D., and Chapman K. (1985) Analysis of microspike movements on the neuronal growth cone. *J. Neurosci.* **5**, 3204–3213.

Bray D., Bunge M. B., and Chapman K. (1987) Geometry of isolated sensory neurons in culture. Effects of embryonic age and culture substratum. *Exp. Cell Res.* **168**, 127–137.

Bueker E. D. (1948) Implantation of tumors in the hind limb field of the embryonic chick and the developmental response of the lumbo-sacral nervous system. *Anat. Rec.* **102**, 369–390.

Calof A. L. and Reichardt L. F. (1984) Motoneurons purified by cell sorting respond to two distinct activities in myotube conditioned medium. *Devel. Biol.* **106**, 194–210.

Calof A. L., and Reichardt L. F. (1985) Response of purified chick motoneurons to myotube conditioned medium: laminin is essential for the substratum-binding neurite outgrowth-promoting activity. *Neurosci. Lett.* **59**, 183–189.

Campenot R. B. (1977) Local control of neurite development by nerve growth factor. *Proc. Natl. Acad. Sci. USA* **74**, 4516–4519.

Campenot R. B. (1986) Retraction and degeneration of sympathetic neurites in response to locally elevated potassium. *Brain Res.* **399**, 357–363.

Carbonetto S., Gruver M. M., and Turner D. C. (1983) Nerve fiber growth in culture on fibronectin, collagen, and glycosaminoglycan substrates. *J. Neurosci.* **3**, 2324–2335.

Carlin B., Jaffe R., Bender B., and Chung A. E. (1981) Entactin, a novel basal lamina-associated glycoprotein. *J. Biol. Chem.* **256**, 5209–5214.

Carrell R., and Travis J. (1985) *alpha*$_1$-antitrypsin and the serpins: variation and countervariation. *Trends in Biochem. Sci.* **10**, 20–24.

Chang S., Rathjen F. G., and Raper J. A. (1987) Extension of neurites on axons is impaired by antibodies against specific neural cell surface glycoproteins. *J. Cell Biol.* **104**, 355–362.

Charonis A. S., Tsilibary E. C., Saku T., and Furthmayr H. (1986) Inhibition of laminin self-assembly and interaction with type IV collagen by antibodies to the terminal domain of the long arm. *J. Cell Biol.* **103**, 1689–1697.

Chiu A. E., Matthew W. D., and Patterson P. H. (1986) A monoclonal antibody that blocks the activity of a neurite regeneration-promoting factor: studies on the binding site and its localization in vivo. *J. Cell Biol.* **103**, 1383–1398.

Chun J. J. M., Nakamura M. J., and Shatz C. J. (1986) Transient fibronectin-like immunoreactivity in the subplate during development of the cat's telencephelon. *Soc. Neurosci. Abstr.* **12**, 502.

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

Chung A. E., Jaffe R., Freeman I. L., Vergnes J.-P., Braginski J. E., and Carlin B. (1979) Properties of a basement membrane-related glycoprotein synthesized in culture by a mouse embryonal carcinoma-derived cell line. *Cell* **16**, 277–287.

Cohan C. S., Connor J. A., and Kater S. B. (1986) Signals which inhibit growth cone motility also increase intracellular calcium levels within the growth cone. *Soc. Neurosci. Abstr.* **12**, 370.

Cohen J., Burne J., McKinlay C. and Winter J. (1987) The role of laminin and the laminin/fibronectin receptor complex in the outgrowth of retinal ganglion cell axons. *Dev. Biol.* **122**, 407–418

Cohen J., Burne J. F., Winter J., and Bartlett P. (1986) Retinal ganglion cells lose response to laminin with maturation. *Nature* **322**, 465–467.

Collins F. (1978) Induction of neurite outgrowth by a conditioned-medium factor bound to the culture substratum. *Proc. Natl. Acad. Sci. USA* **75**, 5210–5213.

Collins F. (1980) Neurite outgrowth induced by the substrate associated material from nonneuronal cells. *Devel. Biol.* **79**, 247–252.

Collins F., and Garret J. E., Jr. (1980) Elongating nerve fibers are guided by a pathway of material released from embryonic nonneuronal cells. *Proc. Natl. Acad. Sci. USA* **77**, 6226–6228.

Collins F., and Lee M. R. (1984) The spatial control of ganglionic neurite growth by the substrate-associated material from conditioned medium: an experimental model of haptotaxis. *J. Neurosci.* **4**, 2823–2829.

Connolly J. L., Seeley P. J., and Greene L. A. (1985) Regulation of growth cone morphology by nerve growth factor: a comparative study by scanning electron microscopy. *J. Neurosci. Res.* **13**, 183–198.

Constantine-Paton M. (1979) Axonal navigation. *Bio-Science* **29**, 526–532.

Constantine-Paton M., Blum A. S., Mendez-Otero R., and Barnstable C. J. (1986) A cell surface molecule distributed ina dorsoventral gradient in the perinatal rat retina. *Nature* **324**, 459–462.

Cornbrooks C. J., Carey D. J., McDonald J. A., Timpl R., and Bunge R. P. (1983) In vivo and in vitro observations on laminin production by Schwann cells. *Proc. Natl. Acad. Sci. USA* **80**, 3850–3854.

Coughlin M. D., Bloom E. M., and Black I. B. (1981) Characterization of a neuronal growth factor from mouse heart cell-conditioned medium. *Dev. Biol.* **82**, 56–68.

Coughlin M. D., Grover A. K., and Jung C. Y. (1986) Determination of the molecular weight of neronectin, a conditioned medium-derived, substrate binding, neurite-extension factor: comparison with laminin using radiation inactivation analysis. *J. Neurosci.* **6**, 1553–1559.

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

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

Davis G. E., Manthorpe M., Engvall E., and Varon S. (1985a) Isolation and characterization of rat Schwannoma neurite-promoting factor: evidence that the factor contains laminin. *J. Neurosci.* **5**, 2662–2671.

Davis G. E., Manthorpe M., and Varon S. (1985b) Parameters of neuritic growth from ciliary ganglion neurons in vitro: Influence of laminin, Sch-

wannoma polyornithinebinding neurite promoting factor and ciliary neurono-trophic factor. *Dev. Brain Res.* **17**, 75–84.

Del Rosso M., Cappelletti R., Viti M., Vannucchi S., and Chiarugi V. (1981) Binding of the basement-membrane glycoprotein laminin to glycosaminoglycans. *Biochem J.* **199**, 699–704.

Drubin D. G., Feinstein S. C., Shooter E. M., and Kirschner M. W. (1985) Nerve growth factor-induced neurite outgrowth in PC12 cells involves the coordinate induction of microtubule assembly and assembly-promoting factors. *J. Cell Biol.* **101**, 1799–1807.

Easter S. S., Bratton B., and Scherer S. S. (1984) Growth-related order of the retinal fiber layer in goldfish. *J. Neurosci.* **4**, 2173–2190.

Edelman G. M. (1985) Cell adhesion and the molecular processes of morphogenesis. *Ann. Rev. Biochem.* **54**, 135–169.

Edgar D., and Thoenen H. (1982) Modulation of NGF-induced survival of chick sympathetic neurons by contact with a conditioned medium factor bound to the culture substrate. *Dev. Brain Res.* **5**, 89-92.

Edgar D., Timpl R., and Thoenen H. (1984) The heparin-binding domain of laminin is responsible for its effects on neurite outgrowth and neuronal survival. *EMBO J.* **3**, 1463–1468.

Engel J., Odermatt E., Engel A., Madri J. A., Furthmayr H., Rohde H., and Timpl R. (1981) Shapes, domain organizations and flexibilities of laminin and fibronectin, two multifunctional proteins of the extracellular matrix. *J. Mol. Biol.* **150**, 97–120.

Engvall E., Davis G. E., Dickerson K., Ruoslahti E., Varon S., and Manthorpe M. (1986) Mapping of domains in human laminin using monoclonal antibodies: localization of the neurite-promoting site. *J. Cell Biol.* **103**, 2457–2465.

Fallon J. F. (1985a) Preferential outgrowth of central nervous system neurites on astrocytes and Schwann cells as compared with nonglial cells in vitro. *J. Cell Biol.* **100**, 198–207.

Fallon J. F. (1985b) Neurite guidance by nonneuronal cells in culture: preferential outgrowth of peripheral neurites on glial as compared to nonglial cell surfaces. *J. Neurosci.* **5**, 3169–3177.

Farrell D. H., and Cunningham D. D. (1986) Human fibroblasts accelerate the inhibition of thrombin by protease nexin. *Proc. Natl. Acad. Sci. USA* **83**, 6858–6862.

Fassner A., Kruse J., Goridis C., Bock E., and Schachner M. (1984) The neural cell adhesion molecule L1 is distinct from the *N*-CAM related group of surface antigens BSP-2 and D2. *EMBO J.* **3**, 733–737.

Ferrari G., Fabris M., and Gorio A. (1983) Gangliosides enhance neurite outgrowth in PC12 cells. *Dev. Brain Res.* **8**, 215–221.

Freeman J. A., Manis P. B., Snipes G. J., Mayes B. N., Samson P. C., Wikswo J. P. Jr., and Freeman D. B. (1985) Steady growth cone currents revealed by a novel circularly vibrating probe: a possible mechanism underlying neurite growth. *J. Neurosci. Res.* **13**, 257–283.

Frost D., and Westerfield M. (1986) Axon outgrowth of embryonic zebrafish neurons is promoted by laminin and inhibited by fibronectin. *Soc. Neurosci. Abstr.* **12**, 1114.

Furcht L. T. (1983) Structure and function of the adhesive glycoprotein fibronectin. *Modern Cell Biol.* **1**, 53–117.

Gloor S., Odink K., Guenther J., Nick H., and Monard D. (1986) A glia-derived neurite promoting factor with protease inhibitory activity belongs to the protease nexins. *Cell* **47**, 687–693.

Gorin P. D., and Johnson E. M. 1979) Experimental autoimmune model of nerve growth factor deprivation: effects on developing peripheral sympathetic and sensory neurons. *Proc. Natl. Acad. Sci. USA* **76**, 5382–5386.

Greenberg M. E., Greene L. A., and Ziff E. B. (1985) Nerve growth factor and epidermal growth factor induce rapid transient changes in proto-oncogene transcription in PC12 cells. *J. Biol. Chem.* **260**, 14101–14110.

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

Greene L. A. (1984) The importance of both early and delayed responses in the biological actions of nerve growth factor. *Trends in Neurosci.* **7**, 91–94.

Greene L. A., and Tischler A. S. (1982) PC12 pheochromocytoma cells in neurobiological research, *Advances in Cellular Neurobiology*, vol. 3, Federoff S. and Hertz L., eds., Academic Press, New York, pp. 373–414.

Greene L. A., Aletta J. M., Burstein D. E., Drexler S. A., Greenberg M. E., Leonard D., and Ziff E. B. (1985) Gene regulation by nerve growth factor,

Colloquium—Mosbach 1985: Neurobiochemistry (Colloquium der Gesellschaft fuer Biologische Chemie), Hamprecht B. and Neuhoff V., eds., Springer -Verlag, Berlin, pp. 1–6.

Greene L. A., Drexler S. A., Connolly J. L., Rukenstein A., and Green S. H. (1986) Selective inhibition of responses to nerve growth factor and of microtubule-associated protein phosphorylation by activators of adenylate cyclase. *J. Cell Biol.* **103**, 1967–1978.

Greve J. M., and Gottlieb D. I. (1982) Monoclonal antibodies which alter the morphology of cultured chick myogenic cells. *J. Cell. Biochem.* **18**, 221–229.

Griffin C. G., and Letourneau P. C. (1980) Rapid retraction of neurites by sensory neurons in response to increased concentrations of nerve growth factor. *J. Cell Biol.* **86**, 156–161.

Grumet M., Hoffman S., Chuong C., and Edelman G. M. (1984) Polypeptide components and binding functions of neuron-glia cell adhesion molecules. *Proc. Natl. Acad. Sci. USA* **81**, 7989–7993.

Guenther J., Nick H., and Monard D. (1985) A glia-derived neurite-promoting factor with protease inhibitor activity. *EMBO J.* **4**, 1963–1966.

Gundersen R. W. (1987) Response of sensory neurites and growth cones to patterned substrata of lamin-in and fibronectin in vitro. *Dev. Biol.* **121**, 423–431.

Gundersen R. W. (1985) Sensory neurite growth cone guidance by substrate adsorbed nerve growth factor. *J. Neurosci. Res.* **13**, 199–212.

Gundersen R. W., and Barrett J. N. (1979) Neuronal chemotaxis: chick dorsal-root axons turn towards high concentrations of nerve growth factor. *Science* **206**, 1079–1080.

Gundersen R. W., and Barrett J. N. (1980) Characterization of the turning response of dorsal root neurites toward nerve growth factor. *J. Cell Biol.* **87**, 546–554.

Gundersen R. W., and Barrett J. N. (1984) Neurite growth cone-substratum adherence increases in vitro. *Dev. Brain Res.* **12**, 21–26.

Hall D. E., Neugebauer K. M., and Reichardt L. F. (1987) Embryonic neural retinal cell response to extracellular matrix proteins: developmental changes and effects of the cell substratum attachment antibody (CSAT). *J. Cell Biol.* **104**, 623–634.

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.

Hammarback J. A., and Letourneau P. C. (1986) Neurite extension across regions of low cell-substratum adhesivity: implication for the guidepost hypothesis of axonal pathfinding. *Dev. Biol.* **117**, 655–662.

Harris W. A. (1986) Homing behavior of axons in the embryonic vertebrate brain. *Nature* **320**, 266–269.

Hassell J. R., Kimura J. H., and Hascall V. C. (1986) Proteoglycan core protein families. *Ann. Rev. Biochem.* **55**, 539–567.

Hatta K., Okada T. S., and Takeichi M. (1985) A monoclonal antibody disrupting calcium-dependent cell –cell adhesion of brain tissues: possible role of its target antigen in animal pattern formation. *Proc. Natl. Acad. Sci. USA* **82**, 2789–2793.

Hawkins R. L., and Seeds N. W. (1986a) Effect of proteases and their inhibitors on neurite outgrowth from neonatal mouse sensory ganglia in culture. *Brain Res.* **398**, 63–70.

Hawkins R. L., and Seeds N. W. (1986b) Protease inhibition can influence the direction of neurite outgrowth by neonatal mouse sensory ganglia. *Soc. Neurosci. Abstr.* **12**, 1117.

Haydon P. G., McCobb D. P., and Kater S. B. (1987) The regulation of neurite outgrowth, growth cone motility, and electrical synaptogenesis by serotonin. *J. Neurobiol.* **18**, 197–215.

Helfand S. L., Riopelle R. J., and Wessells N. K. (1978) Non-equivalence of conditioned medium and nerve growth factor for sympathetic, parasympathetic and sensory neurons. *Exp. Cell Res.* **113**, 39–45.

Hendry I. A. (1975) The response of adrenergic neurons to axotomy and nerve growth factor. *Brain Res.* **94**, 87–97.

Horwitz A., Duggan K., Buck C., Beckerle M. C., and Burridge K. (1986) Interaction of plasma membrane fibronectin receptor with talin—a transmembrane linkage. Nature 320, 531–533.

Horwitz A., Duggan K., Greggs R., Decker C., and Buck C. (1985) The cell substratum attachment (CSAT) antigen has properties of a receptor for laminin and fibronectin. *J. Cell Biol.* **101**, 2134–2144.

Horwitz A. F., Knudsen K. A., Damsky C. H., Decker C., Buck C., and Neff N. T. (1984) Adhesion-related integral membrane glycoproteins identified by monoclonal antibodies, *Monoclonal Antibodies and Functional Cell Lines*, Kennet R., Beditor K., and McKearn T., eds., Plenum, New York, pp. 103–118.

Hsu L., and Chen K. Y. (1986) Induction of neurite outgrowth from ganglia and explants by activators of protein kinase C. *Soc. Neurosci. Abstr.* **12**, 1114.

Hume R. I., Role L. W., and Fischbach G. D. (1983) Acetylcholine release from growth cones detected with patches of acetylcholine receptor-rich membranes. *Nature* **305**, 632–634.

Humphries M. J., Akiyama S. K., Komoriya A., Olden K., and Yamada K. M. (1986) Identification of an alternatively spliced site in human plasma fibronectin that mediates cell-type specific adhesion. *J. Cell Biol.* **103**, 2637–2647.

Hynes R. (1985) Molecular biology of fibronectin. *Ann. Rev. Cell Biol.* **1**, 67–90.

Hynes R. O. (1987) Integrins: a family of cell surface receptors. *Cell* **48**, 549–554.

James R., and Bradshaw R. A. (1984) Polypeptide growth factors. *Ann. Rev. Biochem.* **53**, 259–292.

Johnson J. E., Barder Y.-A., Schwab M., and Thoenen H. (1986) Brain-derived neurotrophic factor supports the survival of cultured rat retinal ganglion cells. *J. Neurosci.* **6**, 3031–3038.

Johnston R. N., and Wessells N. K. (1980) Regulation of the elongating nerve fiber. *Curr. Topics Dev. Biol.* **16**, 165–206.

Jones G. E., Arumugham R. G., and Tanzer M. L. (1986) Fibronectin glycosylation modulates fibroblast adhesion and spreading. *J. Cell Biol.* **103**, 1663–1670.

Kapfhammer J. P., Grunewald B. E., and Raper J. A. (1986) The selective inhibition of growth cone extension by specific neurites in culture. *J. Neurosci.* **6**, 2527–2534.

Kapfhammer J. P., and Raper J. A. (1987) Collapse of growth cone structure on contact with specific neurites in culture. *J. Neurosci.* **7**, 201–212.

Katz M. J., Lasek R. J., and Nauta H. J. W. (1980) Ontogeny of substrate pathways and the origin of the neural circuit pattern. *Neurosci.* **5**, 821–833.

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.

Kleinman H. K., Cannon F. B., Laurie G. W., Hassell J. R., Aumailley M., Terranova V. P., Martin G. R., and Dubois-Dalcq M. (1985) Biological activities of laminin. *J. Cell Biochem.* **27**, 317–325.

Kleinman H. K., McGarvey M. L., Hassell J. R., Martin G. R., Baron van Evercooren A., and Dubois-Dalcq M. (1984) The role of laminin in basement membranes and in the growth, adhesion, and differentiation of cells, *The Role of Extracellular Matrix in Development*, Trelstad R., ed., Alan R. Liss Inc., New York, pp. 123–143.

Kligman D., and Marshak D. R. (1985) Purification and characterization of neurite extension factor from bovine brain. *Proc. Natl. Acad. Sci. USA* **82**, 7136–7139.

Knauer D. J., Thompson J. A., and Cunningham D. D. (1983) Protease nexins: cell-secreted proteins that mediate the binding, internalization, and degradation of regulatory serine protease. *J. Cell. Physiol.* **117**, 385–396.

Kornblihtt A. R., Umezawa K., Vibe-Pedersen K., and Baralle F. E. (1985) Primary structure of human fibronectin: differential splicing may generate at least 10 polypeptides from a single gene. *EMBO J.* **4**, 1755–1759.

Korsching S. (1986) The role of nerve growth factor in the CNS. *Trends Neurosci.* **9**, 570–573.

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

Krotoski D. M., Doming C., and Bronner-Fraser M. (1986) Distribution of a putative cell surface receptor for fibronectin and laminin in the avian embryo. *J. Cell Biol.* **103**, 1061–1071.

Krystosek A., and Seeds N. W. (1981a) Plasminogen activator secretion by granule neurons in cultures of developing cerebellum. *Proc. Natl. Acad. Sci. USA* **78**, 7810–7814.

Krystosek A., and Seeds N. W. (1981b) Plasminogen activator release at the neuronal growth cone. *Science* **213**, 1532–1534.

Kuwada J. Y. (1986) Cell recognition by neuronal growth cones in a simple vertebrate embryo. *Science* **233**, 740–746.

Lance-Jones C., and Landmesser L. (1981) Pathway selection by embryonic chick motoneurons in an experimentally altered environment. *Proc. R. Soc. Lond.* (Biol.) **214**, 19–52.

Lander A. D., Fujii D. K., and Reichardt L. F. (1985a) Laminin is associated with the "neurite outgrowth promoting factors" found in conditioned medium. *Proc. Natl. Acad. Sci. USA* **82**, 2183–2187.

Lander A. D., Fujii D. K., and Reichardt L. F. (1985b) Purification of a factor that promotes neurite outgrowth: isolation of laminin and associated molecules. *J. Cell Biol.* **101**, 893–913.

Lander A. D., Fujii D. K., Gospodarowicz D., and Reichardt L. F. (1982) Characterization of a factor that promotes neurite outgrowth: evidence linking activity to a heparan sulfate proteoglycan. *J. Cell Biol.* **94**, 574–585.

Lander A. D., Tomaselli K., Calof A. L., and Reichardt L. F. (1983) Studies on extracellular matrix components that promote neurite outgrowth. *Cold Spring Harbor Symp. Quant. Biol.* **48**, 611–623.

Landis S. C. (1983) Neuronal growth cones. *Ann. Rev. Physiol.* **45**, 567–580.

Lankford K. L., DeMello F. G., and Klein W. L. (1986) Transient D1 receptor mediates dopamine inhibition of growth cone motility and neurite outgrowth in a subset of vertebrate CNS neurons. *Soc. Neurosci. Abstr.* **12**, 1116.

Leptin M. (1986) The fibronectin receptor family. *Nature* **321**, 728.

Lesakawa K. C., and Hogan E. L. (1985) Quantitation of the in vitro neuroblastoma response to exogenous, purified gangliosides. *J. Neurosci. Res.* **13**, 539–550.

Lesot H., Kuhl U., and von der Mark K. (1983) Isolation of a laminin-binding protein from muscle cell membranes. *EMBO J.* **2**, 861–865.

Letourneau P. C. (1975) Cell-to-substratum adhesion and guidance of axonal elongation. *Dev. Biol.* **44**, 92–101.

Letourneau P. C. (1985) Axonal growth and guidance, *Molecular Bases of Neural Development*, Edelman G. M., Gall W. E., and Cowan W. M., eds., Wiley, New York, pp. 269–294.

Levi-Montalcini R. (1952) Effects of mouse tumor transplantation on the nervous system. *Ann. NY Acad. Sci.* **55**, 330–343.

Levi-Montalcini R. (1976) The nerve growth factor: its role in growth, differentiation and function of the sympathetic adrenergic neuron. *Prog. Brain Res.* **45**, 235–258.

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

Levi-Montalcini R., Meyer H., and Hamburger V. (1954) In vitro experiments on the effects of mouse sarcomas 180 and 37 on the spinal and sympathetic ganglia of the chick embryo. *Cancer Res.* **14**, 49–57.

Liesi P. (1985a) Do neurons in the vertebrate CNS migrate on laminin. *EMBO J.* **4**, 1163–1170.

Liesi P. (1985b) Laminin-immunoreactive glia distinguish regenerative adult CNS systems from nonregenerative ones. *EMBO J.* **4**, 2505–2511.

Lindner J., Guenther J., Nick H., Zinser G., Antonicek H., Schachner M., and Monard D. (1986) Modulation of granule cell migration by a glia-derived protein *Proc. Natl. Acad. Sci. USA* **83**, 45468–4571.

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 neutotrophic factor. *Dev. Biol.* **112**, 319–328.

Lobb R. R., and Fett J. W. (1984) Purification of two distinct growth factors from bovine neural tissue by heparin affinity chromatography. *Biochem.* **23**, 6295–6299.

Lumsden A. G. S., and Davies A. M. (1986) Chemotropic effect of specific target epithelium in the developing mammalian nervous system. *Nature* **323**, 538–539.

Madsen A., Palm S., Furcht L., and Letourneau P. (1986) Distribution of laminin and collagen type IV during early axonal growth in the mouse CNS. *Soc. Neurosci. Abstr.* **12**, 1210.

Malinoff H. L., and Wicha M. S. (1983) Isolation of a cell surface receptor for laminin from murine fibrosarcoma cells. *J. Cell Biol.* **96**, 1475–1479.

Manthorpe M., Engvall E., Ruoslahti E., Longo F. M., Davis G. E., and Varon S. (1983) Laminin pro-

motes neuritic regeneration from cultured peripheral and central neurons. *J. Cell Biol.* **97**, 1882–1890.

Marsh L., and Letouneau P. C. (1984) Growth of neurites without filopodial or lamellipodial activity in the presence of cytochalasin B. *J. Cell Biol.* **99**, 2041–2047.

McCobb D. P., and Kater S. B. (1986) Serotonin inhibition of growth cone motility is blocked by acetylcholine. *Soc. Neurosci. Abstr.* **12**, 1117.

McLoon S. C., McLoon L. K., Palm S. L., and Furcht L. T. (1986) Laminin is transiently expressed in the developing rat optic nerve during the time retinal axons are growing. *Soc. Neurosci. Abstr.* **12**, 1211.

Monard D. (1985a) Implications of proteases and protease inhibitors in neurite outgrowth, *Colloquium —Mosbach 1985: Neurobiochemistry (Colloquium der Gesellschaft f. Biologische Chemie)*, Hamprecht B., and Neuhoff V., eds., Springer-Verlag, Berlin, pp. 7–12.

Monard D. (1985b) Neuronal cell behavior: modulation by protease inhibitors derived from non-neuronal cells. *Cell Biol. Int. Repts.* **9**, 297–305.

Monard D., Niday E., Limat A., and Solomon F. (1983) Inhibition of protease activity can lead to neurtie extension in neuroblastoma cells. *Prog. Brain Res.* **58**, 359–363.

Monard D., Solomon F., Rentsch M., and Gysin R. (1973) Glia-induced morphological differentiation in neuroblastoma cells. *Proc. Natl. Acad. Sci. USA* **70**, 1894–1897.

Morrison R. S. (1987) Fibroblast growth factors: potential neurotrophic agents in the central nervous system. *J. Neurosci. Res.* **17**, 99–101.

Morrison R. S., Sharma A., de Vellis J., and Bradshaw R. (1986) Basic fibroblast growth factor supports the survival of cerebral cortical neurons in primary culture. *Proc. Natl. Acad. Sci. USA* **83**, 7537–7541.

Nakajima J., Tsuji S., and Nagai Y. (1986) Bioactive gangliosides: analysis of functional structures of the tetrasialoganglioside G_{Q1b} which promotes neurite outgrowth. *Biochim. Biophys. Acta* **876**, 65–71.

Oppenheim R. W. (1981) Neuronal cell death and some related regressive phenomena during neurogenesis: a selective historical review and progress report, *Studies in Developmental Neurobiology*, Cowan W. M., ed., Oxford Univ. Press, New York, pp. 74–133.

Ossowski L., Quigley J. P., and Reich E. (1974) Fibrinolysis associated with oncogenic transformation. *J. Biol. Chem.* **249**, 4312–4320.

Ott U., Odermattt E., Engel J., Furthmayr H., and Timpl R. (1982) Protease resistance and conformation of laminin. *Eur. J. Biochem.* **123**, 63–72.

Palm S. L., and Furcht L. T. (1983) Production of laminin and fibronectin by Schwannoma cells: cell-protein interactions in vitro and protein localization in peripheral nerve in vivo. *J. Cell Biol.* **96**, 1218–1226.

Patel N. B., Xie Z.-p., Young S. H., and Poo M.-m. (1985) Response of nerve growth cone to focal electric currents. *J. Neurosci. Res.* **13**, 245–256.

Pearlman A. L., Kim H. G., and Schmitt G. (1986) Early cortical afferents arrive after fibronectin-like immunoreactivity appears in their migratory pathway. *Soc. Neurosci. Abstr.* **12**, 502.

Pittman R. N. (1985) Release of plaminogen activator and a calcium-dependent metalloprotease from cultures sympathetic and sensory neurons. *Dev. Biol.* **110**, 91–101.

Rao C. N., Barsky S. H., Terranova V. P., and Liotta L. A. (1983) Isolation of a tumor cell laminin receptor. *Biochem. Biophys. Res. Comm.* **111**, 808.

Raper J. A., Bastiani M. J., and Goodman C. S. (1984) Pathfinding by neuronal growth cones in grasshopper embryos IV. The effects of ablating the A and P axons upon the behavior of the G growth cone. *J. Neurosci.* **4**, 2239–2345.

Riggott M. J., and Moody S. A. (1987) Distribution of laminin and fibronectin along peripheral trigeminal axon pathways in the developing chick. *J. Comp. Neurol.* **258**, 580–596.

Roberts A., and Patton D. T. (1985) Growth cones and the formation of central and peripheral neurites by sensory neurones in amphibian embryos. *J. Neurosci. Res.* **13**, 23–38.

Roberts D. D., Liotta L. A., and Ginsburg V. (1986) Gangliosides indirectly inhibit the binding of laminin to sulfatides. *Arch. Biochem. Biophys.* **250**, 498–504.

Roberts D. D., Rao C. N., Magnani J. L., Spitalnik S. L., Liotta L. A., and Ginsburg V. (1985) Laminin binds specifically to sulfated glycolipids. *Proc. Natl. Acad. Sci. USA* **82**, 1306–1310.

Rogers S. L., Edson K. J., Letourneau P. C., and McLoon S. C. (1986) Distribution of laminin in the developing peripheral nervous system of the chick. *Devel. Biol.* **113**, 429–435.

Rogers S. L., Letourneau P. C., Palm S. L., McCarthy J., and Furcht L. T. (1983) Neurite extension by peripheral and central nervous system neurons in response to substratum-bound fibronectin and laminin. *Dev. Biol.* **98**, 212–220.

Rogers S. L., Letourneau P. C., Peterson B. A., Furcht L. T., and McCarthy J. B. (1987) Selective interactions of peripheral and central nervous system cells with two distinct cell binding domains of fibronectin. *J. Cell Biol.*, in press.

Rogers S. L., McCarthy J. B., Palm S. L., Furcht L. T., and Letourneau P. C. (1985) Neuron-specific interactions with two neurite-promoting fragments of fibronectin. *J. Neurosci.* **5**, 369–378.

Roisen F. J., Bartfeld H., Nagele R., and Yorke G. (1981) Ganglioside stimulatiion of axonal sprouting in vitro. *Science* **214**, 577–578.

Ruoslahti E., and Pierschbacher M. D. (1986) Arg-gly-asp: a versatile cell recognition signal. *Cell* **44**, 517–518.

Rutishauser U. (1985) Influences of the neural cell adhesion molecule on axon growth and guidance. *J. Neurosci. Res.* **13**, 123–131.

Rydel R. E., and Greene L. A. (1986) Promotion of survival and neurite outgrowth in cultures of newborn rat sympathetic neurons by a cAMP analog. *Soc. Neurosci. Abstr.* **12**, 1103.

Rydel R. E., and Greene L. A. (1987) Acidic and basic fibroblast growth factors promote stable neurite outgrowth and neuronal differentiation in cultures of PC12 cells. *J. Neurosci.*, In press.

Sandrock A. W., and Matthew W. D. (1985) A bioassay for the analysis of neurite-promoting factors in the extracellular matrix of rat peripheral nerve. *Soc. Neurosci. Abstr.* **11**, 592.

Sandrock A. W., and Matthew W. D. (1986) Identification of a peripheral nerve antigen that promotes axonal regeneration. *Soc. Neurosci. Abstr.* **12**, 1505.

Sandrock A. W., and Matthew W. D. (1987) The identification of a peripheral nerve neurite growth-promoting activity by the use of a novel in vitro bioassay. *Proc. Natl. Acad. Sci. USA* In press.

Sanes J. R., Schachner M., and Covault J. (1986) Expression of several adhesive macromolecules (*N*-CAM, L1, J1, NILE, uvomorulin, laminin, fibronectin, and a heparan sulfate proteoglycan) in embryonic, adult, and denervated adult skeletal muscle. *J. Cell Biol.* **102**, 420–431.

Sasaki M., Kato S., Kohno K., Martin G. R., and Yamada Y. (1987) Sequence of the cDNA encoding the laminin B1 chain reveals a multidomain protein containing cystein rich repeats. *Proc. Natl. Acad. Sci. USA* **84**, 935–939.

Schubert D., Ling N., and Baird A. (1987) Multiple influences of a heparin-binding growth factor on neuronal development. *J. Cell Biol.* **104**, 635–643.

Schuerch-Rathgeb Y., and Monard D. (1978) Brain development influences the appearance of glial factor-like activity in rat brain primary cultures. *Nature* **273**, 308–309.

Schwab M. E., and Caroni P. (1986) Rat CNS myelin and a subtype of oligodendrocytes in culture represent a nonpermissive substrate for neurite growth and fibroblast spreading. *Soc. Neurosci. Abstr.* **12**, 12.

Schwarzbauer J. E., Mulligan R. C., and Hynes R. O. (1987) Efficient and stable expression of recombinant fibronectin polypeptides. *Proc. Natl. Acad. Sci. USA* **84**, 754–758.

Scott R. W., Bergman B. L., Bajpai A., Hersh R. T., Rodriquez H., Jones B. N., Barreda C., Watts S., and Baker J. B. (1985) Protease nexin. Properties and a modified purification procedure. *J. Biol. Chem.* **260**, 7029–7034.

Seeley P. J., Rukenstein A., Connolly J. L., and Greene L. A. (1984) Differential inhibition of nerve growth factor and epidermal growth factor effects on the PC12 pheochromocytoma line. *J. Cell Biol.* **98**, 417–426.

Sekiguchi K., Siri A., Zardi L., and Hakomori S. (1985) Differences in domain structure between human fibronectins isolated from plasma and from culture supernatants of normal and transformed fibroblasts. Studies with domain specific antibodies. *J. Biol. Chem.* **260**, 5105–5114.

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

Skene J. H. P. (1984) Growth-associated proteins and the curious dichotomies of nerve regeneration. *Cell* **37**, 697–700.

Smallheiser N. R., Crain S. M., and Reid L. M. (1984) Laminin as a substrate form retinal axons in vitro. *Dev. Brain Res.* **12**, 136–140.

Solomon F. (1984) Determinants of neuronal form. *Trends in Neurosci.* **7**, 17–20.

Steele J. G., and Hoffman H. (1986) Neurite-promoting activity from fetal skeletal muscle: partial purification of a high molecular weight-form. *J. Neurosci. Res.* **15**, 323–339.

Stirling R. V., and Summerbell D. (1985) The behavior of growing axons invading developing chick wing buds with dorsoventral or anteroposterior axis reversed. *J. Embryol. Exp. Morph.* **85**, 251–269.

Taghert P. H., and Lichtman J. W. (1986) Axon outgrowth in vertebrates. *Nature* **320**, 210–211.

Terranova V. P., Rao C. N., Kalebic T., Margulies I. M., and Liotta L. A. (1983) Laminin receptor on human breast carcinoma cells. *Proc. Natl. Acad. Sci. USA* **80**, 444–448.

Thoenen H., and Barde Y.-A. (1980) Physiology of nerve growth factor. *Physiol. Rev.* **4**, 1284–1335.

Thomas K. A., and Gimenez-Gallego G. (1986) Fibroblast growth factors: broad spectrum mitogens with potent angiogenic activity. *Trends in Biochem. Sci.* **11**, 81–86.

Timpl R., Engel J., and Martin G. R. (1983) Laminin—a multifunctional protein of basement membranes. *Trends in Biochem. Sci.* **8**, 207–209.

Timpl R., Rohde H., Risteli L., Ott U., Robey P. G., and Martin G. R. (1982) Laminin. *Meth. Enzymol.* **82**, 831–838.

Timpl R., Rohde H., Robey P. G., Rennard S. I., Foidart J.-M., and Martin G. R. (1979) Laminin—a glycoprotein from basement membranes. *J. Biol. Chem.* **254**, 9933–9937.

Togari A., Dickens G., Kuzuya H., and Guroff G. (1985) The effect of fibroblast growth factor on PC12 cells. *J. Neurosci.* **5**, 307–316.

Tomaselli K. J., Damsky C. H., and Reichardt L. F. (1986) PC12 cells as a model for neuron extracellular matrix interactions. *Soc. Neurosci Abstr.* **12**, 1459.

Tomaselli K. J., Reichardt L. F., and Bixby J. L. (1986) Distinct molecular interactions mediate neuronal process outgrowth on nonneuronal cell surfaces and extracellular matrices. *J. Cell Biol.* **103**, 2659–2672.

Tosney K. W., and Landmesser L. T. (1984) Pattern and specificity of axonal outgrowth following varying degrees of chick limb bud ablation. *J. Neurosci.* **4**, 2518–2527.

Trinkaus J. P. (1985) Further thoughts on directional cell movement during morphogenesis. *J. Neurosci. Res.* **13**, 1–19.

Tsui H.-C.T., Lankford K. L., and Klein W. L. (1985) Differentiation of neuronal growth cones: Specialization of filopodial tips for adhesive interactions. *Proc. Natl. Acad. Sci. USA* **82**, 8256–8260.

Wagner J. A., and D'Amore P. A. (1986) Neurite outgrowth induced by an endothelial cell mitogen isolated from retina. *J. Cell Biol.* **103**, 1363–1367.

Walicke P., and Cowan W. M. (1986) Basic and acidic fibroblast growth factors increase survival of, and neurite outgrowth from, hippocampal neurons in vitro. *Soc. Neurosci. Abst.* **12**, 1101.

Walicke P., Cowan W. M., Ueno N, Baird A., and Guillemin R. (1986) Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension. *Proc. Natl. Acad. Sci. USA* **83**, 3012–3016.

Wan Y. J., Wu T. C., Chung A. E., and Damjanov I. (1984) Monoclonal antibodies to laminin reveal the heterogeneity of basement membranes in the developing and adult mouse tissues. *J. Cell Biol.* **98**, 971–979.

Wewer U. M., Albrechtsen R., Manthorpe M., Varon S., Engvall E., and Ruoslahti E. (1983) Human laminin isolated in a nearly intact biologically active form from placenta by limited proteolysis. *J. Biol. Chem.* **258**, 12654–12660.

Wewer U. M., Liotta L. A., Jaye M., Ricca G. A., Drohan W. N., Claysmith A. P., Rao C. N., Wirth P., Coligan J. E., Albrechtsen R., Mudryj M., and Sobel M. E. (1986) Altered levels of laminin receptor mRNA in various human carcinoma cells that have different abilities to bind laminin. *Proc. Natl. Acad. Sci. USA* **83**, 7137–7141.

Wewer U. M., Tichy D., Damjanov A., Paulsson M. and Damjanov I. (1987) Distinct antigenic characteristics of murine parietal yolk sac laminin. *Devel. Biol.* **121**, 397–407.

Wiegandt H., Kanda S., Inoue K., Utsumi K., and Nojima Sh. (1982) Studies on the cell association

of exogenous glycolipids, *New Vistas in Glycolipid Research (Advances in Experimental Medicine and Biology*, vol. 152), Makita A., Handa S., Taketomi T. and Nagai Y., eds., Plenum, New York, pp. 343–352.

Yamada K. M. (1983) Cell surface interactions with extracellular materials. *Ann. Rev. Biochem.* **52**, 761–799.

Yamada K. M., Spooner B. S., and Wessells N. K. (1971) Ultrastructure and function of growth cones and axons of cultured nerve cells. *J. Cell Biol.* **49**, 614–635.

Yankner B. A., and Shooter E. M. (1982) The biology and mechanism of action of nerve growth factor. *Ann. Rev. Biochem.* **51**, 845–868.

Young S. H., and Poo M.-m. (1983) Spontaneous release of transmitter from growth cone of embryonic neurone. *Nature* **305**, 634–637.