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AXONAL GROWTH-ASSOCIATED PROTEINS

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Elongation of axons and active remodeling of their terminal arbors underlies the assembly of neural circuits during development, determines the success or failure of nerve regeneration, and may contribute to some forms of synaptic plasticity in adult brains. For most neurons, elongation of a principal axon is confined to a few days or weeks during development. Remodeling of axon terminal arbors also is most pronounced during transient "critical periods" late in development, although some forms of synaptic remodeling continue throughout life (Lichtman et al 1987). Once past these epochs of axon elongation and dynamic sorting of synaptic terminals, it might be possible to stabilize principal axon branches or their terminal arbors by inactivating some of the molecular processes required for growth and synaptogenesis. Selective inactivation or retention of some growth-related processes in maturing neurons might then define some limits on the mechanisms available for synaptic remodeling in the adult nervous system.

Studies of axon regeneration in vivo and in tissue culture indicate that some aspects of axon growth are indeed repressed in many adult neurons but can be re-induced under some conditions. Although such studies have considered primarily the elongation of primary axons, they also raise the possibility that neuronal processes underlying more subtle aspects of axon growth and synaptogenesis may be down-regulated chronically in mature neurons. At the molecular level, periods of axon outgrowth during development and re-induction of axon growth for regeneration are correlated with large and specific changes in synthesis of a few proteins transported into the growing axons. This suggests the hypothesis that differentiation to a stable mature state can include the selective repression of genes required for axon growth. The list of genes expressed selectively during

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periods of axon growth is far from complete, but it is already possible to identify a few genes that seem to be tightly correlated with a neuronal "growth state." One of these is a gene encoding an acidic membrane protein designated GAP-43 (also known as B-50, F1, pp46, or p57). Although most neurons selectively reduce GAP-43 expression as they mature, a subset of neurons continue to express high levels of the protein in the adult CNS. Phosphorylation of GAP-43 in adult brain has been correlated with long-term potentiation at some synapses. Biochemical characterization of GAP-43, and emerging evidence of other genes expressed during axon growth, suggest that some "growth-associated" proteins may alter a neuron's responses to extracellular signals by altering intracellular signal transducing systems.

"GROWTH STATE(S)" AND MATURE NEURONS

When developing neurons are explanted from fetal or neonatal animals, the explanted cells can reinitiate neurite outgrowth in the culture dish within a few hours (Argiro & Johnson 1982, Collins & Lee 1982). In contrast, adult neurons explanted to identical culture conditions do not extend neurites for several days (Agranoff et al 1976, Landreth & Agranoff 1976, Argiro & Johnson 1982, Collins & Lee 1982), thus suggesting that some aspects of axon growth are repressed or impaired in the mature neurons and are reinduced slowly in culture. In some cases, maturation may involve selective loss of individual features of axon growth. Chick retinal neurons, for example, lose much of their ability to extend neurites on a laminin substratum between embryonic days E6 and E12, but retain their capacity for elongating neurites on glial cell surfaces (Cohen et al 1986, Hall et al 1987).

For neurons that regenerate their axons successfully in vivo, a nerve injury made several days prior to explanation substantially reduces the lag before outgrowth of neurites in culture (Agranoff et al 1976, Landreth & Agranoff 1976). Similarly the lag before initiation of axon regeneration in vivo is reduced if the same nerve received an earlier "conditioning" lesion, suggesting that nerve injury induces events in the neuronal cell body that prepare the neuron to extend its axon (reviewed in Grafstein & McQuarrie 1978, McQuarrie 1984). All of these observations are consistent with a model in which some of the molecular processes important for axon growth become repressed or down-regulated in many neurons as they mature, but may be reinduced under some conditions in adult animals. The developmentally regulated events in the cell body might include either fundamental alterations in the machinery for neurite extension or, as illustrated for maturing chick retinal cells, more subtle changes in molecules involved in

the recognition and transduction of growth-related signals in the extracellular environment.

MOLECULAR CORRELATES OF AXON GROWTH

The apparent repression of growth-related properties in many neurons during differentiation suggests that some of the genes involved in axon growth might be expressed transiently during development and reinduced during successful axon regeneration. This has prompted a search for genes whose expression is correlated consistently with periods of axon growth. Most of these studies have concentrated on the synthesis of proteins destined for transport into axons and their terminals, the population of neuronal proteins most directly involved in axon functions. In almost all neurons screened in this way, it has been possible to identify one or more axonal proteins whose synthesis is specifically increased an order of magnitude during developmental or regenerative axon growth (Table 1). These proteins are distinguished as rapidly transported or slowly transported proteins, depending on whether they are delivered into the axon within a few hours of synthesis (transport groups I and II or FC; Willard et al 1974, Grafstein & Forman 1980) or move along axons much more slowly as part of a large complex of cytoskeletal and cytoplasmic components (groups IV and V or SC and SCb; Willard & Hulebak 1977, Hoffman & Lasek 1975, Black & Lasek 1980). The small number of proteins transported at an intermediate velocity (group III of Willard et al 1974) have not been examined in detail for growth-related changes.

When the synthesis of individual proteins is normalized to overall protein synthesis, only a small number of proteins in any one neuronal system show large changes in their relative synthesis during axon growth. Even fewer proteins are consistently expressed at elevated levels during both developmental and regenerative axon growth in many different neurons (Table 1). Among these are tubulin and actin, and a rapidly transported membrane protein designated GAP-43. Periods of axon growth also are characterized in many cases by decreased synthesis or transport of neurofilament proteins, particularly the largest of the three neurofilament subunits (Hoffman & Lasek 1980, Shaw & Weber 1982, Willard & Simon 1983, Pachter & Liem 1984, Tetzlaff et al 1987, Kalil & Perdew 1988). It has been suggested that some aspects of axon growth or plasticity may be reduced in maturing neurons by the assembly of an extensive network of neurofilament cross-links mediated by the large neurofilament subunit (Willard et al 1984, Glicksman & Willard 1985).

With the expanding use of subtractive and differential hybridization in screening cDNA libraries, identification of additional "growth-associated"

Table 1 Large changes in synthesis of individual axonal proteins during period of axon growth[a]

	GAP-43	GAP-24 (23–28 K)	Tubulins	Actin	NF decreased transport	Other proteins	References
	(Rapidly transported)		(Slowly transported)				
REGENERATION							
Toad optic n.	●	●	●	●		50 K, 33 K, 42 K	Skene & Willard 1981a
Fish optic n.	●	●	●	●		100–140 K	Benowitz et al 1981, 1983
						250 K	Giulian et al 1980
						68–70 K	Heacock & Agranoff 1976, 1982
						numerous smaller changes	Perry et al 1987a
Frog optic n.	NO (2X)[b]	NO	2X			several smaller changes	Szaro et al 1985
Frog sciatic (DRG)	NO	2X					Perry et al 1987b
							Perry & Wilson 1981
Rat sciatic n.:							
sensory	●	●	<2X	<2X		several smaller changes	Hall 1982
							Redshaw & Bisby 1987
							Basi et al 1987
							Goldstein et al 1987
motor			NO	NO	●		Hoffman & Lasek 1980
Rat facial n.			●	●	●		Tetzlaff et al 1987
Rat hypoglossal n.	●	●					Redshaw & Bisby 1984a
Rabbit hypoglossal n.	●	no change					Skene & Willard 1981b

DEVELOPMENT					
Rabbit optic n.	●	●		●	Skene & Willard 1981b Willard & Simon 1983
Rat optic n.	●	●		●	Freeman et al 1986 Pachter & Liem 1984
Hamster pyramidal tr.	●	NO		●	Kalil & Skene 1986 Kalil & Perdew 1987
Whole cortex	●	NO	●	●	Jacobson et al 1986 Shaw & Weber 1982 Lewis et al 1985 Carden et al 1987
ABORTIVE REGENERATION					
Rabbit optic n.	NO	NO			Skene & Willard 1981b
Rat optic n.	NO ●	NO			Redshaw & Bisby 1984b Freeman et al 1986
Hamster pyramidal tr.	NO	NO			Kalil & Skene 1986 Reh et al 1987

[a] All proteins listed are reported to undergo at least a ten-fold change in synthesis correlated with axon growth in at least one system. All entries refer to specific changes in protein synthesis or mRNA abundance. *Solid dots* indicate that synthesis of the protein increases ten-fold or more, compared to uninjured adult neurons. *Filled dots* indicate order-of-magnitude changes. "NO" indicates that synthesis of the indicate protein was assayed and found not to change significantly during axon growth. *Blank spaces* indicate that the protein was not assayed.

[b] See text for discussion.

proteins or genes seems a likely prospect. In the meantime, proteins such as GAP-43 and the cytoskeletal proteins, whose expression is widely correlated with axon growth, provide a starting point for studying how specific changes in gene expression in the cell body are influenced by changing conditions in distal portions of an axon, and how altered expression of those genes might affect various steps in axon growth.

GAP-43 (A.K.A. B-50/F1/pp46/P-57)

The most extensive correlation with axon growth documented so far is for the synthesis of GAP-43 (Table 1). This assertion rests on evidence, sometimes indirect, that the protein designated "GAP-43" is the same or homologous in each species and neuron type studied. In all cases, "GAP-43" refers to a membrane-bound, rapidly transported (Group I) protein with a very acidic isoelectric point (4.3–4.5) and aberrant migration on SDS gels, such that the protein's *apparent* molecular weight varies from 43–57,000 daltons or greater, depending on the gel concentration used (Jacobson et al 1986, Benowitz et al 1987). Direct electrophoretic comparisons have been used to suggest homologies between the rabbit (Skene & Willard 1981b), rat (Jacobson et al 1986), and hamster (Kalil & Skene 1986) proteins and the definitional toad GAP-43, and between the rat and goldfish proteins (Perrone-Bizzozero et al 1986). Immunological cross-reactivity between the toad and rat proteins has been demonstrated (Jacobson et al 1986). Cloning of rat GAP-43 cDNA (Basi et al 1987, Karns et al 1987, Neve et al 1987) now provides a more definitive basis for identifying GAP-43 in various neurons.

This same protein has been discovered with relentless regularity by investigators working on several aspects of axon growth and synaptic function. A synaptic protein designated B-50 has been suggested as a possible regulator of polyphosphoinositide metabolism (Oestreicher et al 1983, van Dongen et al 1985, van Hoof et al 1988). Other investigators have found that kinase-C-mediated phosphorylation of the same protein is correlated with long-term synaptic potentiation in rat hippocampus (Nelson & Routtenberg 1985, Routtenberg 1986); in that context, the protein was designated F1. A major phosphoprotein of growth cone membranes has been called pp46 (Katz et al 1985, Meiri et al 1986). Finally, an unusual calmodulin-binding protein from bovine and mouse brain, designated p57 (Andreason et al 1983), binds calmodulin preferentially in the absence of calcium; phosphorylation of p57 by kinase C greatly reduces the protein's affinity for calmodulin. Biochemical and immunological evidence that these are all the same protein has been reviewed (Benowitz &

Routtenberg 1987). More recently, direct sequence comparisons (Figure 1) show that mouse and bovine p57 (Wakim et al 1987, Cimler et al 1987) are virtually identical to rat GAP-43 (Basi et al 1987, Karns et al 1987), F1 (Rosenthal et al 1987), and B-50 (Nielander et al 1987).

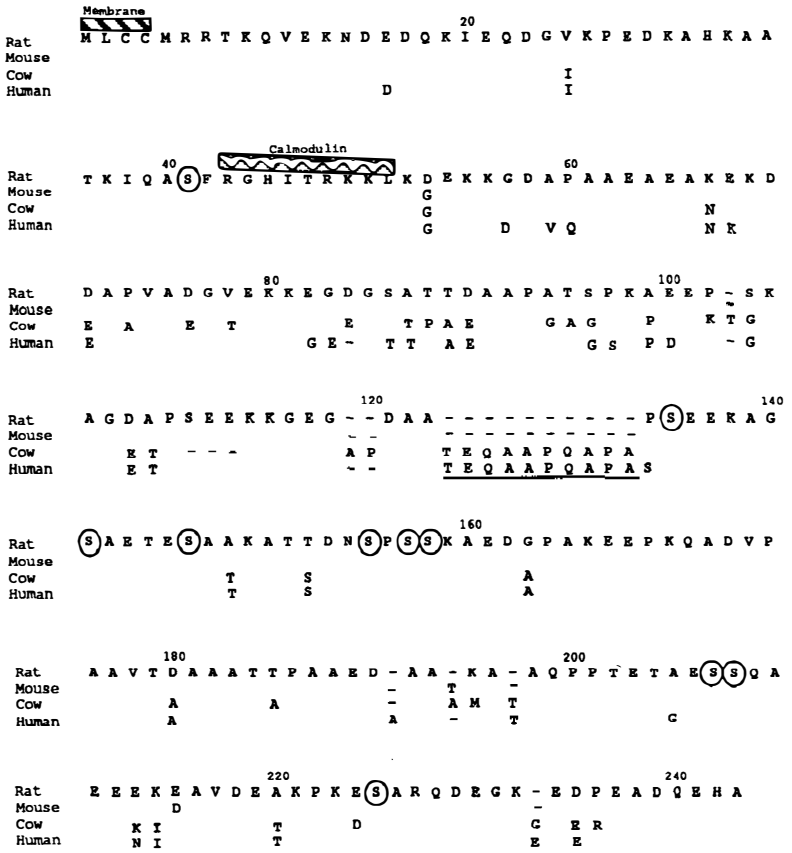

Figure 1 Amino acid sequence of GAP-43. cDNAs corresponding to rat proteins identified as GAP-43 (Basi et al 1987, Karns et al 1987) or protein F1 (Rosenthal et al 1987) yielded identical sequences. The sequences for mouse and cow were determined for the calmodulin-binding protein p57 (Cimler et al 1987, Wakim et al 1987). Human sequences are predicted from cDNAs isolated with a rat GAP-43 cDNA probe (Ng et al 1988) or with antibodies against purified protein B-50 from rat (Kosik et al 1988). *Shaded bars* indicate the putative membrane binding domain (Basi et al 1987) and calmodulin binding site (Wakim et al 1987, Alexander et al 1988). A 10-amino-acid in the cow and human sequence, not found in rodents, is underlined. *Dashes* in the sequence indicate spaces inserted to optimize alignments. Serine residues conserved in all four sequences are *circled.*

Localization of protein B-50 to presynaptic membranes in adult brain (Gispen et al 1985), and correlation of protein F1 phosphorylation with long-term potentiation (Nelson & Routtenberg 1985, Lovinger et al 1986) has expanded speculation about possible roles of GAP-43 to include physiological or structural plasticity of synaptic terminals, in addition to any role the protein might play in elongation of principal axons.

Association with Axon Growth

Consistent with a possible role in axon elongation, GAP-43 has been shown to be a prominent protein component of growth cone membranes. A subcellular fraction from developing rat brain highly enriched for growth cones (Pfenninger et al 1983) is also highly enriched for GAP-43 (Skene et al 1986, Mciri et al 1986); GAP-43 is also a prominent substrate for endogenous kinase(s) in these isolated growth cones (Katz et al 1985, deGraan et al 1985, Jacobson et al 1986). GAP-43 is an extremely abundant protein in growth cones, comprising on the order of 1% of the total protein in growth cones membranes (Skene et al 1986). Immunolocalization of GAP-43 in cultured neurons (Meiri et al 1988) confirms that the protein is most heavily concentrated in growth cones. GAP-43 appears to be present throughout the growth cones, including filopodial extensions. Varicosities along the length of neurites also contained high concentrations of GAP-43. Neuron cell bodies show low levels of diffuse staining for GAP-43, in contrast to the punctate staining of neurites and growth cones (Meiri et al 1988).

A growth cone localization for GAP-43 is equally consistent with the protein's involvement in axon elongation or in the formation and active reorganization of synapses once axons reach their target tissues. The time course of GAP-43 expression during axon regeneration is also consistent with either of these possibilities. In regenerating toad retinal ganglion cells, GAP-43 synthesis begins to increase approximately 4 days after axotomy and reaches a maximum during the second week of regeneration (Skene & Willard 1981a); induction follows a similar time course in goldfish (Benowitz et al 1981, Benowitz & Schmidt 1987). Axon elongation commences approximately 4.5 days after injury of goldfish optic nerves (Grafstein & McQuarrie 1978, McQuarrie & Grafstein 1981), and the initial delay for amphibian optic nerves appears to be similar or slightly longer (Agranoff et al 1976). Thus the initial rise in GAP-43 synthesis coincides with, or slightly precedes, initiation of axon outgrowth in fish and amphibian optic nerves, but the protein's synthesis does not peak or plateau until regeneration is well underway. In rat dorsal root ganglia, induction of GAP-43 mRNA begins between 1 and 2 days after sciatic nerve injury (G. S. Basi and J. H. P. Skene, unpublished), again cor-

responding to the end of the lag period before axon outgrowth (McQuarrie et al 1977, Forman & Berenberg 1978). It is intriguing that the effects of a "conditioning" lesion on subsequent axon regeneration of rat DRG axons are first detectable if the testing lesion is administered 2 days after the conditioning lesion (Forman et al 1980), coincident with the increase in GAP-43 mRNA. Although GAP-43 expression is not fully elevated until axon elongation is underway, induction of GAP-43 does not appear to be a secondary consequence of axon outgrowth. In rat sciatic nerves, application of colchicine at the time of nerve injury or at the end of the lag period (2 days post-crush) should prevent axon outgrowth (e.g. Bamburg et al 1986), but it has no effect on the time course or amplitude of GAP-43 induction (G. S. Basi and J. H. P. Skene, unpublished).

Elevated GAP-43 expression continues throughout the period of axon elongation and synaptogenesis in all developing and regenerating systems examined. In the toad visual system regenerating optic axons begin to reenter their target tissue, the optic tectum, by 3 weeks after nerve crush, but GAP-43 synthesis does not decline until 5–10 weeks after axotomy (Skene & Willard 1981a), during the period when an orderly pattern of retinotectal synapses is reestablished (Freeman 1977). In regenerating goldfish retinal ganglion cells, synthesis of GAP-43 declines slowly beginning 2–3 weeks after a nerve crush, and remains above control levels through 8 weeks, including the period of formation and activity-dependent refinement of the retinotectal projection (Benowitz & Schmidt 1987). The decline of GAP-43 also coincides with synaptogenesis in developing hamster pyramidal tract neurons (Kalil & Skene 1986), and the steady-state amount GAP-43 declines slowly during the "critical period" for plasticity of cat visual cortex (McIntosh et al 1987). The relatively slow decline in GAP-43 synthesis late in axon development or regeneration would therefore permit the protein to play some role in synaptogenesis or in the active sorting out of the terminal arbor.

GAP-43 induction in regenerating systems seems to begin just early enough not to rule out a role in the initial phases of axon outgrowth, and to persist just long enough not to rule out participation in later phases of synaptogenesis and maturation of the axon's terminal arbor. The localization of the protein to growth cones and the distal portions of outgrowing neurites does argue against direct GAP-43 participation in maturation of the axon structure behind the immature axon sprouts, or the slow growth in axon diameter, myelination, and maturation of the axon's electrical properties.

The strong correlation of axon growth and elevated GAP-43 synthesis, and the abundance of GAP-43 in growth cone membranes, suggest that GAP-43 might be essential for some steps in axon growth. However, there

have been some reports of successful regeneration with no discernible increase in GAP-43 synthesis or axonal transport (Szaro et al 1985, Hall 1982, Hall et al 1978, Perry et al 1987b). Most of these studies were made before the aberrant SDS gel behavior of GAP-43 was appreciated, and they did not include known GAP-43–containing samples to identify the position of the protein in the gel systems employed. Inspection of the published data of Szaro et al (1985) suggested that their spot 23, which showed a significant induction during regeneration, might correspond to GAP-43 (Jacobson et al 1986). The comparatively modest (two-fold) induction of the GAP-43–like protein during regeneration of *Xenopus* retinotectal axons—compared to more than ten-fold increases in other systems—might reflect the fact that axons were lesioned directly at the tectum and proteins were analyzed after the reestablishment of retinotectal synapses was underway (Szaro et al 1985). Other studies reported no evidence of GAP-43 induction in rat and frog dorsal root ganglia (Hall 1982, Perry et al 1987b) and rat sympathetic ganglia (Hall et al 1978) during axon regeneration. Those investigators employed isoelectric focusing gels with a narrow pH range (4.5–7) in order to gain high resolution, but the very acidic GAP-43 (pI 4.3–4.5) might be difficult to detect reliably on such a system. Indeed, those investigators reported variable detection of a protein somewhat similar to GAP-43 in frog DRG (Perry et al 1987b), and inspection of the data of Hall (1982) suggests that the protein designated 2F1, which showed a highly variable degree of labeling but appeared to increase during regeneration, might be similar to GAP-43. Direct measurement of GAP-43 in one of these systems—rat dorsal root ganglia—shows definitively that GAP-43 mRNA is elevated during axon regeneration (Basi et al 1987; G. S. Basi and J. H. P Skene, unpublished) and development (Karns et al 1987).

Synaptic Plasticity in Adult Brain

While the correlation of GAP-43 synthesis with axon development and regeneration was being investigated, an independent set of investigations identified the same protein as a potential mediator of synaptic functions in adult brain. Gispen and colleagues described a synaptic membrane protein (B-50) whose phosphorylation was inhibited by an ACTH-derived peptide reported to inhibit performance on a learning task (Zwiers et al 1976). Routtenberg and his colleagues found that phosphorylation of protein F1 was correlated with synaptic long-term potentiation in rat hippocampus (Nelson & Routtenberg 1985, Lovinger et al 1986). In both cases, the phosphorylation was shown to be carried out by the calcium/phospholipid-dependent protein kinase C (Aloyo et al 1983, Nelson & Routtenberg 1985, Akers & Routtenberg 1985).

Long-term potentiation (LTP) occurs at some synapses following a train of high-frequency stimulation. LTP is assayed as an increased post-synaptic response to a given afferent stimulation, and it can be observed for minutes or hours in in vitro preparations and up to several weeks in vivo (reviewed in Teyler & DiScenna 1987, McNaughton & Morris 1987, Smith 1987). Stimulation of LTP can be associative; that is, subthreshold stimulation of one afferent pathway can sum with subthreshold stimulation of a second pathway projecting to the same post-synaptic population to generate LTP. The long duration and associative properties of LTP have made it a popular model for synaptic mechanisms that might be involved in memory (McNaughton & Morris 1987).

Possible roles of protein kinase C in LTP have received increasing attention in recent years. Akers et al (1986) showed that LTP in hippocampus is accompanied by translocation of kinase C from cytosol to membranes. Direct activation of protein kinase C with phorbol esters has been reported to potentiate synaptic transmission in hippocampal slices (Malenka et al 1986), and kinase C activation by phorbol esters or oleic acid has been reported to enhance LTP in intact animals (Routtenberg et al 1986, Linden et al 1986, 1987). Conversely, Lovinger et al (1987) reported that several inhibitors of protein kinase C could inhibit LTP in the intact hippocampus.

In none of these studies could it be determined whether the LTP-related alterations in protein kinase C occurred in the presynaptic terminals or the postsynaptic cells. Although the initial trigger for LTP appears to occur postsynaptically, debate has continued over the proposal that maintenance of LTP occurs through increased presynaptic transmitter release (Teyler & DiScenna 1987, Smith 1987). Preliminary immunolocalization of GAP-43/B-50 by electron microscopy indicates that this protein is exclusively presynaptic in adult brains (Gispen et al 1985, Norden et al 1987). Direct demonstration of kinase-C–mediated phosphorylation of an exclusively presynaptic protein during LTP would show that at least some of the LTP-activated protein kinase C is in presynaptic terminals (Routtenberg 1986). However, assays of GAP-43/F1/B-50 phosphorylation in LTP experiments have been carried out in vitro on homogenized preparations (Akers & Routtenberg 1985, Nelson & Routtenberg 1985, Lovinger et al 1986, Routtenberg 1986); as a result, it has not been shown definitively that phosphorylation of GAP-43 in vitro reflects access of the activated fraction of kinase C to presynaptic GAP-43 in vivo.

GAP-43/F1/B-50 phosphorylation during LTP can be interpreted in several ways. First, kinase C-regulated aspects of LTP might be mediated exclusively through other substrates of the kinase, so that GAP-43 phosphorylation is only coincidental with, and not functionally involved in,

LTP; at least one other kinase C substrate also shows increased phosphorylation during LTP (Nelson et al 1987a). Second, GAP-43 might participate in some steps in activity-stimulated neurotransmitter release. This possibility is not incompatible with a related role in axon growth, since neurotransmitter release and axon elongation have many subcellular mechanisms in common (e.g. vesicle movement, membrane fusion). Finally, if the biochemical actions of GAP-43 pertain exclusively to structural growth of axons, GAP-43/F1 phosphorylated in LTP might participate in structural alterations, such as the growth of additional synaptic terminals during LTP (e.g. Greenough 1984).

Proposals that GAP-43 participates in either axon growth or synaptic plasticity are based so far only on correlative evidence. In the case of developing and regenerating systems, GAP-43 was distinguished from other potential regulators of growth by the consistency with which its synthesis is correlated with growth in many different neuronal systems. GAP-43/F1 phosphorylation during LTP has been investigated primarily in one system, the perforant pathway of rats (Lovinger et al 1986, Routtenberg 1986). Is expression or phosphorylation of GAP-43 generally correlated with synaptic plasticity throughout adult brain?

GAP-43 expression and phosphorylation show a distinctly nonuniform distribution in adult rat and primate brains (Benowitz et al 1988, Neve et al 1987, Kristjansson et al 1982, Oestreicher & Gispen 1986, Rosenthal et al 1987). Both the protein and its mRNA are located predominantly in the prosencephalon, although there are discrete GAP-43-positive nuclei in the medulla and mesencephalon (Benowitz et al 1988). Within neocortex, GAP-43 and is mRNA are both concentrated most heavily in higher order sensory and "associational" cortex, with much lower levels of expression in primary sensory areas and motor cortex (Neve et al 1987, Benowitz et al 1988). Parts of the hippocampus show heavy immunostaining for GAP-43 (Oestreicher & Gispen 1986, Benowitz et al 1988) but low concentrations of GAP-43 mRNA (Neve et al 1987, Rosenthal et al 1987), thus suggesting that the protein is located in synaptic terminals of extrinsic neurons projecting to hippocampus. High concentrations of GAP-43 in adult rat brains also are found in various nuclei of the basal ganglia, thalamus, and hypothalamus (Benowitz et al 1988) and the septal area (Oestreicher et al 1986).

Apparently, GAP-43 is expressed in substantial amounts only in a discrete set of neurons during adult life. Several authors have pointed out that polymodal sensory cortex, the hippocampus, frontal cortex, and many parts of the "limbic system" all show levels of GAP-43 and/or GAP-43 mRNA (Neve et al 1987, Benowitz et al 1988, Nelson et al 1987b), and that these structures are generally thought to be involved in memory and

other higher order processing that might well require a good deal of synaptic plasticity. In contrast, primary sensory areas and motor cortex, along with most of the medulla and spinal cord, contain only low concentrations of GAP-43, and these regions often are considered in terms of rather static circuitry. The gradient from low GAP-43 in primary sensory areas to higher GAP-43 expression in polymodal or association cortex has been reported both in rodents and primates (Neve et al 1987, Nelson et al 1987b, Benowitz et al 1988). This sort of generalization leaves one in a quandary over areas such as somatosensory cortex, which displays remarkable functional plasticity in adult life (Kaas et al 1983, Jenkins & Merzenich 1987) but has a relatively low concentration of GAP-43 (Benowitz et al 1988), and many parts of the thalamus and basal ganglia, which contain very high concentrations of GAP-43 but are not usually considered areas of special synaptic plasticity. The immediate challenge is to identify any anatomical, physiological, or functional characteristics shared by the GAP-43–expressing population of adult neurons. Defining these "high GAP-43" populations of neurons should be facilitated by ongoing studies using in situ hybridization to locate cell bodies containing GAP-43 mRNA to complement the immunohistochemical map of GAP-43-containing synaptic terminals (Benowitz et al 1988). The possibility that the subset of neurons expressing high levels of GAP-43 through adult life has a special propensity for some forms of synaptic plasticity (Routtenberg 1986, Jacobson et al 1986, Benowitz et al 1988, Neve et al 1987) remains appealing, but not established.

Structure and Biochemical Characteristics of GAP-43

Correlative studies have focused attention on GAP-43 as a potential contributor to some forms of axon growth or synaptic plasticity, but the actual effects of GAP-43 on growth cones and synaptic terminals remain unknown. One way to establish the functions of GAP-43 is to build up from its molecular properties a picture of the protein's actions within a neuron. Structural and biochemical studies of GAP-43 reveal a novel protein that appears to interact extensively with several intracellular messenger systems.

The amino acid sequence of GAP-43 is highly conserved among mammals, although cow and human GAP-43 contain a ten–amino acid insert not found in the rodent protein (Figure 1). In Figure 1, the alignment of the bovine and mouse sequences has been altered from the published alignment (Wakim et al 1987) to resemble the alignments of rat and human sequences (Ng et al 1988, Kosik et al 1988). The structural novelty of GAP-43 is underscored by the failure of extensive searches of nucleic acid and amino acid sequence databases to reveal any significant homologies

with previously analyzed proteins (Basi et al 1987, Cimler et al 1987, Karns et al 1987, Rosenthal et al 1987). The predicted molecular weight of the unmodified rat protein is 23.6 kD, consistent with earlier indications that the mature protein is much smaller than it appears on SDS gels (Masure et al 1986, Benowitz et al 1988). Secondary structure predictions agree with circular dichroism studies (Masure et al 1986) in showing a large proportion of the protein in random coil, interrupted by 17 proline residues. GAP-43, then, appears to be an elongated (Masure et al 1986) kinked coil, with limited domains of more ordered secondary structure.

MEMBRANE ASSOCIATION One of the most striking structural features of GAP-43 is its extreme hydrophilicity (Basi et al 1987, Rosenthal et al 1987), somewhat surprising for a protein associated predominantly with membranes. GAP-43 is synthesized as a soluble protein, whose post-translational association with membranes probably is mediated by covalent attachment of fatty acid (J. H. P. Skene and I. Virag, unpublished). A short hydrophobic region at the amino terminus of the protein (Figure 1) is the most likely site of fatty acylation and membrane attachment (Basi et al 1987; J. H. P. Skene and I. Virag, unpublished). Although the major fraction of GAP-43 is membrane-bound, there may exist a soluble form of the protein resulting from reversible removal of the bound fatty acid or irreversible cleavage of the putative membrane-binding domain (Basi et al 1987, Rosenthal et al 1987). The extreme hydrophilicity of GAP-43 and the nature of its membrane attachment are consistent with models that envision the protein extending away from the cytoplasmic surfaces of growth cone and synaptic membranes (Skene & Willard 1981c, Gispen et al 1985, Meiri et al 1988), in a position to interact with cytoplasmic or cytoskeletal proteins on one hand, and reversibly attached to the membrane on the other.

CALMODULIN BINDING GAP-43/p57 was identified by Storm and his colleagues as a unique calmodulin-binding protein, binding calmodulin selectively in the *absence* of calcium, and releasing calmodulin at higher calcium concentrations (Andreason et al 1983). This "reversed" calcium dependence for calmodulin binding is sufficiently rare that it can be used to purify GAP-43/p57 to homogeneity (Andreason et al 1983, Masure et al 1986). On the basis of its abundance, membrane binding, and its "reversed" pattern of calmodulin binding, GAP-43/p57 has been proposed to act under low calcium conditions to sequester calmodulin in certain regions of neuronal membrane, releasing calmodulin upon influx or mobilization of free calcium (Andreason et al 1983, Cimler et al 1987). However, because the calcium-dependent antagonism of GAP-43–calmodulin binding is strongly affected by ionic strength (Alexander et al 1987), it is not clear

whether calcium regulates calmodulin binding to GAP-43 under any or all physiological conditions. An alternative regulator of calmodulin binding to GAP-43 is protein kinase C. Phosphorylation of GAP-43/p57 by protein kinase C strongly inhibits binding of the protein to calmodulin (Alexander et al 1987).

PHOSPHOINOSITIDE METABOLISM The antagonistic interactions of kinase C and calmodulin with GAP-43 are intriguing, because calmodulin and kinase C participate in separate branches of a bifurcating second-messenger system in many cells (e.g. Berridge 1987). The now-classical version of this second-messenger system begins with the receptor-activated hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP_2) to produce diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3). DAG, in turn, specifically activates protein kinase C, while IP_3 stimulates the release of free Ca^{2+} from intracellular stores (Figure 2).

The precursor for these two second messengers, PIP_2 is produced by phosphorylation of phosphatidyl inositol 4-phosphate (PIP). Van Dongen et al (1985) reported that phosphorylated GAP-43/B-50 inhibits the activity of a purified PIP-to-PIP_2 kinase; conversely, antibodies against GAP-43/B-50 stimulated production of PIP_2 in isolated synaptosomal membranes, while blocking phosphorylation of GAP-43/B-50 (Oestreicher et al 1983). It was therefore suggested that GAP-43/B-50 acts as a feedback inhibitor of kinase C activation and calcium mobilization (Jolles et al 1980, Oestreicher et al 1983, van Dongen et al 1985, van Hoof et al 1988). There is, however, evidence that under some conditions PIP can be hydrolyzed directly to yield DAG plus an inositol biphosphate that does not stimulate calcium mobilization (Berridge 1987). Under those conditions, inhibition of PIP_2 production would uncouple kinase C activation from calcium mobilization. In either case, the effect of phosphorylated GAP-43 would be to terminate IP_3-dependent calcium mobilization by preventing replacement of hydrolyzed PIP_2. It remains to be shown whether the actions of phosphorylated GAP-43 on phosphoinositide phosphorylation in vitro have a substantial effect on the production of PIP_2 in vivo.

GAP-43 AND CALCIUM SIGNALING The possible sequestering of calmodulin by GAP-43 and the potential capability of phosphorylated GAP-43 to terminate calcium mobilization suggest that GAP-43 may play a critical role in modulating intracellular signaling by calcium. The reported interactions of GAP-43 with components of the calcium-calmodulin intracellular messenger system are illustrated in Figure 2. An important unanswered question is whether GAP-43 inhibits the activity of bound calmodulin. If calmodulin bound to GAP-43 is effectively prevented from interacting with other calmodulin-binding effector proteins, the limited

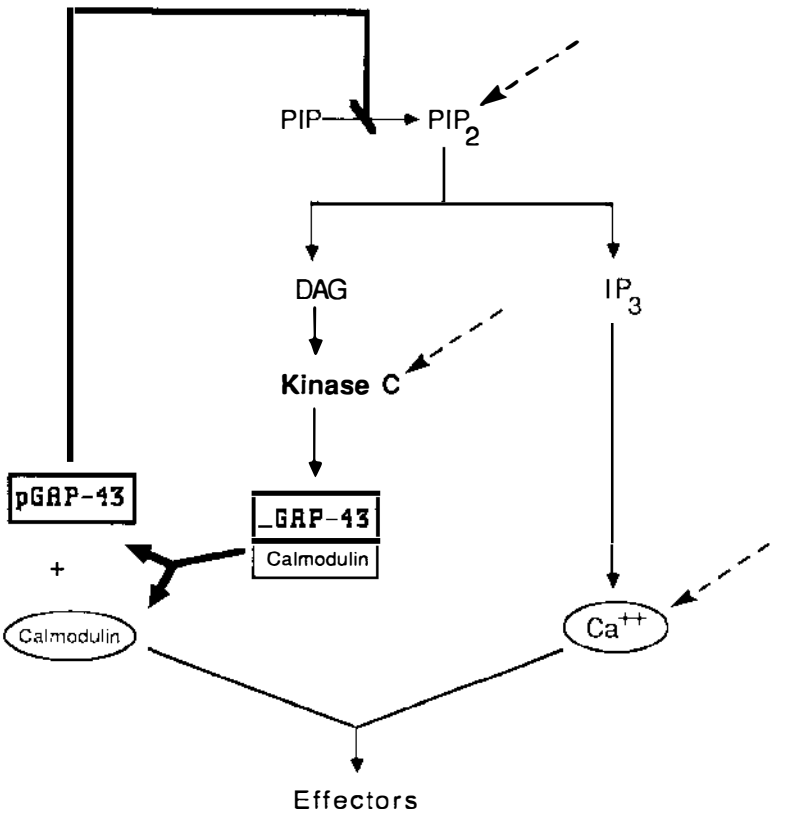

Figure 2 Proposed activities of GAP-43 in signal transduction pathways. The hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP_2) yields diacylglycerol (DAG), an activator of protein kinase C, and inositol 1,4,5-triphosphate (IP_3), which mobilizes intracellular calcium (reviewed in Berridge 1987). *Bold lines* indicate the binding of calmodulin by GAP-43, and release of calmodulin after phosphorylation of GAP-43 by kinase C (Alexander et al 1987), and the inhibition of phosphatidylinositol 4-phosphate (PIP) phosphorylation by phosphorylated GAP-43 (van Dongen et al 1985). *Dashed lines* indicate potential influences by extracellular signals: receptor-stimulated hydrolysis of PIP_2, influx of extracellular calcium, and alternative activation of protein kinase C discussed in the text.

data available can be assembled into a simplistic model in which GAP-43 acts to sharpen the spatial (Andreason et al 1983, Alexander et al 1987) and temporal resolution of intracellular calcium signals.

In an initial or "resting" state, with low intracellular calcium and a small steady-state pool of PIP_2, a large concentration of GAP-43 would constitute a calmodulin buffer, so that calcium-calmodulin mediated pro-

cesses in a growth cone or synaptic terminal would have a reduced response to small influxes or mobilization of calcium. Stimulation of PIP_2 hydrolysis would cause a rise in free calcium (via IP_3) and a parallel activation of protein kinase C. GAP-43 phosphorylation by kinase C would tend to liberate calmodulin from GAP-43, an effect that might be augmented by elevated free calcium. In the presence of GAP-43, then, the cellular response to PIP_2 hydrolysis would rise exponentially because GAP-43 makes both the mobilization of calcium and the concentration of free calmodulin responsive to PIP_2 breakdown. In the meantime, phosphorylation of GAP-43 would inhibit regeneration of PIP_2 from PIP. As a result, mobilization of intracellular calcium would be terminated after hydrolysis of the preexisting pool of PIP_2. The effect of GAP-43, therefore, would be to sharpen the temporal resolution of calcium-calmodulin signals resulting from activation of phospholipase C-coupled receptors.

Similar considerations suggest that GAP-43 could act to sharpen the spatial resolution of calcium signals in synaptic terminals and growth cones. Local activation of phospholipase-coupled receptors in one region of a growth cone membrane, for example, would lead to local phosphorylation of GAP-43, particularly if GAP-43 exists in vivo in a complex with protein kinase C (Zwiers et al 1980, Aloyo et al 1983). Unphosphorylated GAP-43 in other regions of the membrane would tend to buffer those regions of the growth cone against calmodulin-dependent responses to mobilized calcium. At the same time, the locally phosphorylated GAP-43 would terminate the IP_3-dependent calcium signal, possibly reducing its spread to other parts of the growth cone.

In this model, some effects of GAP-43 depend on the availability of nonclassical activators of protein kinase C. In the classical bifurcating pathway, C kinase is activated only by DAG derived from PIP_2. In that case, phosphorylated GAP-43 would be a feedback inhibitor of kinase C. The effect of GAP-43 would be to introduce a (relative) refractory period after phospholipase activation, when neither calcium mobilization nor kinase C activation would occur in response to receptor occupancy, because no PIP_2 would be available for hydrolysis. As kinase C was inactivated, recovery of PIP_2 could proceed at a rate dependent on the rate of GAP-43 dephosphorylation.

On the other hand, there is evidence that kinase C can be activated independently of PIP_2, via hydrolysis of PIP (Berridge 1987), *cis*-unsaturated fatty acids (Murakami & Routtenberg 1985), or proteolytic cleavage of the kinase itself (Kishimoto 1983, Melloni et al 1986). Activation of kinase C by these mechanisms could keep GAP-43 phosphorylated chronically, inhibiting IP_3-dependent calcium mobilization, but providing a large pool of free calmodulin. Under those circumstances, an axon terminal

would be highly responsive to an influx of extracellular calcium. Calmodulin-mediated responses—possibly including neurotransmitter release (Llinas et al 1985, Augustine et al 1987)—would be potentiated so long as kinase C remained activated.

Any account of GAP-43's biological role(s) is almost certain to change substantially with increasing biochemical characterization of this protein. GAP-43 interactions with membranes and possibly with cytoskeletal elements are likely to be important in localizing the protein and may be involved in biological roles beyond any participation in calcium-calmodulin signaling. Nevertheless, the limited biochemical data already available indicate that one consequence of high concentrations of GAP-43 in a growth cone or synaptic terminal is likely to be a substantial alteration in the way an axon terminal responds to environmental stimuli acting through calcium and calmodulin.

OTHER GROWTH-ASSOCIATED PROTEINS

GAP-43 is useful in establishing that selective regulation of neuronal gene expression does occur in correlation with periods of axon growth, but as illustrated by Table 1, GAP-43 is likely to be only one example of an emerging class of growth-associated axonal proteins. Identifying these other GAPs is important for understanding the aspects of axon growth subject to this kind of developmental regulation.

A common, though imperfect, correlate of axon outgrowth is elevated synthesis and axonal transport of tubulin. Recently it has been found that only one of several tubulin genes exhibits elevated expression during development and regeneration of axons (Lewis et al 1985, Miller et al 1987a–c). For neurons in which total tubulin synthesis shows little or no specific increase during periods of axon growth, it would be interesting to know whether the high rate of expression of multiple tubulin genes masks growth-associated regulation of one or more tubulin species. In regenerating nerves, tubulin synthesized in response to an injury does not seem to be required for the initiation of axon outgrowth. The axotomy-induced elevation of tubulin synthesis, when it occurs, begins several days after injury, concurrent with or slightly after the onset of axon elongation (Heacock & Agranoff 1982, Agranoff & Ford-Holevinski 1984, Skene & Willard 1981a). For axons interrupted several millimeters or even several centimeters from their cell bodies, the slowly transported tubulin requires several days more to reach the site of injury. This means that the initial stages of axon regeneration must proceed without benefit of additional tubulin produced in response to axotomy. Nevertheless, an increased supply of a major cytoskeletal protein might contribute to later stages of

axon growth. The rate of delivery of slowly transported axon components, including tubulin, has been proposed to set an upper limit on the rate of axon elongation (Hoffman & Lasek 1980, Komiya 1981, Wujek & Lasek 1983, Cancalon 1983). Where the newly synthesized tubulin reaches the distal segments of a growing axon, it may permit an increased velocity of axon extension. For developing neurons extending axons *de novo*, in which axon elongation is initiated at the cell body itself, elevated tubulin synthesis could easily participate in the initial phases of axon outgrowth. Because of its slow delivery along axons, *synthesis* of tubulin does not appear to be a good candidate for the regulation of synaptic remodeling or terminal sprouting far from the cell bodies of maturing or adult neurons. This does not mean, of course, that local alterations in tubulin assembly/disassembly cannot be intimately involved in controlling these modes of axon growth.

Another correlate of axon growth in many systems is elevated synthesis of a 23–28 kD rapidly transported membrane protein (Table 1). Variations in reported molecular weights and isoelectric points make it difficult to determine whether the 23–28 kD growth-associated proteins induced in different neuronal systems are really homologous proteins. Some indirect evidence suggests that the developmentally regulated 23 kD rabbit protein is related to GAP-24 from regenerating toad optic nerves (Skene & Willard 1981b). The difficulty of assessment of homologies among the 23–28 kD proteins in different neurons is aggravated by the number of rapidly transported proteins in this size range, several of which undergo small changes in synthesis during axon growth (e.g. Skene & Willard 1981a,b, Benowitz & Lewis 1983). It is also difficult to evaluate the apparent absence of growth-associated 23–28 kD proteins in several developing and regenerating neurons (Table 1). The rapid degradation of toad GAP-24 (Skene & Willard 1981c) may make the protein difficult to detect in some samples, although efforts to minimize in vivo and artifactual degradation failed to reveal a GAP-24–like protein in developing pyramidal tracts (Kalil & Skene 1986). A more definitive correlation between GAP-24 expression and some forms of axon growth will require specific probes for the protein or its mRNA.

Analysis of metabolically labeled axonally transported proteins has revealed a small number of proteins whose synthesis is widely correlated with periods of axon growth. However, this approach is limited in its ability to detect relatively minor proteins or proteins whose size or charge puts them outside the resolution of the electrophoretic systems used in the analyses. An alternative approach is to investigate the developmental and injury-induced expression of identified genes or proteins known or suspected to participate in cell or axon growth.

The proto-oncogene c-*src* has been found to resemble GAP-43 in its

localization and growth-associated expression. Immunodetectable c-*src* and *src*-related tyrosine kinase activity are expressed at elevated levels during neural development (Sorge et al 1984, Maness et al 1986, Lev et al 1984, Simon et al 1985), and are reinduced in regenerating rat sciatic nerves (Le Beau & Walter 1987). Like GAP-43, neural c-*src* is highly enriched in a subcellular fraction containing isolated growth cones (Maness et al 1988). Although the role of c-*src* in growth cones is unknown, expression of the oncogenic viral form of the protein (v-*src*) in the pheochromocytoma cell line PC12 induces neurite outgrowth (Alema et al 1985), thus strongly suggesting a regulatory role for c-*src* in some aspects of axon extension. It remains to be established whether the developmental expression of immunodetectable c-*src* reflects developmentally regulated expression of the c-*src* gene, and whether expression of this gene is widely correlated with axon regeneration a number of different neurons.

Another identified protein whose expression may be correlated with axonal growth is the receptor for apolipoproteins B and E (LDL receptor). Immunostaining reveals extremely high levels of the LDL receptor on the distal portions of regenerating axons in rat sciatic nerves and lesser amounts on remyelinating Schwann cells (Boyles et al 1987). A primary ligand for this receptor, apolipoprotein E (apoE) is synthesized and secreted by nonneuronal cells of the distal nerve stump after injury to mammalian peripheral nerves (Skene & Shooter 1983, Ignatius et al 1986, Snipes et al 1986) and in the developing CNS (Muller et al 1985, Freeman et al 1986). Uptake of lipids via the LDL receptor in neuronal growth cones (Ignatius et al 1987a,b) could contribute to axonal outgrowth. As with c-*src*, it is not yet known whether the growth-associated expression of immunodetectable LDL receptor is mediated by altered synthesis of the protein in neuron cell bodies.

One of the clearest illustrations that maturing neurons alter their responsiveness to environmental cues for axon growth is the developmentally decreasing ability of chick retinal neurons to extend neurites on laminin (Cohen et al 1986, Hall et al 1987). The altered responsiveness of these neurons to laminin occurs between embryonic days 6 and 12, and is accompanied by decreased expression of some protein recognized by the CSAT and JG22 antibodies (Hall et al 1987). These antibodies recognize several members of a family of receptors for extracellular matrix proteins (e.g. Hynes 1987), and the antibodies interfere with neurite outgrowth on a laminin substratum (Tomaselli et al 1986, Bozycko & Horwitz 1986). It will be important to determine whether the developmentally regulated CSAT/JG22 proteins are regulated at the level of protein synthesis, and whether growth-associated expression of these proteins is a common feature of many different developing and regenerating neurons.

Neurite outgrowth on cell surfaces involves axonal receptors separate from the extracellular matrix receptors recognized by the CSAT and JG22 antibodies (Tomaselli et al 1986); these additional receptors apparently include proteins in the N-CAM family of cell-adhesion molecules (Bixby et al 1987). It is therefore particularly interesting that a developmentally regulated growth cone membrane protein, the 5B4 antigen (Wallis et al 1985, Ellis et al 1985), is homologous to neural cell adhesion molecules (NCAMs) (Ellis et al 1987). Immunologically detectable N-CAMs also increase dramatically in rat sciatic nerves during regeneration (Daniloff et al 1986). Again, it remains to be established that the developmental and injury-induced appearance of these axonal proteins reflects altered synthesis rather than local modification, although a transcriptional level for N-CAM regulation has been suggested (Daniloff et al 1986).

These studies based on immunological detection of identified axonal proteins during development and regeneration suggest that the responsiveness of many axons to environmental cues may be altered as the cells mature by changes in cell surface receptors and proteins involved in signal transduction.

REGULATION OF GROWTH-ASSOCIATED GENE EXPRESSION

Identification of even a few genes whose expression is tightly linked to axon growth supports the suggestion that the decreased propensity for axon growth and remodeling exhibited by many neurons as they mature can be mediated in part by selective repression of neuronal genes involved in growth. Understanding the molecular mechanisms controlling these growth-associated genes should shed light on the kinds of regulatory signals that control a neuron's propensity for axon growth, and may also provide a basis for identifying other neuronal genes able to respond to those same regulatory signals.

Individual growth-associated proteins are not always expressed coordinately; this indicates that multiple signals mediate the retrograde regulation of these genes. In toad optic nerve, for example, synthesis of the protein designated GAP-24 rises sharply 2–4 days after nerve injury, whereas synthesis of GAP-43 and tubulin increases more slowly (Skene & Willard 1981a). In some PNS neurons, the homologue of GAP-24 seems to be expressed throughout adult life, whereas GAP-43 is induced only after nerve injury (Skene & Willard 1981b). Noncoordinate regulation of growth-associated proteins is particularly striking in goldfish retinal ganglion cells whose axons are deprived of their normal synaptic targets by removal of the optic tectum. Tectal removal has no effect (Benowitz et al 1983) or

only a small effect (Grafstein et al 1987) on the return of GAP-43 synthesis to pre-injury levels beginning 3–4 weeks after nerve crush. In contrast, synthesis of a 26 K protein, which is induced during regeneration and ordinarily declines 3–4 weeks post-crush, remains elevated if synaptic targets have been removed (Benowitz et al 1983). Tubulin synthesis not only fails to return to prelesion levels, but continues to rise in regenerating neurons deprived of their normal tectal targets (Grafstein et al 1987). Noncoordinate regulation of individual growth-associated proteins indicates that there are likely to be multiple signaling pathways by which events in and around an axon can influence gene expression in neuronal cell bodies. This, in turn, suggests that not all aspects of axon growth must be expressed or repressed coordinately in neurons.

Several kinds of retrogradely transported signals might regulate neuronal gene expression in response to changing conditions in the axon or its synaptic terminals. Signals might originate in synaptic target tissues or from glial cells along the length of axons. Signal molecules might be among those that are transported anterogradely into axons and then return to their own cell bodies of origin by retrograde transport (Bisby 1980, 1984); such molecules could serve as useful signals if they underwent some modification in the distal axon that reflected conditions in the axon. Whatever their source, signals regulating growth-associated gene expression might be negative—chronically repressing GAP expression in uninjured mature neurons, or positive—produced in developing or injured nerves to induce GAP expression. In rat sciatic nerves, removal of the distal nerve stump or blockade of axonal transport with colchicine does not alter the time course and magnitude of GAP-43 mRNA induction in dorsal root ganglia (G. S. Basi and J. H. P. Skene, unpublished), thus indicating that inducers produced distal to the site of nerve injury are not critical for GAP-43 induction. Co-culture of DRG neurons with potential synaptic target cells does reduce GAP-43 synthesis in vitro (Baizer & Fishman 1987), consistent with a target-derived GAP-43 repressor.

Synaptic targets do not seem to play a dominant role in GAP-43 regulation in the CNS. Removal of synaptic target neurons has no effect (Benowitz et al 1983) or has a small effect (Grafstein et al 1987) on the recovery of GAP-43 expression to pre-injury levels after optic nerve regeneraion in goldfish. In the mammalian CNS, injury of axons many millimeters away from their cell bodies does not re-induce GAP-43 expression (Skene & Willard 1981b, Kalil & Skene 1986, Reh et al 1987), despite disconnection of the axons from their synaptic targets. One possibility is that chronic repression of the GAP-43 gene by target-derived signals is replaced or supplemented in some mammalian CNS neurons by inhibitory signals derived from glial cells along the length of the axons. In

that case, axotomy far from the cell body, leaving a long segment of the surviving axon in contact with CNS glia, would leave GAP-43 synthesis repressed. Consistent with this explanation, injury of adult rat optic nerves very close to the retina has been reported to induce GAP-43 expression in retinal ganglion cells (Lozano et al 1987). The retinal cells injured close to their cell bodies also showed a far greater propensity to extend axons when provided with favorable environmental conditions in the form of a peripheral nerve graft (Vidal-Sanz et al 1987).

SUMMARY AND FUTURE PROSPECTS

Axon growth and synaptogenesis involve extensive interactions between an axon and multiple environmental cues, ranging from the availability of glial cell surface molecules extracellular matrix components (Bozycko & Horwitz 1986, Tomaselli et al 1986, Vidal-Sanz et al 1987, Schwab & Caroni 1988) to patterns of neuronal activity (Meyer 1983, Schmidt & Edwards 1983, Fawcett & O'Leary 1985, Stryker & Harris 1986). A neuron's ability to respond to any of these environmental cues, and the nature of its responses to any particular cue, depend on the neuron's expression of relevant receptors, signal transducing proteins, and the structural materials to execute a response—its "preparatory set." The evidence reviewed here indicates that expression of some of the neuronal genes involved in axon growth and/or synaptogenesis decreases sharply as neurons mature, so that extracellular cues that stimulate axon elongation in developing neurons evoke different responses by mature neurons. To borrow a term from neurophysiologists (Evarts et al 1984), the sum of these developmentally regulated genes may constitute a "preparatory set" for axon elongation or synaptic modification. Differential expression of individual growth-associated proteins might create a range of preparatory sets among different neurons. This view of axon growth regulation emphasizes the need to consider not only the "triggering stimuli" that elicit overt axon growth or synaptic remodeling, but also the regulation of internal neuronal events that represent a predisposition for certain forms of growth.

The genes for GAP-43 and a few other axonal proteins are strong candidates for individual elements of a preparatory set for axon growth and/or synaptogenesis. It is not at all clear, however, whether GAP-43 itself is critical for axon elongation or for some other steps in the growth and remodeling of synaptic connections. One important challenge is to identify the full set of genes whose expression confers on developing neurons their predisposition for axon growth and synaptic remodeling, and to determine which genes are involved in individual phases or steps

in axonal and synaptic development. A second challenge is to understand the mechanisms by which these genes become repressed during neuronal maturation and conditions under which they can be re-induced in adults.

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