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Lesion-induced synthesis and secretion of proteins by nonneuronal cells resident in frog peripheral nerve

(nerve degeneration/nerve regeneration/neuroma/Schwann cells/lipoproteins)

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ABSTRACT **Transection of a peripheral nerve results in Wallerian degeneration of the nerve segment distal to the lesion site and the initiation of axonal regeneration just proximal to it (neuroma site). Nonneuronal cells resident in peripheral nerve are suggested to play an important role in neural repair mechanisms through diffusable molecules that they synthesize and secrete. We examined the array of proteins synthesized and secreted by nonneuronal cells resident in the frog peripheral nerve, which is known for its high regenerative capacity. Nerve segments were incubated in medium containing [^{35}S]methionine, and the secreted radioactively labeled proteins were analyzed by gel electrophoresis. Nerve injury resulted in the complete down-regulation of a group of proteins synthesized and secreted by nonneuronal cells in intact nerve. At the same time, the synthesis and secretion of several proteins were up-regulated in the neuroma and degenerating nerve segments, proximal and distal to the axotomy site, respectively. Proteins secreted by the proximal segment were of apparent kDa/pI (mass/isoelectric point) values of 215/5.6, 76/6.7, 73/7.0, 44/5.2, 36.5/5.6, 35.5/6.0, and 32/6.0. Similar proteins were secreted by the degenerating distal segment but with the exception of variable reductions in the 44- and 32-kDa proteins and increases in proteins of apparent kDa/pI values of 39/5.2 and 29/7.3–7.4. Step gradient ultracentrifugation suggested that the latter two are apolipoproteins. Comparison with plasma apolipoproteins further indicated that nerve and plasma apolipoproteins differ. The up-regulation of the synthesis and secretion of these proteins concurrently with nerve degeneration and regeneration strongly imply that these molecules are involved in neuronal repair mechanisms.**

Peripheral nerve injury is followed by Wallerian degeneration of the nerve segment distal to the site of the axotomy. Nerve fibers and myelin sheaths break down and become phagocytized by proliferating Schwann cells and invading macrophages (e.g., for reviews see refs. 1 and 2). Nonneuronal cells and extracellular matrices in degenerating nerve segments then form an environment that supports the growth of regenerating axons of both peripheral and central nervous system origin (e.g., see refs. 1, 3, and 4). In contrast, degenerating central nervous system nerve segments do not support axonal growth. Indeed, they may even play an active role in inhibiting growth (e.g., see refs. 1, and 5–7).

The properties of supporting and directing growth can be mediated from a distance by degenerating peripheral nerve segments (e.g., see refs. 8 and 9). Such observations led to the search for diffusible mediator molecules synthesized and secreted by resident nonneuronal cells. Proteins identified thus far are nerve growth factor (NGF) (10), apolipoprotein E (apoE) (11–14), and apolipoprotein A-I (apo A-I) (15). NGF is a well-defined growth-promoting factor that supports the growth of sympathetic and dorsal root ganglion cells (e.g., see ref. 16). It is clear, however, that additional growth-promoting molecule(s) must be present in degenerating peripheral nerves since antibodies to NGF do not abolish their biological activity (e.g., see ref. 8). Apolipoproteins are thought to play a role in the enhanced metabolism of lipids resulting from the breakdown of myelin and reutilization of lipids during regeneration and remyelination (17, 18).

The frog peripheral nerve is known for its high regenerative capacity. We therefore examined the profiles of proteins synthesized and secreted by nonneuronal cells that reside in intact segments and in segments of nerve distal and proximal to a site of lesion. The synthesis and secretion of several proteins increased manyfold in degenerating nerve stumps and in nerve segments that contain neuromata and are located just proximal to lesion sites. At the same time and in the same nerve segments, the synthesis and secretion of several other proteins were significantly down-regulated.

METHODS

Experimental Animal. Frogs (*Rana pipiens*) were kept at room temperature and fed twice a week.

Surgical Procedures. Frogs were anesthetized by placing them in water containing 0.1% tricain methanesulfonate (Sigma). The sciatic nerve was either transected or crushed unilaterally. In nerve-transected animals, a segment of nerve 1 cm long was removed to retard regeneration. To attain long-term denervation, the lesion was repeated every 3–4 weeks.

Metabolic Labeling. In each experiment, nerve segments were removed from two to five animals. Nerve segments (2–4 cm long) were cut into small pieces (2–3 mm long) and incubated in medium (1 ml per nerve) containing [^{35}S]methionine (50–100 μCi per nerve; 1 Ci = 37 GBq) for 16–18 hr at room temperature (frog ambient temperature) under continuous gentle agitation. The medium was first adjusted to frog osmolarity (220 mosM) and pH (7.2–7.4; 10 mM Hepes buffer). After incubation, the medium was separated from the tissue by centrifugation (12,000 × *g*). Proteins released into the medium by nonneuronal cells that reside in the nerve segments were lyophilized after dialyzing the medium against 50 mM ammonium acetate at 4°C (three or four changes of $\times 10^3$ vol). In several experiments, the incubation period was reduced to 4 hr and proteins were rapidly precipitated (10% trichloroacetic acid). Electrophoretic analyses revealed the same pattern of proteins whether short or prolonged incubation times were used or rapid precipitation or prolonged dialysis was performed. Thus, electrophoretic analyses revealed authentic proteins.

Lipoprotein Isolation. This was carried out by high-density ultracentrifugation (e.g., see ref. 19). Medium was first dia-

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Abbreviations: NEPHGE, nonequilibrium pH gradient gel electrophoresis; apo, apolipoprotein; NGF, nerve growth factor.

lyzed against 50 mM sodium phosphate/140 mM sodium chloride, pH 7.6, at 4°C (three or four changes of $\times 10^3$ vol). The dialyzed sample was then adjusted to a density of 1.21 g/ml with potassium bromide and added to a centrifuge tube (total vol, 5 ml) and either overlaid by or mixed with a solution of potassium bromide (1.21 g/ml) and 2 mM EDTA. After 48–72 hr of centrifugation at 250,000 × *g* at 4°C, 1-ml fractions were collected. The top 2 ml contained the lipoprotein complexes. In some experiments, lipoproteins were enriched with lipids by incubating the medium prior to ultracentrifugation with either frog serum (1:1) or 1% Intralipid (KabiVitrum, Stockholm) for 1 hr. In the latter case, excess lipids were removed after a 30-min centrifugation at 250,000 × *g*. In several experiments the top 1 ml of solution (containing lipoprotein complexes) was added to an 11-ml centrifuge tube; overlaid by a step gradient of 1.21, 1.06, 1.02, and 1.006 g of potassium bromide per ml; and centrifuged for 48 hr at 250,000 × *g*. Then 1-ml fractions were collected and their densities were determined by weight. Similar procedures were carried out for the isolation of frog plasma lipoproteins.

Electrophoretic Analysis of Proteins. Analysis from desired fractions was carried out after proteins were either precipitated (10% trichloroacetic acid) or lyophilized. Proteins were separated by the one-dimensional SDS/PAGE, the two-dimensional steady-state SDS/PAGE, and the two-dimensional nonequilibrium pH gradient gel electrophoresis (NEPHGE) methods (20–22). In the majority of cases, 10% polyacrylamide gels were used. To determine the pH gradient after electrofocusing, the pH of 0.5-mm segments of the isoelectric focusing tubes was measured after a 2-hr incubation period in 25 mM KCl. Sigma (nonradioactive) and Amersham (^{14}C labeled, rainbow) molecular weight standards were used. Gels were dried after autoradiographic image enhancement by Ampliphy (Amersham) and were exposed on Kodak XAR-5 x-ray film.

RESULTS

In this initial study, protein synthesis was examined in lesioned sciatic nerve in the absence of nerve regeneration. The lesioned nerve was divided into three domains. A segment was obtained from where the nerve exits the spinal cord up to 5 mm from the site of axotomy, another was from the site of the axotomy to 5 mm proximal to the lesion, and another was from the degenerating nerve segment distal to the lesion. The segment just proximal to the axotomy contained the neuroma where regeneration began. A control segment of nerve was obtained from an intact sciatic nerve. Protein synthesis was examined in the control and lesioned nerve segments (2–8 weeks after nerve section) by incubating in tissue culture medium containing [^{35}S]methionine. Trichloroacetic acid-precipitable and nondialyzable radioactivity was observed in the medium, indicating that the nonneuronal cells that reside in the nerve segments synthesized and secreted proteins. The number of radioactive counts per mg of wet tissue was highest from medium conditioned by either the neuroma nerve segment just proximal to the lesion site or the degenerating nerve segment distal to it.

The proteins synthesized by the nonneuronal cells in the various nerve segments were analyzed by SDS/PAGE, and representative results from 10 experiments are shown in Fig. 1. Of the proteins synthesized and secreted by intact nerve, ≈30 were detected. The four major ones were of apparent molecular weights 86,000, 85,000, 63,000, and 46,000 (Fig. 1, lane A). These are referred to as the 86-, 85-, 63-, and 46-kDa protein species, and the same nomenclature will be used for all other identified proteins. The same results were obtained from the nerve segment closest to the spinal cord in a lesioned nerve (data not shown). Interestingly, all these proteins, as well as others, were absent in medium conditioned by segments taken immediately proximal or distal to the site of lesion (lanes B and C). Medium conditioned by the nerve segment located 5 mm proximal from the lesion site was enriched with the 215-, 76-, 73-, 44-, 36.5-, 35.5-, and 32-kDa proteins (lane B). This proximal segment contained the neuroma where axonal regeneration began. While the degenerating distal nerve segment conditioned medium contained the same proteins as the proximal segment, some were present in either reduced or very small amounts—i.e., the 44-, 36.5-, 35.5-, and 32-kDa species. The most dramatic change, however, was the appearance of the 39- and the 29-kDa proteins (lane C). Nerve lesion clearly results in the repression of the synthesis of a significant number of proteins by nonneuronal cells and the expression of new ones. Moreover, nonneuronal cells that reside in proximal and degenerating distal nerve segments differ in their response to axotomy.

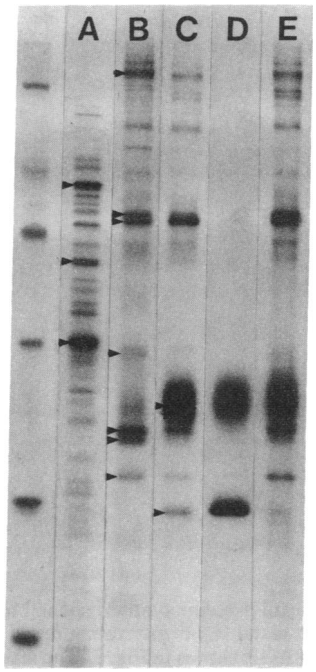

FIG. 1. Lanes A–C, the array of proteins synthesized and secreted by nonneuronal cells differ between cells residing in intact nerve segments (lane A), in nerve segments at sites of neuromata just proximal to regions where nerves were sectioned (lane B), and in degenerating nerve segments distal to sites of axotomy (lane C). Arrowheads mark protein sizes of 86/85, 63, and 46 kDa in lane A; 215, 76, 73, 44, 36.5, 35.5, and 32 kDa in lane B; and 39 and 29 kDa in lane C. Nerve segments were removed 2–8 weeks after axotomy and incubated in medium containing [^{35}S]methionine for 16–18 hr. Radioactive-labeled proteins secreted into the medium were analyzed by SDS/PAGE and visualized by autoradiography. The left lane contains radioactive-labeled molecular mass standards of 200, 92.5, 69, 46, 30, and 21.5 kDa. All experimental lanes were loaded with equal numbers of nondialyzable counts. Lanes D and E, high density ultracentrifugation of proteins synthesized and secreted by nonneuronal cells residing in degenerating nerve segments (lane C) results in two fractions. One (lane D) is composed of the 39- and 29-kDa proteins floated in the top 2 ml of the centrifuge tube, and the second (lane E) was in the bottom 3 ml of the centrifuge tube.

The conditioned medium from the three nerve segments were further analyzed by two-dimensional NEPHGE and two-dimensional steady-state PAGE. The two-dimensional NEPHGE analysis of the proteins synthesized and released by nonneuronal cells of intact nerve segments resolved ≈40 species and revealed that their pI values all fell in a narrow range between 5 and 6 (Fig. 2*A*). The two arrows in this figure indicate the positions of the 86/85- and 46-kDa proteins resolved in the one-dimensional analysis (Fig. 1, lane B). In contrast, the proteins synthesized and secreted by the resi-

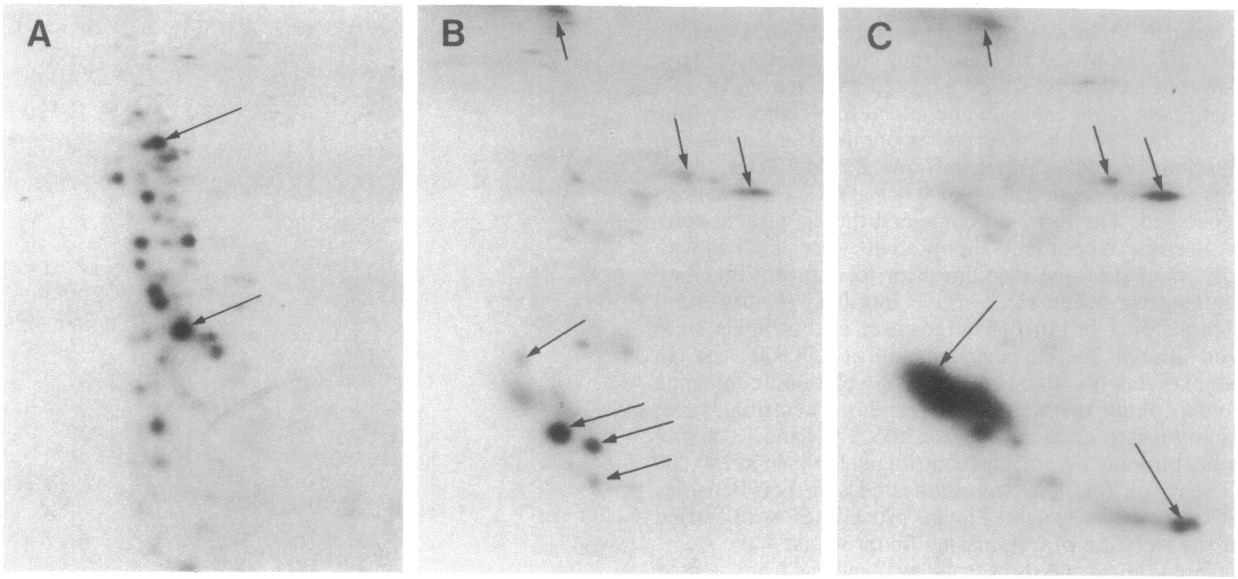

FIG. 2. The array of proteins synthesized and secreted by nonneuronal cells residing in intact nerve segments (*A*), and following nerve section in proximal (*B*) and distal (*C*) degenerating nerve segments were analyzed by two-dimensional NEPHGE. The samples are identical to those analyzed by SDS/PAGE shown in Fig. 1. The arrows mark the 86/85- and 46-kDa proteins (*A*); the 215-, 76-, 73-, 44-, 36.5-, 35.5-, and 32-kDa proteins (*B*); and the 215-, 76-, 73-, 39-, and 29-kDa proteins (*C*).

dent nonneuronal cells from the proximal and distal segments of lesioned nerve showed a much wider range of pI values (Fig. 2 *B* and *C*). Medium conditioned by the nerve segment located 5 mm proximal to the lesion site was enriched by the 215-, 76-, 73-, 44-, 36.5-, 35.5-, and 32-kDa proteins that had the respective apparent pI values of 5.6, 6.7, 7.0, 5.2, 5.6, 6.0, and 6.0 (Fig. 2*B*). The similarities and differences between the analyses of these two arrays of proteins seen in the conditioned medium from the proximal and distal nerve segments in the one-dimensional electrophoretic analysis (Fig. 1, lanes B and C) were maintained in two-dimensional NEPHGE. Thus, the 44-, 36.5-, 35.5-, and 32-kDa species were reduced in the medium from the distal compared to the proximal segment (Fig. 2 *B* and *C*). In some instances, in medium conditioned by distal degenerating segment, the 32-kDa protein consisted of two species of the same molecular mass but different isoelectric points. The 39-kDa molecule showed heterogeneity in molecular mass in the one-dimensional analyses and this extended to a heterogeneity in pI in the two-dimensional analyses. As a consequence, it showed a significant diagonal trail with two apparently major components of slightly different molecular masses and pI values of 5.2 and 5.6. The 29-kDa protein also showed trailing because of heterogeneity of both molecular mass and pI. In some experiments, this species was observed as two almost equally intense spots with pI values of 7.3 and 7.4.

Of particular interest was the steady-state analysis of the 39-kDa protein. The extension of a relatively small pH range over a relatively long distance achieved in this analysis led to the observations that the 39-kDa species has several isoforms that range in pI values from 4.9 to 5.95 (Fig. 3*B*).

Nonneuronal cells resident in degenerating nerve segments from rat sciatic nerve were previously shown to synthesize and secrete an apolipoprotein, apoE (e.g., see ref. 13). Moreover, in two-dimensional analyses, apoE showed the same characteristic trailing as the 39-kDa species described above (13). Lipoprotein complexes were therefore isolated from the medium conditioned by frog nerve segments by flotation during ultracentrifugation (e.g., see ref. 19). In nine experiments with the medium from the distal degenerating segment, the fraction of nondialyzable radioactive counts in the top fraction was 14.1% of the total radioactivity recovered. To ensure that the majority of lipoproteins were recovered, samples were enriched with lipids by incubating them with either frog serum (two experiments) or a preparation of 1% Intralipid (two experiments). Similar procedures were previously shown to bring up the yield of lipoprotein recovery to better than 95% (23). The yield of radioactive counts recovered after lipid enrichment averaged 19.4% in the four experiments. The recovery of radioactive counts incorporated into lipoprotein complexes from medium conditioned by intact nerve segments averaged 2.6% and from nerve segments proximal to sites of lesion was 2.1% (three and two experiments, respectively).

The various fractions of medium that were conditioned by degenerating nerve segments and subjected to high-density ultracentrifugation were then analyzed by one-dimensional

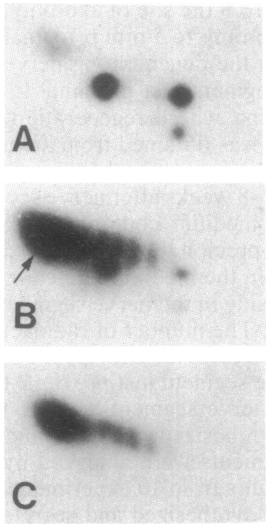

FIG. 3. The array of proteins synthesized and secreted by nonneuronal cells residing in lesioned nerve in proximal (*A*) and degenerating distal (*B*) segments were analyzed by two-dimensional steady-state PAGE. (*A*) In proximal segments, the 36.5-, 35.5-, and 32-kDa proteins are clearly observed. (*B*) In degenerating nerve, the 39-kDa protein (arrow) appears with a tail composed of several isoforms. (*C*) The 39-kDa protein was also isolated by high density ultracentrifugation from a sample identical to that seen in (*B*).

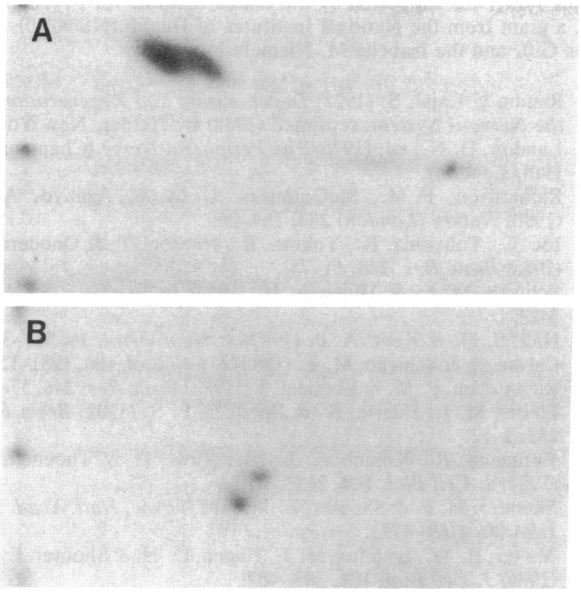

FIG. 4. Lipoproteins synthesized and released by degenerating nerve segments (*A*) differ from plasma lipoproteins (*B*). Radiolabeled nerve lipoproteins and plasma lipoproteins were isolated by high density ultracentrifugation, analyzed together on the same gels by two-dimensional NEPHGE, and visualized by fluorography and Coomassie blue stain, respectively. The molecular mass markers shown are 46, 30, and 21.5 kDa.

PAGE (Fig. 1, lanes C and D), two-dimensional steady-state PAGE (Fig. 3*C*), and two-dimensional NEPHGE (Fig. 4). Of the proteins characteristic of degenerating nerve segments, only the 39- and 29-kDa species were present in the top fraction. In two experiments, the top fraction was further subjected to a step gradient ultracentrifugation. These proteins were then found distributed in the range of high density lipoproteins (1.08–1.16 g/ml). Proteins present in the bottom fraction after high density centrifugation were also analyzed electrophoretically (Fig. 1, lane E). All the proteins present in the sample before centrifugation (lane C) were also present in this fraction with the exception of the almost complete absence of the 29-kDa species and a significant reduction in the amount of the 39-kDa species. The inability to float all the isoforms of the 39-kDa protein can be explained in either of two ways. First, the 39-kDa species could form aggregates that sediment rather than float during high density centrifugation. Second, it is possible that not all the proteins within the 39-kDa species are lipoproteins. This second possibility seems less likely in view of the fact that the analysis of the 39-kDa protein after isolation by flotation (Fig. 3*C*) shows exactly the same distribution of isoforms as it does before centrifugation (Fig. 3*B*).

The frog plasma lipoproteins were isolated by the same centrifugation procedure. Analysis by two-dimensional NEPHGE revealed two major high density plasma apolipoproteins of apparent molecular weights 28,000 and 25,000 with pI values of 6.0 and 6.5, respectively (Fig. 4*B*). Other minor bands of molecular weights 71,000, 65,000, 52,000, 40,000, 21,000, and 18,000 were also observed (data not shown). Thus, the lipoproteins involved in plasma lipid transport differ from those participating in lipid transport from peripheral nerve.

The time course for the development of observed changes in the synthesis and secretion of proteins by nonneuronal cells in the absence of regeneration was studied in three experiments in which degenerating nerve segments were removed from animals at various time intervals after nerve transection: 2–3 days; 6–7 days; and 2, 3, 4, and 8 weeks. The first appearance of the proteins characteristic of the degenerating segment was observed 6–7 days after lesion. The amounts of these proteins increased further 2 weeks after lesion and this high level was then maintained for a further 4 weeks.

DISCUSSION

Nonneuronal cells resident in intact or injured peripheral nerve segments synthesize and release [^{35}S]methionine-labeled proteins into serum-free tissue culture medium within 16–18 hr. These proteins were detected by autoradiography after analysis by one-dimensional SDS/PAGE, two-dimensional steady-state PAGE, and two-dimensional NEPHGE.

The data show that nerve injury induces changes in the synthesis and secretion of proteins by nonneuronal cells resident in peripheral nerve and that three different types of responses are observed depending on which nerve segment is examined. No changes in protein synthesis are seen after nerve injury in the segment from the spinal cord to within 5 mm of the lesion site. Significant changes occur in the segment just proximal to the lesion site, which includes the neuroma, and in the distal degenerating segment. The responses of the nonneuronal cells in these two segments are not identical.

The nonneuronal cells in intact frog sciatic nerve synthesize and secrete a considerable number of proteins. None of these proteins has been identified and their functions are unknown. It is possible that these proteins mediate interactions between the nonneuronal cells or between them and the axons they ensheath. Interestingly, whatever their functions might be in intact nerve, these functions are not required in the degenerating nerve or at the site of initial nerve regeneration (neuroma). Instead, the nonneuronal cells in the two nerve segments, immediately proximal and distal to the site of lesion, turn on a new program of protein synthesis and secretion that persists for many weeks after nerve injury in the absence of nerve regeneration. It is unlikely that proteolysis induced by enzymes from invading macrophages can entirely account for the loss of the proteins characteristic of the nonneuronal cells of intact nerve, although these enzymes probably play a role in removing the last traces of these proteins, as their synthesis is down-regulated.

The proteins whose synthesis and secretion are up-regulated by nonneuronal cells that reside in the proximal segment containing the neuroma are the 215-, 76-, 73-, 44-, 36.5-, 35.5-, and 32-kDa species. A similar array of proteins is observed from the cells resident in the degenerating segment. They differ, however, from nonneuronal cells in the proximal segment in that they secrete much lower amounts of the 44- and 32-kDa species but at the same time large amounts of the 39- and 29-kDa apolipoproteins. The biological role of the non-apolipoproteins is as yet unknown. Their possible role in the various aspects of nerve degeneration and nerve regeneration is strongly implied by the manyfold increase in their synthesis and secretion by nonneuronal cells at sites of axonal growth (neuromata) and in degenerating nerve stumps. A similar distribution was observed for the up-regulation of the synthesis of NGF following nerve lesion (10). The metabolic labeling method used in this study is not sensitive enough to detect the up-regulation in the levels of NGF that presumably also occur in the frog sciatic nerve after injury.

In the degenerating nerve segment, one or more cell types synthesize and secrete two apolipoproteins identified as the 39- and 29-kDa species, with the former being in considerable excess over the latter. In the proximal segment, where little nerve degeneration occurs but rather regeneration commences, only a small amount of the 39-kDa species appears and, apparently, none of the 29-kDa species. Thus, the signal

for the production of these two apolipoproteins lies in the degeneration process itself. In the degenerating rat sciatic nerve, apoE is produced in abundance and the invading macrophages that are activated by the lipid released from the degenerating axon are its source (24). The frog 39-kDa species shows the same heterogeneity of molecular mass and pI and the same average values for these two parameters as does rat apoE, suggesting that the 39-kDa species is frog apoE. If this is so then, as in the rat, it is likely that the 39-kDa species is synthesized and secreted by macrophages invading the nerve segment. A lack of cross-reactivity between the 39-kDa species and antibody to rat apoE has prevented confirmation of this idea (data not shown).

Although the 29-kDa lipoprotein has the same molecular mass as rat and other apoA-Is (19), it is considerably more basic than these proteins and its identification requires further work. It is likely that the heterogeneity of the 29-kDa apolipoprotein also results from different degrees of sialylation. It is of interest that two lipoproteins appear in frog sciatic nerve after injury. To the best of our knowledge, this is the only example in which two different types of apolipoproteins are synthesized simultaneously by nonneuronal cells residing in the same nerve segment. In the two other species that have been studied, only one apolipoprotein appears—either apoE in the rat (18) or apoA-I in the chicken (15). Moreover, unlike the two species described above, the two apolipoproteins that appear in frog nerve after injury are quite different from the major apolipoprotein species in frog plasma, making the point that the nervous system in the frog evolved a different set of apolipoproteins for lipid transport.

The observation that apolipoproteins are synthesized and secreted by nonneuronal cells in degenerating nerve segments was anticipated, since these molecules are known to serve as lipid carriers. As such, they are likely to play a role in myelin degradation during degeneration and lipid reutilization during regeneration and remyelinization. The present observations of the up-regulation of the synthesis and release of the 39- and 29-kDa apolipoproteins in degenerating peripheral segments, and to a lesser extent of the 39-kDa lipoprotein in the proximal segment, agree very well with this concept. In support of the view that local utilization of triglycerides can take place in nerve segments is our preliminary observation of lipoprotein lipase activity in peripheral nerve (S.R. and T. Hachek, unpublished results).

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1. Ramon y' Cajal, S. (1928) *Degeneration and Regeneration of the Nervous System*; reprinted (1968) by Hafner, New York.
2. Landon, D. N., ed. (1976) *The Peripheral Nerve* (Chapman & Hall, London).
3. Richardson, P. M., McGuinness, U. M. & Aguayo, A. J. (1980) *Nature (London)* **284,** 264–265.
4. Ide, C., Tohyama, K., Yokota, R., Nitatori, T. & Onodera S. (1983) *Brain Res.* **288,** 61–75.
5. Schwab, M. E. & Thoenen, H. (1985) *J. Neurosci.* **5,** 2415–2423.
6. Hall, S. M. & Kent, A. P. (1987) *J. Neurocytol.* **16,** 317–331.
7. Caroni, P. & Schwab, M. E. (1988) *J. Cell Biol.* **106,** 1281–1288.
8. Richardson, P. M. & Ebendal, T. (1982) *Brain Res.* **246,** 57–64.
9. Politis, M. J., Ederle, K. & Spencer, P. S. (1982) *Brain Res.* **253,** 1–12.
10. Heumann, R., Korsching, S., Bandtlow, C. & Thoenen, H. (1987) *J. Cell Biol.* **104,** 1623–1631.
11. Skene, J. H. P. & Shooter, E. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4169–4173.
12. Muller, H. W., Ignatius, M. J., Hagen, D. H. & Shooter, E. M. (1986) *J. Cell Biol.* **102,** 393–402.
13. Ignatius, M. J., Gebicke-Harter, P. J., Skene, J. H. P., Schilling, J. W., Weisgraber, K. H., Mahley, R. W. & Shooter, E. M. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 1125–1129.
14. Snipes, G. J., McGuire, C. B., Norden, J. J. & Freeman, J. A. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 1130–1134.
15. Dawson, P. A., Schechter, N. & Williams, D. L. (1986) *J. Biol. Chem.* **261,** 5681–5684.
16. Thoenen, H. & Barde, Y. A. (1980) *Physiol. Rev.* **60,** 1284–1325.
17. Ignatius, M. J., Gebicke-Harter, P. J., Pitas, R. E. & Shooter, E. M. (1987) *Prog. Brain Res.* **71,** 177–184.
18. Ignatius, M. J., Shooter, E. M., Pitas, R. E. & Mahley, R. W. (1987) *Science* **236,** 959–962.
19. Segrest, J. P. & Albers, J. J. (1986) *Methods Enzymol.* **128,** 155–189 and Table 12.
20. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
21. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250,** 4007–4021.
22. O'Farrell, P. Z., Goodman, H. M. & O'Farrell, P. H. (1977) *Cell* **12,** 1133–1142.
23. Eisenberg, S., Bilheimer, D. W. & Levy, R. I. (1972) *Biochim. Biophys. Acta* **280,** 94–104.
24. Boyles, J. K., Zoellner, C. D., Anderson, L. J., Kosik, M. L., Pitas, R. E., Weisgraber, K. H., Hui, D. Y., Mahley, R. W., Gebicke-Haerter, P. J., Ignatius, M. J. & Shooter, M. E. (1989) *J. Clin. Invest.* **83,** 1015–1031.