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MACROMOLECULAR TRANSFER FROM GLIA TO THE AXON

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SUMMARY

Axons do not contain polysomes and therefore are unable to synthesize proteins. On the other hand, the nerve cell body has a well-developed protein synthesizing capacity, and proteins are conveyed into the axon from the cell body by orthograde axonal transport. Studies on vertebrate neurones demonstrate that the proteins conveyed from the cell body constitute all of the major structures of the axon including: membranous vesicles, the endoplasmic reticulum, mitochondria, and the cytoskeleton which consists of microtubules, neurofilaments, and microfilaments. Although the nerve cell body is the major source of the macromolecules which provide the structural integrity of the axon, in some axons the glial cells surrounding the axon also supply proteins to the axon. The transfer of newly synthesized proteins from glial cells to the axon is documented most completely in the case of the squid giant axon. The transfer of proteins from glial cells to the neurone can be studied in the giant axon by incubating axons, which have been disconnected from their nerve cell bodies, in a solution which contains labelled amino acids. The glial cells transfer as much as 40% of their newly synthesized proteins to the giant axon. These transferred proteins can be studied in axoplasm which is separated from the giant axon by extrusion.

What are the comparative roles of proteins supplied to the axon by the glial cells and the nerve cell body? To begin to answer this question, we have compared the glial-transfer proteins with the proteins which constitute whole axoplasm. The proteins were analysed by two-dimensional polyacrylamide gel electrophoresis, and axoplasmic proteins were detected by staining the gels with Coomassie blue, whereas the labelled proteins were detected by fluorography of the same gels. The glial-transfer proteins differ substantially from the stained axoplasmic proteins. For example, neurofilament proteins and tubulin are major proteins of the axoplasm but are not represented among the labelled proteins which are transferred from the glial cells. In fact, these proteins are not synthesized to any significant degree by the glial cells. One of the glial-transfer proteins (molecular weight 70000) stands out because it is heavily labelled and is well resolved on the gels. This protein which we have named traversin is present among the stained proteins of whole axoplasm but is a relatively minor component of the axoplasm. In order to determine whether traversin is supplied to the axon by the nerve cell body we analyzed the proteins synthesized in the stellate ganglion which contains the nerve cell bodies of the giant axon. The pattern of proteins synthesized by the stellate ganglion was similar to that of whole axoplasm,

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but was very different from the constellation of proteins transferred from the glial cells. Traversin was not among the major labelled proteins synthesized by the ganglion and is at most a minor synthetic product of the ganglion. Because the ganglion contains glia, we can not ascertain whether the small amount of labelled traversin in the ganglion was synthesized in the glia or the nerve cell bodies. However, these results suggest that traversin is synthesized in much greater amounts by glial cells than by nerve cells. If, as our results suggest, traversin is supplied to the axon principally by the glial cells, then the proteins transferred from the glial cells are likely to be specialized in function and not simply an accessory source which supplements the proteins supplied by the nerve cell body.

Although some of the proteins transferred from the glial cells are special and are probably not supplied to the axon by the nerve cell body, we have also found proteins which are supplied by both the nerve cell body and the glial cells. One of these proteins is actin. The presence of actin in the population of proteins which are transferred from the glial cells is particularly interesting because of the known contractile function of this protein. Actin could be involved in the mechanism of protein transfer from the glial cells. The transferred proteins apparently exist in the form of a complex in the axoplasm. This complex of proteins can be separated from axoplasm because of its chromatographic behaviour of Sephadex G-200. These results suggest that the glial-transfer proteins are conveyed from the glial cell to the axon in the form of a particle which includes actin in its structure.

INTRODUCTION

Neurones and glia are sister cells arising from the same embryonic precursor, the neuroectoderm (Jacobson, 1978). This common origin brings these cells into close proximity even at the earliest stages of their development. The direct physical relationship between neurones and glia is characteristic of all organisms with discrete nervous systems. These include all of the metazoa except the sponges and possibly the coelenterates, which are in an indeterminate position with regard to the evolution of nervous systems (Bullock & Horridge, 1965). Thus, the physical relationship between neurones and glia is essentially a constant in the evolution of nervous systems. This morphological relationship between glia and neurones suggests that these two cell types are functionally interrelated. Neurones are clearly the central characters in nervous system function while the glial cells act as supporting elements for the neurones. The glial cells can be compared with the nurse cells that surround and support the developing oocytes of many species. As nurse cells, glia appear to have two primary functions. One of their roles is to isolate neurones from each other. A hallmark of this functional role in the nervous system is the fact that glial cells are universally inexcitable (Kuffler & Nicholls, 1966). This contrasts with and counterbalances the excitability of neurones. The other role of the glial cells is to support the neurone metabolically. Glia may support neurones by supplying metabolites such as glucose (Kuffler & Nicholls, 1966) or lipid precursors (Gould, Lasek & Spenser, 1978). Another particularly interesting possibility is that the glia provide macromolecules, such as proteins, to the neurone.

Although the concept that glia supply molecules to the neurone has been popular

Direct evidence for molecular transfer has been relatively difficult to obtain (Kuffler & Nicholls, 1966). Recently investigators have recognized that the axon represents a particularly favourable part of the neurone for investigating the transfer of molecules from the glial cells to the neurone. This is the case, because the elongate geometry of the axon places its cytoplasm at a great distance from the nucleated cell body. The axon does not appear to have the capacity to synthesize proteins except for the possibility of a very small subset of proteins which are synthesized within the mitochondria (Lasek, Dabrowski & Nordlander, 1973; Barondes, 1974; Gambetti *et al.* 1972). The essential absence of protein synthesis in the axon necessitates that the axonal proteins are provided by the neurone cell body or from the Schwann cells surrounding the axon. A vast amount of evidence demonstrates that the nerve cell body synthesizes axonal proteins and that these proteins are conveyed into the axon by axonal transport (Grafstein & Forman, 1980). A remarkable variety of proteins are supplied by the nerve cell body and these proteins may be sufficient to account for the supply of all of the structural elements of the axon (Willard Cowan & Vagelos, 1974; Tytell *et al.* 1981). The essential role of the cell body in the support of the axon is clearly documented by studies of explanted neurones in tissue cultures which demonstrate that neurones are capable of extending an axon without the assistance of supporting cells (Harrison, 1910; Bray, 1973).

Although the supply of proteins from the neurone cell body is sufficient for the maintenance of the axon in tissue culture the possibility remains that, in the organism, the glial cells provide proteins to the axon. One line of evidence that has been interpreted as support for the role of glia as suppliers of macromolecules to the axon comes from studies on the prolonged survival of severed giant axons in arthropods (Bittner, 1973). The studies demonstrate that certain giant axons can survive for many months after being disconnected from their cell bodies (Hoy, Bittner & Kennedy, 1967; Nordlander & Singer, 1972; Sarne, Neale & Gainer, 1976). It has been proposed that because other axons degenerate after they are disconnected from their cell bodies, the giant axons are maintained by their surrounding glial cells (Bittner, 1973; Nordlander & Singer, 1972). This argument rests on the assumptions that the proteins supplied to the axon by the nerve cell body are relatively short lived and that the axon degenerates if these proteins are not renewed. The proteins which are carried to the axon by fast axonal transport are rapidly turned over and have a lifespan which can be less than a few hours (Grafstein & Forman, 1980; Tytell *et al.* 1980). However, proteins are relatively stable molecules and many proteins in the axon (particularly those of the cytoskeleton) can have life spans measured in years (Lasek & Hoffman, 1976). In addition, the degeneration of axons after they are severed is an active process in which the Schwann cells normally participate (Nordlander & Singer, 1972). It may be that the prolonged survival of the crustacean giant axons results principally from the absence of a catabolic response by the Schwann cells (Bittner, 1973). Thus, the prolonged survival of the giant axons is not in itself sufficient evidence to implicate the glia as suppliers of proteins to the axon.

More substantial evidence in support of the supply of proteins from glial cells to the axon has been provided by radioisotopic tracer experiments in which labelled amino acids are employed as precursors. The first study of this type was carried out

by Singer & Salpeter (1966). They found that when amino acids were injected systemically into newts, radioactivity appeared in the Schwann cells before it appeared in the axons. On the basis of these results, Singer (1968) proposed that the Schwann cells synthesized proteins which were subsequently transferred to the axon. These studies were extended to the squid giant axon by Fischer and Litvak (1967) and Giuditta, Dettbarn & Brzin (1968). The squid-giant axon has the advantage that the axoplasm can be separated from the axon for subsequent chemical analysis. When labelled amino acids were applied to isolated giant axons, labelled proteins appeared in the axoplasm. Because the axons were severed from their cell bodies when the isotope was applied, these proteins must have been synthesized at the level of the axon. Although these studies rigorously demonstrated that newly synthesized proteins could appear in the axon, when it was disconnected from its nerve cell body, the location of protein synthesis was not clear. While Fischer & Litvak (1967) suggested that Schwann cells supply the proteins, Giuditta, Dettbarn & Brzin (1968) favoured the interpretation that the proteins were synthesized in the axon rather than in the glial cells.

The question – are proteins synthesized in the axon? – has been addressed by analyzing the types of RNA which are present in the axoplasm. Protein synthesis requires three forms of RNA: transfer RNA, messenger RNA,and ribosomal RNA. If protein synthesis occurs in the axon, all three forms of RNA must be present. Analyses of the RNA in axoplasm demonstrate that it consists principally of 4s RNA (Lasek *et al.* 1973; Giuditta *et al.* 1980; Koenig, 1979), and that this is functional transfer RNA (Black & Lasek, 1977). Studies of axons in the goldfish and the rat indicate that the 4s RNA is synthesized in the nerve cell body and then carried to the axon by axonal transport (Ingoglia & Tulizewski, 1976; Ingoglia, 1979; Lindquist & Ingoglia, 1979; Politis & Ingoglia, 1979; Por *et al.* 1978). A small amount of ribosomal RNA has been found in the giant axon of one species of squid (Giuditta *et al.* 1980) and in the Mauthner axons of goldfish (Koenig, 1979). The discovery that the axon contains ribosomal RNA leaves open the possibility that protein synthesis occurs in the axon. However, most cells which actively engage in protein synthesis (including the nerve cell body) have about five times more ribosomal RNA than 4s RNA (Lasek, Dabrowski & Nordlander, 1973). The axon is unusual in that more than 95% of the RNA is 4s RNA (Lasek *et al.* 1973; Giuditta *et al.* 1980; Koenig, 1979). The large proportion of transfer RNA in the axon suggests that this RNA may be involved in some process other than protein synthesis (Black & Lasek, 1977). The relative lack of ribosomal RNA in the axoplasm reduces the possibility that active proteins synthesis occurs in the axoplasm. Furthermore, no direct evidence for protein synthesis in the axon has been obtained; instead, the available evidence indicates that when radioactive precursors are applied locally to the squid giant axon, essentially all of the radioactivity which is incorporated into axoplasmic proteins results from protein synthesis in the Schwann cells and subsequent transfer of these labelled proteins to the axon (Lasek *et al.* 1977; Gainer *et al.* 1977).

EVIDENCE SUPPORTING THE GLIA-NEURONE PROTEIN TRANSFER HYPOTHESIS

The hypothesis that proteins are transferred from the glial cell to the axon is supported principally by experiments on the squid giant axon. The isolated squid giant fibre represents a particularly favourable preparation for these studies because 20–40% of the labelled proteins synthesized by the glia appear in the axoplasm (Lasek, Gainer & Przybylski, 1974). This provides sufficient quantities of radioactively labelled axoplasmic proteins for biochemical analysis (Lasek *et al.* 1974). Axoplasm is easily separated from the giant fibre by simply cutting the fibre at one end and extruding the axoplasm in a manner that can be compared with squeezing tooth paste from a tube. The axoplasm can be extruded without any contamination from the surrounding sheath. However, a small amount of axoplasm remains behind with the plasma membrane. This subaxolemmal rim of axoplasm appears to be firmly attached to the plasma membrane, so that, when the axoplasm is extruded it shears along a plane which is about 5 μm deep to the plasma membrane. Thus, the axoplasm can be removed without contamination from the elements of the sheath, but the sheath contains about 1% of the total axoplasm and the axolemma.

Several lines of evidence support the hypothesis that proteins are transferred from adaxonal glia to the giant axon. First, the Schwann cells surrounding the axon have the cytological characteristics of cells which secrete proteins. The Schwann cells contain large numbers of polysomes and a relatively dense cytoplasm (Hodge & Adelman, 1980). Autoradiographic analysis of the giant fibre demonstrate that the Schwann cells actively incorporate amino acids into protein (Lasek *et al.* 1973). These observations indicate that the Schwann cells are a possible source of the labelled proteins which occur in the axoplasm. The strongest support for the hypothesis that the labelled proteins which appear in the axon are supplied by the Schwann cells is provided by the following evidence. Axoplasm removed from the giant axon is incapable of incorporating any significant amount of labelled amino acids into protein (Fischer & Litvak, 1967; Lasek *et al.* 1977). It might be suggested that the axoplasm is damaged by removal from the giant fibre. This seems unlikely because the isolated axoplasm is capable of carrying out complex activities (Larrabee & Brinley, 1968). For example, axonal transport has been observed in axoplasm removed from the squid giant axon and it can continue for periods of hours. (Brady, Lasek & Allen, 1981).

Studies on squid giant axons perfused with artificial buffers provide added evidence that the newly synthesized proteins which appear in the axon are not synthesized in the axoplasm (Gainer *et al.* 1977). When the axon is perfused with artificial buffers and labelled amino acids are provided in the solution surrounding the axon, labelled proteins appear in the perfusate. The appearance of labelled proteins follows linear kinetics over a period of 8 h. During this 8 h period, it is probable that most of the soluble factors, including those required for protein synthesis, are leached out of the axon by the substantial volume of fluid which passes through the axon. Yet the appearance of labelled proteins in the axon continued linearly through this period. Perfusion also permits the introduction of protein synthesis inhibitors directly into the axon. Ribonuclease is a protein synthesis inhibitor which does not readily cross the plasma membrane because it is a protein. Ribonuclease interferes with protein syn-

thesis by degrading all of the major species of RNA. Perfusion of the axon with solution containing 1 mg/ml ribonuclease had no effect on the appearance of newly synthesized proteins in the axoplasm (Gainer *et al.* 1977). This result effectively eliminates the possibility that endogenous protein synthesis contributes to the newly synthesized proteins which appear in the giant axon.

The evidence supporting the glia–neurone protein transfer hypothesis, which has been obtained by studying the squid giant axon, does not constitute a definitive proof of this hypothesis. However, the evidence is completely consistent with the hypothesis and the only alternative, that the proteins are synthesized in the axon, has been effectively eliminated. Thus, until evidence is put forth which challenges this hypothesis, it can be considered the best hypothesis to explain the appearance of large amounts of labelled proteins in giant axons which have been severed from their cell bodies. Many questions arise about the transfer of proteins between the glial cells and the axon. For example, what is the mechanism by which these proteins are conveyed from one cell to another? Another issue concerns the role of these proteins in the axon. One way to obtain information that can shed light on these questions is to determine the composition of the transferred proteins. Recent advances in electrophoretic methods, in particular two-dimensional gel electrophoresis (2D-PAGE) (O'Farrell, 1975), permits a high-resolution analysis of radioactive proteins in the squid giant axon. We have undertaken such an analysis of the labelled proteins in the squid giant axon and compared them with the proteins of the sheath.

MATERIALS AND METHODS

Squid with mantle lengths ranging between 15 and 25 cm were maintained in a sea-water tank and used within 48 h of capture. The paired giant fibres associated with the stellar nerves were carefully dissected from squid which had been killed by decapitation. The fibres were tied at each end, removed from the animal and any remaining small fibres removed over a distance of 3 cm from the proximal ligature. We cleaned only 3 cm of the axon because it is difficult to routinely clean more distal regions of the giant fibre without damaging it. Care was taken not to pull off any of the small branches emanating from the giant axon. Holes in the axon can be detected by the presence of white spots in the axoplasm, apparently due to entry of Ca^{2+} into the axons. Axons with large white spots were discarded. The temperature during the dissection and subsequent incubation was 17–20 °C.

The axons were incubated in a trough containing 0·3 ml filtered sea water. The troughs were constructed from cleaned microscope slides on which an elliptical barrier was formed with petroleum jelly. Evaporation was minimized by placing the trough in a petri dish with a piece of moistened tissue paper. Incubation media consisted of Millipore-filtered sea water (Millipore Corp., Boston, Mass) to which L[4,5-^{3}H]leucine (30–50 Ci/m-mol) was added by rapidly drying the amino acid in a test tube under vacuum and adding filtered sea water. The incubations were terminated after 4 h by immersing and giant fibre in a large volume of filtered sea water at 4 °C. Excess seawater was removed from the giant fibre by touching it to a microscope slide several times. Axoplasm was extruded with a length of polyethylene tubing from the proximal 3 cm of fibre measured on a rule under a microscope. Care w

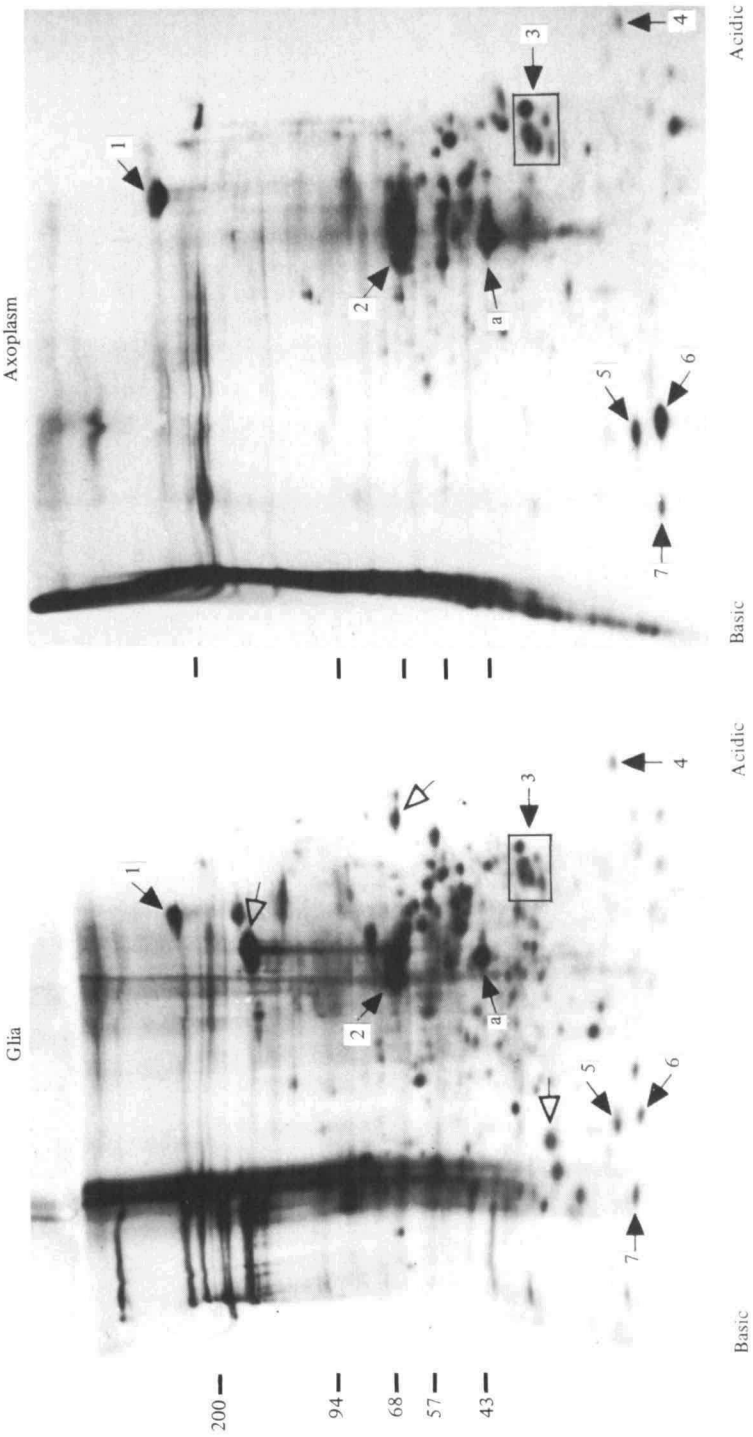

Fig. 1. Fluorographs of 2-dimensional polyacrylamide gels of labelled polypeptides made in the glial cells (left) and those transferred from the glia to the axoplasm (right). The approximate molecular weights ($\times 10^{-3}$) are indicated along the left edge of each gel and the pH gradient was from about 6·8 at the basic end to about 4·3 at the acidic end. Several transferred polypeptides are illustrated by the numbered arrowheads pointing to identical spots in the two fluorographs. Arrowhead A indicates the spot corresponding to actin in each fluorograph. The open arrows in the fluorograph of the glial cells indicate examples of glial polypeptides that are not transferred in detectable amounts and thus are not present in the fluorograph of axoplasm.

taken not to contaminate the axoplasm with material which moves along the surface of the sheath in association with the polyethylene tubing. The axoplasm was quickly drawn up into a capillary tube and transferred to a homogenizer (4 °C) containing 0·2 ml of BUST (2% 2-mercaptoethanol, 8 M urea 1% sodium dodecyl sulphate, 0·2 M Tris HCl, pH 7·3).

Two-dimensional gel electrophoresis was performed by the method of O'Farrell (1975). Samples in BUST were diluted with 1·5 vols of high NP-40 lysis buffer which contained 8% Nonidet P-40 (Particle Data Laboratories), 1·6% pH 5–7 ampholines, 0·4% pH 3–10 ampholines (all ampholines were obtained from LKB Instruments Inc.), 5% 2-mercaptoethanol, 0·9 M urea. Samples could then be run in isoelectric focusing in the first dimension and SDS gel electrophoresis on 4–17·5% gradient slab gels with 4% stacking gels in the second dimension. Proteins were visualized by staining with 0·1% Coomassie blue in 35% methanol and 7% acetic acid. The radioactive peptides were visualized by fluorography according to the method of Bonner & Laskey (1974). Gels were prepared for fluorography by dehydration in dimethyl sulfoxide, impregnation with 2,5-diphenyloxazole (New England Nuclear), and vacuum drying on to filter paper. The fluorographs were exposed at −70 °C.

RESULTS

Fig. 1 compares the labelled proteins which appear in the axon (subsequently referred to as the transferred proteins) and those which remain associated with the sheath. The two-dimensional polyacrylamide gels (2D-PAGE) separate the proteins both on the basis of charge and molecular weight. The first dimension, which runs across the gels, involves isoelectric focusing and separates proteins on the basis of their charge. The isoelectric gradient in Fig. 1 runs from approximately pH 6·8 to 4·3 and therefore displays those proteins which are neutral or acidic in their isoelectric point. Most cytoplasmic proteins fall into this category. In the second dimension the proteins are denatured with SDS so that they run in accordance with their molecular weight.

The pattern of transferred proteins is rather complex, confirming earlier observations with one-dimensional PAGE (Lasek *et al.*, 1977). The transferred proteins consist of about a dozen major labelled proteins and more than 50 other more lightly labelled proteins. Comparison of the transferred proteins with those in the sheath reveals that essentially all of the transferred proteins are also present in the sheath. The pattern in the sheath is more complex than that of the axoplasm and a number of heavily labelled proteins of the sheath do not appear in the axoplasm. Several proteins that do not appear in the axoplasm are noted in Fig. 1 by open arrows. These results do not appear in the axoplasm are noted in Fig. 1 by open arrows. These results demonstrate that the transfer of proteins from the glial cells to the axon is a selective process. The selectivity of the transfer process is further indicated by comparison of the labelling intensity of the proteins in the sheath and axoplasm. Protein number 2 stands out particularly. This protein, which has a molecular weight of approximately 70000, is one of the most heavily labelled proteins in the axon. However, in the sheath this protein contains only a small fraction of the radioactivity.

Two of the glial transferred proteins correspond to previously characterized proteins. One of these is the protein designated *a*, which corresponds in its electrophoretic behaviour with squid actin. The other protein, labelled 1, corresponds to a major stained protein in the axoplasm. Protein 1 resembles the cytoskeletal protein fodrin (Levine & Willard, 1981) in its electrophoretic behaviour (Tytell & Lasek, unpublished observation). However, further work will be required to rigorously demonstrate that this protein is fodrin. Protein 1 and *a* correspond to axoplasmic proteins, which can be detected on gels by staining with Coomassie blue. However, most of the labelled proteins cannot be detected in the axoplasm by staining with Coomassie blue (Tytell & Lasek, 1980). This observation indicates that the transferred proteins do not correspond to the major structural proteins that constitute axoplasm. Instead, most transferred proteins are minor constituents of the axoplasm, which are revealed because of their rapid synthesis and labelling to a high specific activity. In fact, some of the major proteins of the axoplasm are not detected among the synthetic products of the sheath. Therefore, these proteins are not supplied to the axon by the glial cells. For example, neurofilament proteins are major constituents of the axoplasm (Lasek, Krishnan & Kaiserman-Abramof, 1979); however, these proteins could not be found among the labelled proteins of the sheath, even when the fluorographs were exposed for extended periods. This observation indicates that the neurofilament proteins are not synthesized by the glial cells and is consistent with studies which indicate that neurofilament proteins are synthesized specifically by nerve cells.

DISCUSSION

The 2D-PAGE method permits a rigorous comparison of the transferred proteins in the axoplasm with those which remain in the sheath. Our results demonstrate that many of the proteins synthesized in the sheath cells do not appear in the axoplasm. However, essentially all of the proteins which appear in the axoplasm can be found in the sheath. This observation clearly demonstrates that transfer of proteins from the glial cells to the axon is a selective process. The specificity of this process is further illustrated by comparison of the relative labelling of the transferred proteins in the axon with their counterparts in the sheath. A number of the transferred proteins contain a disproportionately large amount of the radioactivity. Protein 2 in Fig. 1 is particularly noteworthy. Protein 2 is the most heavily labelled protein in the axoplasm. In contrast, protein 2 is a relatively minor component among the proteins found in the sheath. These results can be explained if protein 2 is rapidly transferred from the glial cells to the axon so that very little accumulates in the sheath. This possibility is consistent with the observation that the interval between synthesis of the proteins in the sheath and their appearance in the axon is relatively short, occurring within a few minutes (Lasek *et al.* 1977). Another possible explanation is that this protein is processed from a larger precursor protein. Protein 2 might be synthesized as a larger precursor and be processed either in the glial cells or the axon. Our studies have not identified such a precursor–product relationship; however, further analysis is required to distinguish these possibilities. In either case, protein 2 undergoes a selective process that results in its appearance as the most heavily labelled protein in the axon. Because

of the selective movement of this protein from the glial cells to the axon, we have named it 'traversin'.

Our preliminary studies suggest that traversin and a number of the other transferred proteins are supplied to the axon principally by the glial cells rather than by axonal transport from the nerve cell body. Although we have not been able to directly study the axonal transport of radioactive proteins in the squid giant axon, we have analysed the proteins synthesized in the stellate ganglion, which contains the cell bodies of the giant axon. Analyses of the labelled proteins from the ganglion by 2D-PAGE demonstrate that rraversin is a relatively minor component of these labelled proteins (Tytell & Lasek, 1980). This observation suggests that traversin is not among the major constituents supplied to the axon by the nerve cell body.

By determining the relative contribution that the nerve cell body and the glial cells make to the axon, we can gain a greater understanding of the possible roles of the glial transferred proteins in axonal function. For example, actin is supplied to the axon by the nerve cell body (Black & Lasek, 1979; Willard *et al.* 1974) and our studies demonstrate that actin is also supplied by the glial cells. Actin is transported into the axon in the slow components of axonal transport, specifically in the transport component that is designated SCb (Black & Lasek, 1979) or group IV (Willard *et al.* 1979). Actin is a cytoskeletal protein and is associated with the microfilament complex and axoplasmic matrix. Actin is involved in muscle contraction and is important in intracellular transport within the neurone. For example, actin has been implicated in the process of fast axonal transport (Isenberg, Schubert & Kreutzberg, 1980). The supply of actin to the axon by both the cell body and the glial cells raises the following possibilities. Actin which is transferred from the glial cells to the axon may provide a supplemental source to that which is supplied by the cell body. Another possibility is that the actin transferred from glial cells remains specifically associated with the transferred proteins and that its principal role is in the motile mechanism conveying the transferred proteins from the glial cells into the axon. That is, glial-transferred actin may not exchange with actin supplied to the axon by the cell body.

Subcellular fractionation studies of the axoplasm support this possibility. When the glial-transferred proteins are subjected to centrifugation on a sucrose gradient, more than half of the labelled proteins sediment as if they were complexed together in the form of a particle (Tytell & Lasek, 1981). These observations indicate that the glial-transferred proteins represent a special class of proteins that differs from those that constitute the bulk of axoplasm. We propose that the glial-transferred proteins play a special role in the neurone and that they do not simply supplement the proteins supplied by the nerve cell body.

MECHANISM OF TRANSFER FROM THE GLIAL CELLS TO THE AXON

One of the fundamental issues raised by the observation that newly synthesized proteins are transferred from the glial cell to the axon concerns the mechanism of macromolecular transfer between these cells. In Fig. 2 we list four of the most plausible models that could account for the movement of proteins between cells. Each of these models is based on a mechanism proposed in the literature for the

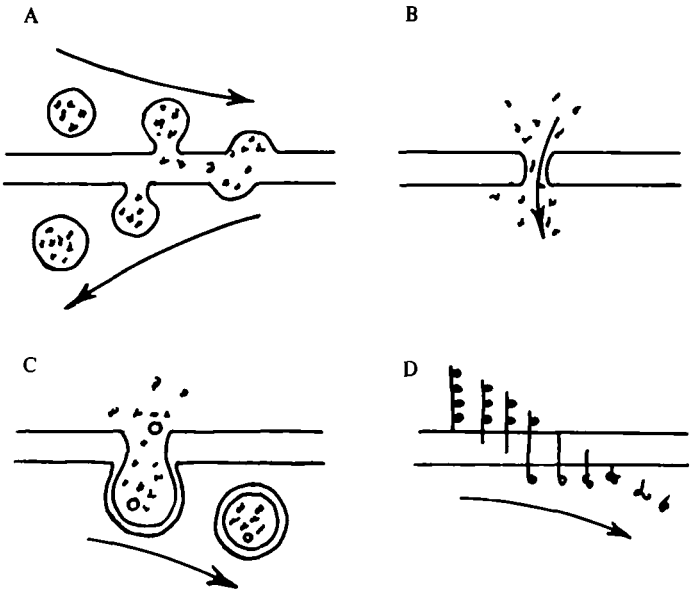

Fig. 2. Illustrates four possible mechanisms for the transfer of proteins between cells: (A) coupled exocytosis–pinocytosis; (B) direct communication; (C) modified phagocytosis and (D) protein translocation.

movement of materials between cells or across membranes. In the following we review the evidence for and against each of these models.

The first model, coupled exocytosis and pinocytosis, is a demonstrated mechanism for the selective movement of materials between cells. This is illustrated in the case of secretory proteins such as yolk proteins, which are secreted by exocytosis from hepatocytes and then carried in the blood stream to the ovary, where they are sequestered into oocytes by pinocytosis. This is just one of a large number of examples of intercellular transfer of macromolecules by exocytosis and pinocytosis.

The giant axon is capable of pinocytosis. If radioactive albumin is placed in the media surrounding the giant axon it rapidly appears in the axoplasm by an energy-dependent process probably involving pinocytosis (Giuditta, D'Udine & Pepe, 1971; Gainer *et al.* 1977). The uptake of exogenous molecules such as albumin into the axon differs from the transfer of protein from the glial cells, in that the uptake of albumin levelled off after 1 h. Suggesting that with albumin some aspect of the process rapidly saturates. By contrast the uptake of glial transfer proteins continues linearly for more than 4 h (Gainer *et al.* 1977). A difficulty with the exocytosis–pinocytosis model is that it does not readily account for the transfer of actin. Actin is a cytoskeletal protein and is localized in the non-membranous cytoplasmic compartment of the cell. There is no evidence that actin enters the membranous compartment such as the SER so that it can be incorporated into secretory vesicles.

On the other hand, both the mechanisms of direct communication and modified phagocytosis could account for the transfer of proteins such as actin into the axon. In these cases cytoplasm enters the receiving cell. An example of direct communication is pores connecting the glial cells with the axon that have been noted in crayfish

ant axons (Perachia, 1981). These structures have not been noted in other axons and there is the reservation that similar structures can be produced by glutaraldehyde fixation. Although this is an interesting model, direct evidence for it in the squid giant axon is lacking. Morphological profiles at the axo-glial boundary have been observed that are consistent with modified phagocytosis (Henkart, 1975). Fingers of the glial cell protrude into the axon where they are enwrapped by the axolemma (Henkart, 1975). The detachment of one of these glial extrusions to form a double-walled vesicle has not been reported; however, double-walled vesicles can be found in the giant axon. Modified phagocytosis can explain a number of properties of the glial-transfer process, including the observation that many of the glial-transferred proteins are apparently in a particulate form in the axoplasm (Tytell & Lasek, 1981)

The final model, protein translocation, is based upon the signal hypothesis, which holds that secretory proteins have a hydrophobic lead sequence which permits the passage of proteins into the endoplasmic reticulum (Blobel & Dobberstein, 1975). This mechanism applies to proteins crossing the membrane immediately after they are synthesized on the rough endoplasmic reticulum and proteins crossing the membrane after synthesis. Specialized contacts have been observed between the glial axolemmal interface, where the membranes of the cells are in close opposition. These could represent regions specialized for communication between the cells (Villegas, 1969; Villegas & Villegas, 1976). Although this is an interesting possibility, this mechanism has not been implicated in the extrusion of proteins through the plasma membrane. Furthermore, it seems unlikely that this mechanism can account for the transfer of the large variety of proteins across the membrane, including actin, which does not appear to be a candidate for membrane translocation.

At present these models are valuable because of their heuristic value. More information will be required before any of them can be eliminated. If we had to choose between these models, either modified phagocytosis or direct communication most effectively explain the available data. Both of these models can explain the entry of actin into the axon. A significant fraction of the glial-transferred proteins behave as if they are particulate when subjected to centrifugation. Modified phagocytosis would produce a particle. However, in the case of direct communication the proteins would have to be conveyed in a vesicle and/or complexed together by intermolecular bonds.

It will be of interest to find out how the proteins are transferred from the glial cells because such information can lead to the identity of the particular cytoplasmic compartment from which these proteins are delivered by the glial cell. The possible role of these proteins will be different if the proteins come from the golgi complex and smooth endoplasmic reticulum than if they are all cytoplasmic such as actin.

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