Local Gene Expression in Axons and Nerve Endings: The Glia-Neuron Unit

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Giuditta A, Chun JT, Eyman M, Cefaliello C, Bruno AP, Crispino M. Local Gene Expression in Axons and Nerve Endings: The Glia-Neuron Unit. *Physiol Rev* 88: 515–555, 2008; doi:10.1152/physrev.00051.2006.—Neurons have complex and often extensively elongated processes. This unique cell morphology raises the problem of how remote neuronal territories are replenished with proteins. For a long time, axonal and presynaptic proteins were thought to be exclusively synthesized in the cell body, which delivered them to peripheral sites by axoplasmic transport. Despite this early belief, protein has been shown to be synthesized in axons and nerve terminals, substantially alleviating the trophic burden of the perikaryon. This observation raised the question of the cellular origin of the peripheral RNAs involved in protein synthesis. The synthesis of these RNAs was initially attributed to the neuron soma almost by default. However, experimental data and theoretical considerations support the alternative view that axonal and presynaptic RNAs are also transcribed in the flanking glial cells and transferred to the axon domain of mature neurons. Altogether, these data suggest that axons and nerve terminals are served by a distinct gene expression system largely independent of the neuron cell body. Such a local system would allow the neuron periphery to respond promptly to environmental stimuli. This view has the theoretical merit of extending to axons and nerve terminals the marginalized concept of a glial supply of RNA (and protein) to the neuron cell body. Most long-term plastic changes requiring de novo gene expression occur in these domains, notably in presynaptic endings, despite their intrinsic lack of transcriptional capacity. This review enlightens novel perspectives on the biology and pathobiology of the neuron by critically reviewing these issues.
I. INTRODUCTION

Neurons relay signals among different regions of the body that are often distant from each other. Accordingly, to perform their function, many neurons need to grow long and branching processes. During the course of phylogenetic evolution, this led to the formation of nerve cells whose cytoplasm resides largely in the axon domain, at distances up to meters away from the nucleus. As a result, the combined mass of cell processes markedly exceeds that of the cell body in many neuronal types and many animal species (Fig. 1). Accordingly, neurons account for half the mammalian brain mass, although their number is only a small fraction of the number of glial cells. The markedly imbalanced cytoplasmic distribution between soma and axon domain could not be without consequence on the trophic support of the axonal periphery. How could a cell body metabolically sustain an axon comprising more than 100-fold its mass? How could a perikaryon synthesize all the axonal, presynaptic, and dendritic proteins in addition to its own? How could it deliver them to the right place at the right time? These considerations may not have been in the mind of the scientists formulating the neuron doctrine (17).

A key problem of cell biology was thus set forth from the very beginning in terms of two opposing points of view, two sides of the same coin. One of them centered on the axon’s trophic dependence on the cell body. The evidence for such dependence was so compelling that for a long time it obscured the other horn of the dilemma, that is, the limited capacity of the neuron soma to satisfy the trophic needs of the increasing axonal mass (315). As a result, the cell body was assigned the role of the only trophic center of the neuron, most people not realizing that such a view was strongly biased towards one side of a coin. But the other side did in fact exist, as “not to see belongs to the observer, while not to exist belongs to the object” (4). In fact, with time, the initial view started to be challenged by theoretical considerations and by experimental evidence, and the biological issue moved towards a more balanced assessment.

This review is an attempt to place in perspective the historical roots and branches of the main features of the local gene expression in the axon domain. We will critically describe early and recent evidence, including the prevailing views and counter criticisms. In our opinion, this approach will provide a more balanced and realistic perspective that is likely to lead to a better understanding of the biology of neurons and glia. The novel concept prompted by available data suggests that the trophic capacity of the neuron soma may satisfy the needs of its cytoplasmic mass only up to a certain limit depending on the ratio of the perikaryal trophic capacity to the cytoplasmic mass and needs. Beyond this limit, which is often exceeded in many mature neuronal types such as vertebrate sensory and motor neurons, the trophic needs of the axon domain must be supplemented by periaxonal and perisynaptic glial cells. Our discussion will be more focused on the latter issue, that is, on the glial synthesis and delivery of transcripts (as well as proteins and other cellular components) to the translation system of axonal sites. Accordingly, data regarding neurons with a limited axon domain, such as developing or cultured nerve cells, will be mentioned only within the scope of the review. Likewise, we will only briefly discuss the growing literature regarding protein synthesis in dendrites (for reviews, see Refs. 141, 222, 276, 291).

The validity of this view depends on the following experimental evidence: 1) that protein synthesis occurs in axons and nerve terminals, 2) that axonal and presynaptic RNAs are synthesized in periaxonal and perisynaptic glial cells.

![Fig. 1. Schematic representation of an adult vertebrate neuron whose axon corresponds to a length of ~1 cm. Several neurons have axons longer than 1 m and may form thousands of synapses in their terminal arborization. [Modified from D’Angelo and Peres (64).] (This image is not present in the text, but it is mentioned.)](http://physrev.physiology.org/)
II. ORIGIN OF AXONAL AND PRESYNAPTIC PROTEINS: THE CELL BODY HYPOTHESIS

One of the main experimental bases of the neuron doctrine (17) regarded the rapid degeneration of mammalian nerves disconnected from their cell bodies. As degenerating axonal segments could be selectively stained, this feature was widely exploited to identify the connections among brain centers, thereby contributing to the general acceptance of the unique trophic role of neuronal perikarya. In those early times, the persisting survival of centrally disconnected axons from crustacean nerves (30, 147) and from a mouse mutant (212) was not known.

The concept of the exclusive trophic role of the neuron soma gained a clearer meaning with the formulation of the basic dogma of molecular biology. The identification of DNA as the undisputed genetic repository of protein primary structure, and of mRNA as its molecular envoy, paved the way to the belief that neuron somas were uniquely responsible for the synthesis of all axonal and presynaptic proteins. The main foundation of this hypothesis rested on the visualization of the Nissl substance in nerve cell bodies and large dendrites (87) but not in axons. The Nissl substance was later identified as a complex of riboliponucleoproteins using the newly developed cytoelectrophoretic method (for a review, see Ref. 45), and eventually as the ordered aggregate of free and membrane-bound polysomes by EM analyses (248). These key discoveries confirmed the high rate of protein synthesis of the neuron cell body, and further supported the view that axons were lacking protein-synthesizing machinery, inasmuch as they lacked Nissl bodies. Curiously enough, an impressive number of authors referred to the Palay and Palade paper as demonstrating the lack of axonal ribosomes. The presumed lack of axonal ribosomes was one of the criteria used to discriminate a central nervous system (CNS) axon from a dendrite. Obviously, such an assumption prevented the identification of axonal ribosomes. The presumed lack of axonal ribosomes remained for a long time the single most important reason to object to the existence of axonal protein synthesis. As a corollary, rRNA and mRNA were also assumed to be absent from the axon domain. Indeed, when axoplasmic 4S RNA was identified by its electrophoretic mobility in squid (Loligo pealii) and Mixycola (a nematelmith) giant axons (31, 190), the presence of minor amounts of RNA was overlooked. As a result, the widely publicized presence of only 4S RNA in axons became an additional strong reason to believe in their incapacity to synthesize proteins.

This established opinion was substantially strengthened by the demonstration that axoplasmic organelles and matrix were slowly moving distally, as shown by their massive accumulation on the proximal side of nerve constrictions (325). These key observations stirred a flurry of experiments aimed at the characterization of what was known as axoplasmic flow or transport (125). Of the main features of this complex process, we will only note its gross distinction in two main rate groups, ranging from a few millimeters per day (slow axoplasmic transport; Ref. 32) to several hundred millimeters per day (fast axoplasmic transport). Cytoskeletal and cytosolic proteins were believed to reach the axon by the slow flow, while smooth vesicles, mitochondria, and other cell organelles targeting nerve endings traveled with the fast flow (125). While the mechanism of the fast transport was elucidated in detail (142, 266), the interpretation of the experimental data for the slow axoplasmic flow remained controversial (8). In the experiments using radiolabeled protein precursor, the slow anterograde flow of radioactivity was initially attributed to the movement of newly synthesized somatic proteins. This interpretation was based on the unproven assumption that axons lacked the capacity to synthesize proteins. However, after the demonstration of an axonal system of protein synthesis (7, 120, 183, 262, 306), these data could be explained in a radically different scenario, involving 1) the incorporation of radiolabeled amino acids into the proteins of nerve cell bodies and initial segments of the axon, 2) the release of radiolabeled amino acids from the degraded axonal proteins, and 3) the distal diffusion and reincorporation of the radiolabeled amino acids into the proteins of more distal axon segments. This explanation was a better fit for the progressive changes in the distribution of radiolabeled axonal proteins as the radioactive wave became wider and flatter with time and kept losing most of its initial radioactivity. For an extensive discussion of this alternative explanation, see Alvarez et al. (7).
The dogma of an exclusive somatic origin of axonal and presynaptic proteins was also weakened by two theoretical objections. One of them regarded the survival of slowly transported axonal proteins, notably cytoskeletal proteins, which would have required months or even years to reach their distal-most targets. These expected delays strongly clashed with the half-life of the transported proteins, which was not longer than a few days. As a result, these proteins were bound to disappear from the axon according to a proximo-distal gradient approaching zero at relatively short distances from the neuron soma. Such an expectation was clearly at odds with all available evidence. The attempt to bypass this objection by the ad hoc assumption that slowly transported proteins were selectively endowed with longer half-lives was proven untenable by the demonstration that their average half-life was not different from the accepted values.

The second objection concerned the rapidly transported presynaptic proteins. If they originated exclusively from the cell body, their participation in plastic events would require short delivery times and a two-way signaling system operating between nerve terminals and neuron soma. In view of the impressive terminal branching of axons and of the thousands of its diverging synaptic connections, any presynaptic ending would need to instruct the cell body to synthesize and send the set of proteins it required, and concurrently provide the spatial coordinates required for a correct delivery. In turn, proteins synthesized by the cell body would likewise be expected to contain enough information to correctly guide them through the multiple axonal branches they needed to cross before reaching their targets.

Neither set of signals has been proven so far, and signals of such complexity may hardly be compatible with our present view of the neuron. Nonetheless, in an ingenious attempt to solve this problem, it was postulated that activated synaptic terminals might be tagged by an unspecified local process not requiring protein synthesis. Tagging would eventually allow the selective capture of appropriate proteins synthesized by the cell body and delivered to the entire neuronal cytoplasm. Such a mechanism has been proposed to explain long-term synaptic changes in activated synapses of Aplysia sensory neurons in culture. Although plausible, this hypothesis has not received convincing experimental support in other animal models. In addition, it does not seem to be readily reconciled with the relatively frequent modifications of synaptic strength, nor with the possible concurrent requirement of different protein sets by different nerve endings of the same neuron. Tagging would also impose a heavy metabolic load onto the synthetic capacity of neuronal perikarya.

These two substantial objections may be readily resolved by the alternative possibility that, in well-differentiated neurons, local systems of gene expression are distributed throughout the axon domain. In this model, cells synthesize and transcellularly deliver coding and modulating transcripts to axons and nerve terminals where these transcripts are eventually translated into proteins. Compared with the previous view, this mechanism appears well tuned, efficient, and highly selective in the spatial and temporal supply of the required gene products. It is also much less demanding on neuron metabolism. The experimental evidence supporting such a model is described in the following sections.

However, it bears emphasis that this conceptual framework does not exclude the role of the neuron cell body in the modulation of local translation processes. Several considerations suggest the contrary. From a general perspective, the correlation between cell body size and the overall mass of neuronal processes may be taken to reflect such involvement. It should also be noted that the fast delivery of soma-derived proteins and organelles to axons and nerve terminals might contribute key controlling factors to local translation processes. Whether soma-imported proteins are directly involved in the regulation of local protein synthesis is yet to be established. However, there has also been more direct evidence for the transfer of cell body RNA to the axon: 1) the targeting of soma-derived BCI RNA to the axonal translation sites (periaxoplasmic plaques) of the goldfish Mauthner axon, and 2) the colocalization of β-actin mRNA and its binding protein ZBP1 to the periauxoplasmic plaques of goldfish and mammalian axons. The targeting of soma-derived RNA-containing vaults to the Torpedo nerve terminals, and the demonstration that, in well-differentiated Aplysia neurons, the synapse-specific long-term facilitation induced at a distal synapse requires axoplasmic transport and somatic protein synthesis in addition to local protein synthesis.

On the other hand, earlier experiments designed to verify the cell body origin of axonal RNAs using radiolabeled precursors consistently yielded controversial results. More recently, RNA granules were identified and characterized in neurons (reviewed in Ref. 171). The cell body origin of the granules localized in the axon domain was demonstrated in cultured or developing neurites but not in mature axons. In fully differentiated neurons, soma-derived RNA granules appear to be exclusively delivered to dendrites. The topic of the cell body origin of axonal and presynaptic RNAs will be discussed in more detail in section v.

III. LOCAL PROTEIN SYNTHESIS IN AXONS

A major technical difficulty encountered in the experimental demonstration of protein synthesis in axons...
derivatives from their strict association with glial cells. This close anatomical relationship required that the translation capacity of axons be distinguished from that of glial cells by autoradiographic methods or microdissection procedures combined with biochemical analyses. A more incisive alternative was offered by axons of unusually large size, such as the giant axon of the squid and the Mauthner (M-) axon of the goldfish, as they could be analyzed with conventional biochemical methods.

A. Early Evidence

In the initial pioneering studies, the dogma of the exclusive somatic origin of axonal proteins was tested and found to be inadequate to explain the resulting observations. According to the cell body hypothesis (seesect., ii), the irreversible inactivation of a neuronal enzyme would lead to its reappearance in the axon according to a proximo-distal gradient (49, 184, 185). An experimental protocol was then devised to implement the inactivation of acetylcholinesterase (AChE) in cat cholinergic neurons by the parental injection of diisopropylfluorophosphate (DFP) (184, 185). However, at variance with expectations, proximal and distal segments of hypoglossal and cervical sympathetic nerves exhibited virtually equal rates of enzyme recovery after the elimination of AChE activity. As the AChE inactivation by DFP was irreversible, the recovery of nerve AChE could not be due to reactivation of the enzyme but rather to its de novo synthesis. The uniform rate of AChE recovery in the hypoglossal nerve was confirmed even when the content of the perikaryal enzyme was either substantially decreased by intracranial injections of DFP throughout the recovery period or kept normal by selectively inactivating the nerve enzyme with diisopropylphosphostigmine iodide, a compound unable to cross the blood-brain barrier.

In a comparable series of experiments, these results were confirmed using a more sensitive AChE assay (174). In addition, they demonstrated that in untreated cats, 1 day after axotomy of the hypoglossal nerve, the level of AChE markedly increased in the most distal portion of the regenerating nerve stump. As this increase was inhibited by puromycin, the effect reflected a local process of protein synthesis. A more conspicuous increment of AChE was observed when the preexisting enzyme activity was suppressed by DFP before axotomy. Under the latter conditions, a previously decentralized nerve segment retained the capacity to synthesize AChE, as the enzyme level significantly increased after an additional axotomy of the decentralized stump. Interestingly, the latter effect was completely blocked by the local application of either RNase or 5-fluororotate, a compound interfering with RNA synthesis. Likewise, the recovery of AChE in a DFP-treated intact nerve was prevented by the local administration of 5-fluororotate.

Additional key observations were obtained following the local application of actinomycin D to the central stump of a DFP-treated and axotomized hypoglossal nerve (176). Compared with the contralateral DFP-treated and axotomized nerve, the level of AChE determined 4 days after axotomy was twice as high in the nerve treated with actinomycin D. Comparable effects were present in the intact nerve, irrespective of its previous treatment with DFP. Moreover, in the untreated intact nerve, the increment of AChE induced by actinomycin D was prevented by the local application of 5-fluororotate or puromycin. These results were interpreted to suggest that the synthesis of nerve AChE was locally regulated at the level of transcription by a repressor RNA whose synthesis was blocked by actinomycin D. Comparable, seemingly paradoxical effects of actinomycin D had previously been reported in the induction of liver enzymes (108).

Following the publication of these data, several groups started to report the local incorporation of radioactive amino acids into axonal proteins of mammals, amphibians, fish, and cephalopods, as described in the following subsections.

B. Model Systems

The incorporation of radioactive amino acids into axonal proteins was demonstrated in model systems under conditions excluding the contribution of nerve cell bodies.

1. The squid giant axon

The unusually large size of this axon (diameter up to 0.5 mm or more) accounts for the relative ease with which axoplasm may be extruded or axon may be internally perfused. These features have long made it a favorite object of neurophysiological studies (reviewed in Ref. 51) and molecular biological investigations (97, 103, 114, 200, 266). The giant axon generally used is the most medial, longest, and largest one of ~10 giant axons associated with the corresponding number of stellate nerves (Fig. 24). The many cell bodies of each giant axon are segregated in a special lobe of the stellate ganglion (the giant fiber lobe). During development, the tiny axons of these neurons fuse together to give rise to the giant axon (225, 335).

Synthesis of axoplasmic proteins by isolated squid giant axons (i.e., separated from their cell bodies) was independently demonstrated by two research groups using different routes of administration of radiolabeled amino acids. In one approach, the giant axon of the squid Loligo pealei was incubated in artificial seawater containing a mixture of $^{14}$Camino acids that were incorporated into axoplasmic proteins at an approximately linear rate for several hours (114). The proteins of the axon sheath were synthesized at a markedly faster rate, as expected
from the presence of resident glial and connective tissue cells. The synthesis of axoplasmic and sheath proteins was strongly inhibited by puromycin and cycloheximide (CXM), which indicated the involvement of a eukaryotic translation system. In the other approach, the synthesis of axonal proteins was demonstrated by microinjecting radiolabeled amino acids into giant axons of the squid Dosidicus gigas (97). Interestingly, the stimulation of the axon at 100 stimuli/s during the first 10 min of the 100-min incubation period increased the incorporation about two-fold. Conversely, when action potentials were abolished by stretching or puncturing the axon, the incorporation fell to negligible values.

In later studies, the synthesis of axoplasmic proteins by the isolated giant axon of Loligo pealeii was confirmed (3), and the question of their cellular localization was addressed (104, 200, 201, 277, 303). At the end of a series of experiments, the conclusion was reached that the axoplasmic proteins synthesized by the isolated giant axon were entirely derived from periaxonal glia (the glia-neuron protein transfer hypothesis). The hypothesis was entirely based on the presumed translational incapacity of the axon, offering a sharp alternative interpretation of the local synthesis of axonal proteins. The main observations supporting this claim included the following: 1) extruded axoplasm was unable to synthesize proteins, 2) newly synthesized proteins accumulated in the perfusate of giant axons even when RNase was added to the internal perfusing solution, and 3) most newly synthesized proteins were localized in the peripheral axon rim, as shown by autoradiographic analyses (104, 200, 201, 303). These data became one of the fundamental underpinnings of the prevalent opinion rejecting the concept of axonal protein synthesis. However, as detailed in a review article (112), the same data allowed alternative interpretations, and did not appear compelling with regard to the lack of protein-synthesizing machinery in the giant axon.

An additional stronghold of the general disclaim of local protein synthesis in axons was the demonstration that squid giant axons only contained tRNA (31, 199). The apparent lack of other cytoplasmic RNAs, notably rRNAs, confirmed that axons could not synthesize proteins and suggested that axoplasmic tRNA comprised only a few species serving a different function such as addition of activated amino acids to the native proteins (161). These data were contradicted by two independent observations. First, selective assays of single tRNAs and aminoacyl-tRNA synthetases demonstrated that the giant axon contained as many as six different tRNAs and the corresponding synthetases. In other words, all the tRNA species assayed for were in the axoplasm (161). As these components could not be involved in other activities but translation processes, the findings contributed to tilt the balance in favor of axonal protein synthesis. Second, with the use of microelectrophoretic methods, axoplasm was shown to contain sizable amounts of rRNAs in addition to an unusually large 4S RNA component and minor amounts of other small RNAs (113). Furthermore, a number of independent analyses demonstrated that extruded axoplasm also contained all the hundred species of soluble proteins and RNAs required for eukaryotic protein synthesis, including initiation and elongation factors, and all tRNAs and aminoacyl-tRNA synthetases (122).
Final proof of axonal protein synthesis was nonetheless delayed until two additional sets of data became available to demonstrate that the axoplasm of the giant axon contained both a population of mRNAs and active polysomes. The first piece of evidence was based on the specific mRNA assay testing the capacity of purified RNA to specify the synthesis of proteins in a cell-free rabbit reticulocyte lysate supplemented with [35S]methionine (251). Unexpectedly, when purified axoplasmic RNA was used as a template and the translation products were fractionated by SDS-PAGE, newly synthesized radiolabeled proteins were found to exceed 50 different species, indicating that at least as many different translatable mRNAs were present in the axoplasm (118, 119). The mobility of the major bands corresponded to the axon-abundant cytoskeletal proteins, such as actin, the two tubulins, and neurofilament (NF) proteins. Notably, a few radiolabeled axoplasmic proteins were missing in the translation pattern of cell bodies RNA (and vice versa). Substantial differences in the relative abundance of axoplasmic and cell body mRNAs were later detailed using the method of competitive RT-PCR (48). In conclusion, at variance with all expectations, the giant axon did contain a large population of mRNAs that specified the synthesis of proteins ranging from ~10 kDa to several hundred kDa, some of which were absent in the cell bodies. Hence, axoplasmic mRNA could not be considered the consequence of passive diffusion of cell body transcripts. These data were confirmed and extended by kinetic hybridization assays of polyadenylated axoplasmic RNA with its cDNA. This raised the estimated number of axoplasmic mRNAs to at least 200 different species (256). Such a high number was remarkable for an axon, even if kinetic hybridization analyses indicated that polyadenylated RNA from the axon cell bodies displayed much greater sequence complexity, comprising several thousand mRNAs. In situ hybridization assays established that poly(A)$^+$ mRNA was distributed throughout the axoplasm of the giant axon. These observations paved the way to the preparation of the first cDNA library of axoplasmic poly(A)$^+$ mRNAs that allowed comparisons with a cDNA library from the cell bodies (169).

Several clones of the axoplasmic library were identified by DNA sequencing, and the corresponding radiolabeled probes were used to confirm the presence of individual mRNAs in the axon by in situ hybridization. The list included the mRNAs encoding β-actin and β-tubulin (169), the heavy chain of kinesin (109), squid enolase (47), and a polypeptide sharing partial homology with a calcium channel of the mammalian sarcoplasmic reticulum (46, 326). In addition, using a riboprobe to mouse NF68 mRNA, the squid giant axon was shown to contain an homologous NF mRNA (121). Later immunoadsorption analyses with squid specific NF antibodies demonstrated the axonal synthesis of NF proteins (M. Crispino, B. B. Kaplan, and A. Giuditta, unpublished data).

The presence in the giant axon of all the components needed for eukaryotic protein synthesis (113, 118, 119, 122, 161, 256) strongly suggested that active polysomes were also present. This possibility was tested by incubating with [35S]methionine intact stellate nerves still connected to stellate ganglia and mantle muscle. Polysomes purified from extruded axoplasm, axonal sheath and the cell bodies of the giant axon were then displayed by sedimentation on linear sucrose gradients (121). This experiment demonstrated that radiolabeled proteins from each sample were almost exclusively localized in the polysomal region of the gradient. In addition, pretreatment of polysomes with EDTA or RNase induced a drastic shift of the radiolabeled proteins to the lighter regions of the gradient, thus proving their nature of nascent peptide chains. The latter data dissipated remaining skepticism and represented the most compelling demonstration of axonal protein synthesis.

The presence of polysomes in the squid giant axon was also confirmed by ultrastructural analyses based on electron spectroscopic imaging (ESI) methods (121) and immunoelectron microscopy (284). In the ESI method, substances containing phosphorus are selectively visualized as the electron beam impinging on phosphorus atoms loses a certain amount of energy. Hence, in view of their high phosphate content, ribosomes emit very strong signals (247). With ESI, large aggregates of polysomes were found in the cortical axoplasmic layer, in close association with mitochondria, as well as in the axoplasm core (33, 121, 224, 226). While the previous arguments against the axonal presence of polysomes were based on negative data, the ESI method and immunoelectron microscopy provided definitive evidence for their presence. The translation of several axoplasmic mRNAs in the giant axon was proved by RT-PCR analysis of purified axoplasmic polysomes. The list includes β-actin and β-tubulin mRNAs (169) as well as the mRNA coding for the heavy chain of kinesin (109).

2. The Mauthner axon

One of the first observations demonstrating the local synthesis of axonal protein was obtained from the Mauthner (M-) neurons of the carpfish (Carassius carassius). The large cell bodies of these two neurons are located on either side of the rostral medulla from which their myelinated fibers decussate and course the entire length of the spinal cord (78). With the use of autoradiographic methods, 1 day after the intracranial injection of [14C]lysine, axons and myelin sheaths of M-fibers were found to contain radiolabeled proteins. Their distribution in the initial 2-mm axonal segment followed a proximodistal gradient that moved distally and flattened out in the course of
several days. Hence, proteins synthesized in the cell body appeared to move into the proximal axon at a rate of 0.1–3.0 mm/day. Interestingly, smaller but significant amounts of radiolabeled proteins were also detected in the more distal regions of axon and myelin sheath throughout the 4-cm length of the M-fiber as early as 1 h after $[^{14}\text{C}]$lysine administration. This quick appearance of radiolabeled proteins could hardly be attributed to the axonal flow. The concentration of these proteins markedly increased after 3 h, and their distribution did not follow a rostrocaudal gradient. Myelin was consistently more labeled than the axon at all times. The latter data suggested that a local system of protein synthesis was present throughout the M-fiber.

Strong support for this interpretation was obtained by incubating spinal cord segments with $[^{35}\text{S}]$methionine (78). Under these conditions, isolated M-axons and myelin sheaths contained radiolabeled proteins, whose synthesis could not be attributed to the cell bodies that were dissected away with the medulla. The local synthesis of M-fiber proteins was strongly inhibited by puromycin, which indicated the involvement of a local ribosomal system. With the use of a similar experimental protocol, the newly synthesized proteins of the M-axon were eventually fractionated by gel electrophoresis, and the major bands were shown to correspond to the axon-enriched cytoskeletal proteins, including actin, $\alpha$- and $\beta$-tubulins, and NF proteins. Their synthesis was strongly inhibited by chloramphenicol (181). Interestingly, the DNA-binding transcription inhibitor actinomycin D markedly decreased the incorporation of radiolebelled leucine, lysine, or an amino acid mixture in the M-fiber (79). The data implied that a large part of axonal protein synthesis requires local DNA templates localized in glial nuclei, and indicated the marked metabolic instability of the transcribed mRNAs. These observations were in general agreement with the high RNA turnover of the M-axon (77, 162).

Isolated M-fibers containing myelin but not glial cell bodies were still capable of synthesizing proteins at a linear rate for several hours. Synthesis of the new protein was partly sensitive to puromycin and acetoxycycloheximide, but virtually insensitive to actinomycin D and to the bacterial translation inhibitor chloramphenicol (CAP) (78). Under these conditions, one-fourth of the radiolabeled proteins were present in the axon, in agreement with the comparable distribution in M-fibers dissected from incubated spinal cord pieces, in which glial cell bodies were still present. The lack of CAP inhibition suggested the involvement of an extramitochondrial translation system. This suggestion was confirmed by the prevalent recovery of the newly synthesized radiolabeled proteins of isolated M-fibers in the microsomal and cytosol fractions (81).

The local synthesis of M-axon proteins was later confirmed by microinjection of a radiolabeled amino acid into the M-axon of live fish (6). The process was sensitive to inhibitors of protein synthesis (5). In these experiments, newly synthesized axonal proteins were 5% of those produced in the perikaryon if referred to unit mass, but the value became 50-fold greater than in the perikaryon when calculated in terms of overall axonal mass. Protein synthesis appeared to occur in the axon itself, as under these conditions myelin contained a much lower amount of radiolabeled proteins. Interestingly, low-frequency stimulation of the M-neuron increased axonal protein synthesis twofold if the stimulation lasted 18 h, but 4-h stimulation produced no effect. The enhanced synthesis was no longer present 1 day later (90). A comparable effect had been previously reported in frog sciatic nerves subjected to high-frequency stimulation (213). The significant decrease in protein content of rat sciatic nerves after in vitro electrical stimulation or following a period of prolonged swimming (163) suggests that the turnover of axonal proteins is a local process.

The M-axon has also been a model system in studies of the components of the protein synthesis machinery. As mentioned earlier, the most important single reason to consider the axon incapable of protein synthesis was the alleged absence of ribosomes, as determined by conventional EM methods. This view only regarded mature axons, as ribosomes were detected in developing axons (40, 199). A pivotal turn drastically revising these views took place when RNA was identified in the adult axons of goldfish, cat, crustaceans, and squid. In these axons, the concentration of RNA was consistently much smaller than in the cell bodies, but its total content was several times higher in the total axonal mass (9, 76, 83, 85, 126, 175).

Axonal RNA was initially detected and characterized by microdissection of fixed M-axons (76, 83). In microchemical analyses, the average RNA content of goldfish (*Carassius auratus*) M-axon corresponded to 0.15 pg/μm. As a result, in a 4-cm axon, total axonal RNA amounted to 6,000 pg, while the cell body only contained 2,000 pg. As expected, RNA was highly concentrated in the perikaryon (1% wt/vol). RNA was also present in the myelin sheath, with 30% higher amounts than in the axon. Interestingly, axon and myelin RNA shared a similar base composition, suggesting a common origin. Nonetheless, the adenine/guanine (A/G) ratio was slightly but significantly higher in the myelin (0.6) than in the axon (0.56), irrespective of the distance from the cell body. Both compartments contained a substantial amount of a nucleotide fraction rich in A, raising the possibility that they might contain some mRNA.

Several RNA species were eventually identified in the unfixed M-axons by electrophoretic fractionation (179).
They included a prevalent 4S RNA, cytoplasmic 26S and 18S rRNA, and minor amounts of unidentified 15S and 8S components that were not present in myelin. As also noted for squid (113), the 4S peak of M-axons included other small RNAs, such as a 7S and a 5S RNA. The relative content of these small RNAs was markedly higher than in the M-cell bodies. This unusual abundance was tentatively attributed to the need to maintain a sufficiently high concentration of tRNAs throughout the axoplasm to ensure optimal rates of protein synthesis for polysomes residing in restricted axonal domains. With time, the apparent lack of morphologically identifiable ribosomes led to a patent discrepancy with the evidence of eukaryotic rRNA in large model axons (76, 80, 113, 179). The apparent absence of axonal ribosomes was likewise contradicted by the local cytoplasmic synthesis of proteins. Thus, for those supporting the axonal location of a protein synthetic machinery, axonal ribosomes were assumed to be in an occult form. A number of hindering conditions were thought to be responsible for the difficulty of detecting them by conventional EM methods, such as their limited number or their association with unusual structures.

The puzzle of vertebrate axons containing rRNA but not allowing the visualization of ribosomes (80, 179) was eventually resolved by staining whole mounts of native M-axons with YOYO-1, a nucleic acid probe that becomes highly fluorescent when it binds to RNA (186). With the use of this procedure, RNase-sensitive fluorescent structures containing more intensely fluorescent “puncta” were identified at the surface of M-axons and other goldfish spinal nerve fibers. When examined by phase-contrast or differential interference contrast microscopy, these newly defined structures (periaxoplasmic plaques or PARPs) protruded from the axon surface and were distributed at irregular intervals along the entire axon. As shown by colabeling with rhodamine-conjugated phalloidin, PARPs were embedded in the peripheral F-actin cytoskeleton. With ESI, the fluorescent puncta present inside the plaques and in the underlying axoplasm were identified as polysomal aggregates. This conclusion was confirmed with immunocytochemistry using ribosome-specific antibodies (186).

The presence of individual mRNAs in M-axons (e.g., the mRNA of the medium-sized NF protein) was demonstrated by RT-PCR methods (324), while in situ hybridization showed that β-actin mRNA is preferentially distributed in goldfish and mammalian PARPs. Myosin Va and kinesin II proteins also coincide with PARPs (285). Besides these molecular motor proteins, axoplasmic plaques appear to contain BC1 RNA, a brain-specific transcript of RNA polymerase III. Following its microinjection in the M-cell body, radiolabeled BC1 RNA was detected in the M-axon (and in M-dendrites) where it was intermittently located according to the characteristic PARP distribution pattern (242). Because BC1 RNA is associated with a 10S RNP (173) and implicated in protein synthesis (296), PARPs may serve as axonal translational centers targeted by the involved RNAs.

The cortical zones of the M-axon and mammalian root axons also contain 7S RNA, a key component of the signal recognition particle (SRP) that correctly addresses the synthesis of secretory and integral membrane proteins (316). Using immunocytochemical methods, the SRP 54 protein has been shown to colocalize with PARPs in rabbit ventral root fibers (297). These observations suggest the intriguing possibility that axons may be able to synthesize secretory or integral membrane proteins, even if the Golgi apparatus has not been documented in axons. Evidence supporting the axonal synthesis of this class of proteins has been reported in decentralized cultured snail axons microinjected with the conopressin receptor mRNA (289).

C. Vertebrate Axons

In the newt Triturus viridescens, 45 min to 18 days after the intraperitoneal injection of [3H]histidine, the radiolabeled amino acid was detected in brachial nerves examined by light and EM autoradiography (281). Silver grains were localized in Schwann cell, myelin lamellae, and axons. According to a semi-quantitative analysis, the level of radioactivity was highest in Schwann cell bodies and myelin during the first few hours, but progressively increased in the axon. Interestingly, the intensity and pattern of the radiolabeled signals were not affected by decentralizing the nerve, or by incubating dissected nerves with the radioactive precursor. The latter observations and the presence of silver grains throughout the length of the nerve suggested that the data were due to the activity of a local system of protein synthesis.

As described in section A.A, the most incisive and productive line of research on axonal protein synthesis started with the demonstration of AChE resynthesis in cat nerves (174, 176, 184, 185). Soon after, a small amount of RNA was identified in the axons of cranial nerve roots of adult cats (175). The concentration of this RNA was only 0.2% of that in the neuron soma, and its A/G ratio was 0.57 just like in the M-axon. Not much later, axonal protein synthesis was directly demonstrated in the rabbit accessory spinal roots incubated with [3H]leucine. In these experiments, the labeled amino acid was incorporated into the proteins of microdissected axons (177). The incorporation proceeded at an almost linear rate for ~8 h, after an initial lag period of ~2 h. The lack of CAP inhibition excluded the involvement of bacteria and mitochondria and suggested the participation of a eukaryotic ribosomal system. As prolonged preincubation with actinomycin D did not modify the rate of protein synthesis, the involved mRNAs appeared to be metabolically
stable. In a later study, electrophoretic fractionation of axonal proteins synthesized in vitro by rat spinal ventral roots showed that axon-enriched cytoskeletal proteins are among the newly synthesized proteins, such as actin, tubulins, and NF proteins (181). This pattern was similar to that of the nerve cell bodies, but differed from the pattern of newly synthesized myelin proteins. In all cases, the synthesis of these proteins was markedly inhibited by CXM. The distinctive pattern of newly synthesized myelin proteins indicated that a local system of protein synthesis is also present in myelin. As myelin represents the overwhelming cytoplasmic periphery of the Schwann cell, its local system of protein synthesis may play similar roles to those played by the axon translation system with respect to the nerve cell body.

The key data demonstrating a local increase of AChE in the proximal stump of axotomized cat hypoglossal nerves (174, 176, 184, 185) prompted further studies of this effect. In the axotomized hypoglossal nerve of the rabbit, in vitro analyses of [3H]leucine incorporation into axonal proteins revealed a dramatic burst of protein synthesis in a narrow axonal segment proximal to the lesion site (298, 299). When the data were normalized with the concurrent accumulation of proteins transported by the axoplasmic flow, the peak of protein synthesis occurred 18 h after axotomy, marking a 20-fold increase. The specific activity of the newly synthesized proteins was consistently higher in the axon than in the myelin, as the latter did not exhibit a significant response to axotomy. Interestingly, in analogy with the axonal synthesis of AChE (see sect. mA), this striking effect was still present in the decentralized nerve stumps (299). CAP was not inhibitory, but CXM strongly inhibited axonal protein synthesis in both intact nerves and axotomized nerve tips. Microelectrophoretic fractionation of the proteins synthesized by the latter axonal segment indicated that they were substantially different from newly synthesized proteins released in the incubation medium (98). Hence, protein synthesis in the decentralized nerve was independent of the cell body. This important set of observations further supported the existence of protein synthesis in the myelinated mammalian axons and its transcription modulation by periaxonal glial cells.

Local protein synthesis was also studied in sciatric nerve, the biggest nerve in the body of mammals. In vivo incorporation of locally applied radiolabeled amino acids in the axons of lesioned and unlesioned rat sciatric nerves was demonstrated by autoradiographic methods (25, 53). More recently, with the use of RT-PCR methods, intact and axotomized sciatric nerves of adult rats were shown to contain the mRNAs encoding all NF proteins. In addition, the content of the light NF mRNA was higher in the proximal stumps of axotomized nerves than in control nerves (286). Interestingly, in situ hybridization analyses demonstrated that the light NF mRNA was also localized in Schwann cell cytoplasm, myelin sheath, and in between the inner myelin lamella, as well as in the axon. The identification of the newly synthesized light NF protein by immunoadsorption and two-dimensional gel electrophoretic methods demonstrated that the corresponding mRNA was translated in the nerve. These observations were consistent with the CXM-sensitive synthesis of NF proteins in axons of rat spinal roots and in M-axons (181). Altogether, these results suggested that some transcripts encoding neuron-specific proteins such as NF may be transferred from glia and translated in the axon.

This idea was further supported by other investigations using in situ hybridization and EM methods. The mRNAs of the medium and light NF proteins were unexpectedly detected in the Schwann cells of young rats examined under a variety of conditions, including differentiation (170), demyelination, or unilateral nerve transaction (271). In the latter condition, the light NF mRNA was also detected in the contralateral nerve, implying that it also occurred in normal nerves. Medium and light NF mRNAs were also transiently expressed in Schwann cells resident in the distal stump of lesioned sciatric nerves during axon degeneration and remyelination of ingrowing axons. Interestingly, translation of these mRNAs was random and unpredictable in Schwann cells, as shown by immunocytochemical analyses (95). These results further support the idea that NF transcripts are not intended for translation in glial cells but are rather destined to be transported and translated in regenerating axons. Identification of NF mRNA sequences in the Schwann cell cDNA library further confirmed that some neuron-specific proteins are transcribed in glial cells (170).

Most recently, the modulation of protein synthesis in the sciatric nerve was neatly demonstrated by RNA interference methodology (240). According to this work, adult mouse sciatric nerve contains several protein components of the RNA-induced silencing complex (RISC), including Argonaute2 nuclease, fragile X mental retardation protein, p100 nuclease, and Gemin3 helicase. Hence, the result extended the previous findings that the same modular complex is present in neurons (189), in developing axons and growth cones (135), and in dendrites (276). Interestingly, local application of short-interfering RNAs (siRNA) against neuronal β-tubulin triggered RISC formation, and specifically produced marked decrease in both mRNA and protein levels of β-tubulin in the nerve fibers (Fig. 3). The reduction of axonal tubulin content was confirmed by the significant decrement in retrograde axonal transport assessed by reduced fluorogold labeling of spinal motoneurons. The existence of such a siRNA-based regulatory mechanism in the axon raises the question of whether this mechanism might be used by the periaxonal glial cells to selectively modulate the synthesis of axonal proteins.
Axonal mRNAs were also found in the mammalian CNS. Notably, studies with rats revealed that several mRNAs encoding peptide hormones of the posterior hypophysis were present in mature axons of the hypothalamic vasopressin and oxytocin neurons (165, 166, 232, 233, 300, 301). Under conditions of functional load, the content of some axonal mRNAs increased much more than in the neuron cell bodies. Furthermore, following the upregulation of the galanin peptide elicited by salt loading, preprogalanin mRNA was identified in neurohypophysial axons (198). Besides the transcripts for the hormones, the mRNA encoding the light NF protein was also detected in the axons of the same neurons (232). Ribosomes have not been reported in neurohypophysial axons of control rats, but they are present in the hypertrophic vasopressin-containing axons following chronic stimulation by a salt-loading regime (223). In addition, the mRNAs encoding olfactory receptors and the olfactory marker protein were identified in the axons of olfactory receptor neurons (70, 268, 307, 328).

Following the demonstration of PARPs in the cortical layer of M-axon (see sect. mB2), analogous formations were identified in axons of rabbit and rat ventral root fibers stained by YOYO-1 and antiribosomal antibodies (187). These structural correlates were likewise protruding from the axon surface, and the fluorescent puncta were identified as polysomal clusters by ESI analyses (Fig. 4, A–D). Compared with M-axon, the mammalian PARPs are quite thin (~1 μm) and occur with variable length (usually ~10 μm) at irregular intervals along the axon (from 10 to 100 μm; average 25 μm). Most PARPs are located at internodes. In view of their large variability in size and morphology, PARPs are likely to represent a dynamic biological structure that changes its location and distribution along the axon, in agreement with the dynamic features of the F-actin layer in which they are embedded (293). The F-actin cytoskeleton may be involved in the assembly and distribution of mRNAs and ribosomes in the axon, as it does in other cells (21, 139, 279). In support of the functionality of cytoskeleton-anchored ribosome and mRNA, elongation factor 1 was found to be bound and regulated by F-actin (52, 208). F-actin may be also involved in the radial displacement of axonal components that are longitudinally transported along microtubules. Although more has to be known about the precise biological role of the mammalian periaxoplasmic plaque, its identification as a ribosome-containing axonal structure has made an important contribution to the hypothesis of axonal protein synthesis. Interestingly, the presence of ribosomes in periaxoplasmic domains was witnessed ante litteram more than a decade ago. In an EM study of myelinated axons of rabbit dorsal root ganglia, ribosomes were mainly distributed in the cortical axoplasm of a few axons. Some ribosomal aggregates were associated with ER (250; Fig. 4E). This observation is one of the few direct EM evidences for the presence of axonal ribosomes in mature neurons, foreshadowing the discovery of PARPs.

The existence of a local system of protein synthesis in mature mammalian axons is now generally accepted (reviewed in Ref. 262), as also demonstrated by studies dealing with its participation in nerve regeneration. The requirement of the local synthesis of proteins for axonal sprouting was initially shown in mouse sciatic nerve regenerating in vitro (75) and later confirmed in vivo. In a live rat, the regeneration of the crushed peroneal nerve was markedly reduced by the local application of CXM, as elongation of the axonal sprout was reduced by 58% during the 3-day recovery period (101). This effect occurred irrespective of the absence of live Schwann cells, confirming the axonal localization of the system of protein synthesis.

The axonal synthesis of proteins is crucial in nerve regeneration. In an incisive experiment, the neurite extension of NGF-differentiated PC12 cells was shown to depend on the translation of a short-lived mRNA encoding the ribosomal L4 protein (302). Administration of antisense oligonucleotides for L4 protein inhibited neurite outgrowth in both PC12 cells and in adult rat sensory.....
neurons cultured after a crush to the sciatic nerve. Further studies of the latter preparation demonstrated that regenerating axons contain L4 and other ribosomal proteins, translation factors, rRNA and mRNA, and are capable of protein synthesis (339). Blocking protein synthesis led to a rapid retraction of growth cones, provided that axonal sprouts were separated from cell bodies by either axotomy or colchicine-based inhibition of axonal transport. Hence, axonal synthesis of protein appears crucial in maintaining growth cones and other intrastructure of regenerating axon. Translation factors, ribosomal proteins, and rRNAs were also detected in motor axons of ventral spinal roots examined 7 days after in vivo lesion of the peripheral nerve. The role of axonal protein synthesis in regeneration was also highlighted by the presence of proteins with nuclear localization signals (importins) in the distal regions of axons. Axonal β-importin increases after nerve lesions by the local translation of its encoding mRNA. The complex of β-importin and dynein with other axonal proteins leads to its retrograde transport into the neuron soma that enables the central response to axonal regeneration (133, 134). Forty proteins that were retrogradely transported have been identified by two-dimensional PAGE and mass spectrometry in regenerating axons of the snail Lymnea stagnalis. Several of them comprise posttranslationally modified proteins and calpain cleavage products of a 51-kDa intermediate filament protein (254, 255). Similar analyses made on purified axons of injury-conditioned dorsal root ganglion neurons led to the identification of cytoskeletal proteins and a large number of other proteins, including heat shock proteins, endoplasmic reticulum proteins, enzymes, and proteins associated with neurodegenerative diseases (330). Interestingly, RT-PCR analyses of the same preparation indicated that regenerating axons also contain mRNAs encoding these proteins, suggesting their translation in the axon. Treatment of regenerating axons with nerve growth factor (NGF) or brain-derived neurotrophic factor (BDNF) markedly enhanced the content of axonal mRNAs encoding cytoskeletal proteins, as a result of their increased rate of delivery via axoplasmic transport. No significant effect regarded other axonal mRNAs.

Further insights into the mechanism of the Wallerian degeneration of decentralized axons were provided by analyses of the Wld^s mutant mouse which undergoes a markedly delayed degeneration. The protective mutant gene encodes a chimeric protein resulting from the fusion of nicotinamide mononucleotide adenylyltransferase-1 (NAD synthetase) with the NH2-terminal fragment of the ubiquitination assembly factor Ube4b (216). In Drosophila, this mouse protein suppresses self-destruction signals originating in transected axons and activating Draper engulfment glial receptors that mediate the elimination of injured axons by glial cells (146, 215). The extensive changes in glial morphology elicited by lesioned axons through the upregulation of these glial receptors did not occur in Draper mutants. As a result, decentralized axons were not eliminated from the nervous system. Interestingly, the Wld^s protein remained ineffective in the clearing...
of supernumerary axons during the development of flies and mice, although Draper receptors and the ubiquitin-proteasome system were needed to implement both injury-induced and development-induced axon degeneration (for reviews, see Refs. 23, 331). Altogether, these studies imply that periaxonal glial cells may play an important role in the physiology of degenerating and regenerating axons.

D. Other Axons

The presence of axonal RNA was also demonstrated in other animal models. In the crayfish *Procambarus clarkii*, the concentration of RNA in giant axons is 0.02% (wt/vol), compared with 0.22% in periaxial glia and 5.7% in neuronal perikarya (9). Accordingly, in a 4-cm axon containing 1.67 pg RNA/μm, the total amount of RNA corresponds to 67,000 pg, compared with ~1,000 pg in the cell body. Crayfish axonal RNA is largely extramitochondrial. The different CG/AU ratios of axonal, glial, and perikaryal RNAs (0.53, 0.77, and 0.97, respectively) were attributed to the relative rRNA content of these tissue samples. Autoradiographic analyses of nerve chords incubated with tritiated uridine showed that newly synthesized RNA was initially present in Schwann cell nuclei, but became progressively more concentrated in Schwann cell cytoplasm and axons with longer incubation times. In the axon, most radiolabeled RNA was localized near the axon, most radiolabeled RNA was localized near the axolemma, notably in the vicinity of abutting Schwann nuclei, suggesting its derivation from periaxonal Schwann cells.

In the slowly adapting stretch receptor neurons of the lobster (*Homarus gammarus*), RNA was also detected in the axons at a concentration of 0.06%, while the concentration of cell body RNA was 0.5% (85, 126) (see also sect. vaB1). More recently, in the snail *Lymnaea stagnalis*, ultrastructural in situ hybridization methods demonstrated that axons and nerve terminals of the neurons secreting the egg-laying hormone contained the corresponding mRNA in secretory granules (72).

E. Neurons in Culture

Protein synthesis was clearly demonstrated in regenerating axons of the cultured ganglion cells from goldfish retina (182). Axons severed from the original explant without extraneous cells incorporated radiolabeled amino acids at an approximately linear rate for 3 h. Intact axons exhibited similar rates. This incorporation did not reflect bacterial or mitochondrial protein synthesis as it was inhibited by CXM and emetine, but not by CAP. Microelectrophoretic fractionation of the proteins synthesized in the axonal fields indicated that most radioactivity was associated with β-tubulin while actin and other proteins were labeled to a lesser extent. In contrast, α-tubulin was not labeled (180). In the intact preparation, the depolarizing condition induced by 100 mM KCl was more inhibitory to protein synthesis in the axonal fields than in the ganglion cell bodies, in analogy to the comparable effects reported for the squid giant axon and its cell bodies (252). Local synthesis of β-tubulin and β-actin was also demonstrated in intact growing axons of rat sympathetic ganglion cells using immunoabsorption methods (88).

Axons of cortical neurons from embryonic rat brain (22) and chick sympathetic neurons (246) contain poly(A)+ mRNA, as shown by in situ hybridization or RT-PCR methods. In the latter study, mRNA associated with granules was present at axon branch points, varicosities, and growth cones, but α-tubulin mRNA was absent, in agreement with data derived from growing axons of goldfish retinal explants (181). mRNAs coding for the Tm-5 tropomyosin isoform and the SCG10 protein were identified in the growing axons of developing rat cerebellar neurons or PC12 cells (131, 132). SCG10 is believed to be an actin-binding protein that modulates membrane-cytoskeletal interactions.

In cultured neurons derived from rat brain, growth cones contain polyribosomes, as shown by EM analyses (24). In addition, using in situ hybridization methods, axonal granules and growth cones were found to contain β-actin mRNA. The association of these granules with microtubules suggests that β-actin mRNA is transported along these cytoskeletal elements. The selectivity of this process was highlighted by the lack of γ-actin mRNA in the axonal granules, in analogy to the lack of α-tubulin mRNA in the axons of sympathetic neurons (246).

In an elegant experiment with cultured neurons of the snail *Limnea stagnalis*, a heterologous mRNA microinjected into isolated neurites was translated into its encoded protein, the egg-laying hormone (305). These observations were the first to demonstrate the axonal translation of a single exogenous mRNA. In later work, isolated snail axons plated in cultured dishes were microinjected with the heterologous mRNA coding for the conopressin receptor (289). One day later, the receptor protein was identified in the microinjected axons by electrophysiological and immunocytochemical methods, showing that this axon-made protein is functional. As the conopressin receptor is coupled to G protein, the depolarizing response elicited by conopressin was successfully blocked by the nonhydrolyzable analog guanosine 5′-O-(2-thiodiphosphate) (GDPβS).

For a detailed discussion of the differences between growing and mature axons, readers are referred to Alvarez et al. (7).
IV. LOCAL PROTEIN SYNTHESIS IN NERVE TERMINALS

A. Model Systems

1. Squid nerve endings

Nerve terminals can be enriched in subcellular fractions containing synaptosomes, which are plasma membrane-enclosed structures derived from synapses. When protein synthesis was demonstrated in the mammalian synaptosomes (see sect. IVB), their heterogeneous composition drew some doubts on the nature and origin of the synaptosomal translation system. While most studies attributed the presence of a cytoplasmic (and a mitochondrial) system of protein synthesis to nerve terminals (14, 329), outlandish explanations were advanced with regard to the nature of the synaptosomal protein synthesizing machinery, or claims were made of its prevalent or almost exclusive localization in postsynaptic and/or glial elements. Valid arguments against these positions will be addressed in the following pages. Nonetheless, the heterogeneous composition of mammalian synaptosomes still allows controversial interpretations of the data. Some of the problems raised by their diverse cellular origin have been solved by studies of the model synaptosomal fraction of squid optic lobes.

The axons of squid retinal photoreceptor cells innervate the cortical layer of the optic lobe where they make synaptic contacts with the resident amacrine neurons through unusually large, carrotlike nerve endings (Fig. 5A). Upon homogenization of the optic lobe, these nerve endings give rise to large presynaptic synaptosomes (Fig. 5B), which are the most abundant components of the synaptosomal fraction. As the optic lobes do not harbor additional nerve terminals of comparable size (50, 336), large synaptosomes of squid optic lobe are exclusively derived from the nerve terminals of photoreceptor neurons.

Investigations of this unique preparation provided conclusive evidence that nerve endings contain active systems of cytoplasmic and mitochondrial protein synthesis (26, 56–58, 73, 137, 226). At variance with the mammalian preparations, the synaptosomes of cephalopod optic lobes are obtained as a floating layer by their differential centrifugation in slightly hypotonic sucrose homogenates. This preparative method leads to better preservation of the...
synaptosomes, and may in part be responsible for their high rate of protein synthesis (57, 137). In most other respects, squid synaptosomes resemble mammalian synaptosomes.

The essential purity of squid optic lobe synaptosomes was first suggested by the electrophoretic pattern of their newly synthesized proteins, which substantially differed from those of neuronal cell bodies or glial cells (56, 57). The low level of contaminating structures was confirmed by light and EM autoradiographic analyses, which also demonstrated the nearly exclusive localization of newly synthesized proteins in large synaptosomes (58). Polysomes purified from optic lobe synaptosomes incubated with [35S]methionine were shown to contain nascent peptide chains (57), and to be free from microsomal polyribosomes (110). Polysomes were likewise identified in the large optic lobe synaptosomes using ESI (Fig. 5C) (58) and in the carotid nerve endings of intact squid optic lobes using ESI and YOYO-1 fluorescent staining (226).

Proteins synthesized by optic lobe synaptosomes or by purified synaptosomal polysomes include several members of the NF protein family (56), as well as calaxitin, a learning-related protein (98). RT-PCR analyses of synaptosomal polysomes have led to the identification of mRNAs coding for cytoskeletal and ribosomal proteins, translation factors and, most notably, mitochondrial proteins encoded by nuclear DNA (110, 111). The association of these mRNAs with purified polysomes indicates that the corresponding proteins are synthesized in presynaptic synaptosomes. These data were confirmed and extended using two-dimensional electrophoretic methods that demonstrated the presynaptic synthesis of ~80 different protein species, several of which were identified by mass spectroscopy (164). They include cytoskeletal proteins, enzymes, novel proteins, and mitochondrial proteins encoded by nuclear DNA. The synthesis of mitochondrial proteins in nerve terminals has physiological significance, as it adds a new dimension to the concept of presynaptic autonomy. Indeed, presynaptic (and axonal) mitochondria can no longer be considered exclusively dependent on nuclear-encoded mitochondrial proteins derived from the distant neuron soma, as these proteins are locally synthesized.

Protein synthesis in presynaptic synaptosomes may be under the control of cell signaling pathways, as shown by its strong modulation by cytosolic Ca\(^{2+}\)). With the use of intracellular and extracellular Ca\(^{2+}\) ionophores, chelators, and other modulators, protein synthesis of optic lobe synaptosomes was shown to proceed at nearly optimal rates when the intraterminal Ca\(^{2+}\) level was maintained within the normal physiological range. When this level increased or decreased, protein synthesis was markedly depressed (26, 27, 57). As strong inhibition was also induced by calphostin, a selective inhibitor of protein kinase C (26), modulation of presynaptic protein synthesis by Ca\(^{2+}\) may be in large part funneled through protein kinase C (PKC) and its downstream targets, eIF-2 or eEF-2. The data suggest that incidental surge of Ca\(^{2+}\) at the nerve endings (such as elicited by ischemic or other degenerative insults) may have an additional deleterious consequence. When presynaptic protein synthesis is inhibited by high levels of cytosolic Ca\(^{2+}\), the repressed local synthesis of nuclear-encoded mitochondrial proteins may lead to the reduced supply of mitochondrial energy. Consequently, further inhibition of presynaptic protein synthesis may follow. Such a feedback loop might eventually cause degeneration of the nerve terminal and possibly lead to “dying back” of the related axonal segment.

2. Aplysia nerve endings

In elegant experiments with cultured Aplysia neurons, the long-term potentiation of a sensory-motor synapse was subjected to a series of detailed manipulations to elucidate the underlying molecular mechanisms. In the intact animal, this synapse is responsible for the facilitation of the gill withdrawal reflex elicited by nociceptive stimuli. The experiments regarded cultured sensory neurons endowed with a bifurcated axon establishing separate synapses with two different motor neurons. As long-term facilitation (LTF) may be mimicked in vitro by repeated spaced administration of serotonin to the sensory nerve terminals, such treatment at one synapse induced LTF in that synapse, but not in the other synapse. These experiments demonstrated the great spatial selectivity of LTF, and the essential role of presynaptic protein synthesis in allowing LTF. Indeed, local protein synthesis at the sensory nerve terminal increased threefold in the potentiated synapse but not in the control synapse. In addition, local inhibition of presynaptic protein synthesis completely abolished LTF (44, 220, 221). Interestingly, transcription and translation events triggered in the cell body by serotonin administration to the presynaptic sensory terminal were also essential for LTF. This suggested the activation of the somatic gene expression system by retrograde signals and the delivery of newly synthesized somatic gene products to the same terminal.

Comparable data demonstrating the importance of both somatic and local protein synthesis in synapse-specific LTF were more recently described in well-differentiated Aplysia ganglia mediating the syphon withdrawal reflex (128). These authors demonstrated that in a distal sensory-motor synapse a similar interplay between somatic and local systems of protein synthesis was necessary to allow LTF. The involvement of axonal transport was proven by the nocodazole block of LTF due to its disruption of axonal neurotubules. Nonetheless, the large distance between the synapse and the cell body (2–3 cm) induced strains in the attempt to fit the known rates of...
retrograde transport, somatic transcription and translation, and anterograde transport into the time frame allotted by the progressive cell body-dependent stabilization of LTF. Among several alternative explanations, the authors mentioned “different mechanisms could mediate LTF at mature, intact synapses, compared with mechanisms at newly formed synapses in dissociated cell cultures.” These mechanisms might regard the intercellular interplay of the presynaptic terminal with the perisynaptic glia.

Local presynaptic protein synthesis is also needed for synapse formation between cultured neurons. Removal of the cell body of *Aplysia* presynaptic sensory neurons and of postsynaptic target motor neurons does not interfere with the formation of synaptic connections. Under these conditions, synaptic efficacy increases for 2 days and is reversibly blocked by anisomycin, an inhibitor of protein synthesis (274). Isolated sensory synapses deprived of their cell bodies are still able to express a form of LTF that is dependent on local protein synthesis and is even greater than that produced when the cell bodies are present. However, this form of LTF decays after 1 day, thereby showing the relevance of the cell body for longer-term memory (210). Changes in synaptic efficacy are associated with the accumulation of presynaptic sites of the sensory neuron-specific neuropeptide sensorin and sensorin mRNA. Selective interference with sensorin mRNA by dsRNA does not decrease the level of presynaptic sensorin but prevents LTF, suggesting the need for its local translation (214, 275). The target-dependent synthesis and release of sensorin acting on autoreceptors mediates the increase in synaptic efficacy (148). These effects also require the activation of multiple protein kinases and their complex interactions (149, 209).

On the whole, these data demonstrate that local translation processes are absolutely required for implementing long-term plastic changes on the synapses established between cultured neurons or well-differentiated neurons. They also raise intriguing questions on the complex interactions between somatic and local gene expression processes.

**B. Vertebrate Nerve Endings**

Nonmitochondrial organelles originally detected in the mitochondrial fraction of rat brain (259) were eventually purified by sedimentation on sucrose density gradients and largely identified as pinched-off nerve terminals (synaptosomes) on the basis of their content of synaptic vesicles and mitochondria (67, 127). Since then, mammalian brain synaptosomes purified with different protocols have been found to contain other minor particulates such as free mitochondria and fragments of axons, dendrites, and glial processes (54, 105, 124, 136, 234, 264, 310; for a review, see Ref. 329). In parallel with these observations, synaptosomes were examined for their capacity to synthesize proteins, as previously proposed for axons (184, 185). These investigations were prompted by the unusually high rate of protein synthesis displayed by brain mitochondria that otherwise appeared to share the same features of liver mitochondria (43). An important clue emerged when protein synthesis by the brain mitochondrial fraction was unexpectedly found to be inhibited by CXM, in addition to CAP (334). The data implied that the brain mitochondrial fraction contained nonmitochondrial structures capable of cytoplasmic protein synthesis.

The suggestion that these structures were synaptosomes came from experiments with rat brain slices incubated with a radiolabeled amino acid (14). The subcellular fractionation of the radiolabeled slices indicated that the specific activity of synaptosomal proteins increased linearly with time from the very beginning of the incubation. The absence of a lag period, also lacking in vivo (74; for a review, see Ref. 18), suggested that newly synthesized synaptosomal proteins originated locally. This interpretation was proven correct by the demonstration that purified synaptosomes catalyzed the incorporation of a radiolabeled amino acid into protein (235–237). The reaction did not require addition of exogenous soluble factors, was insensitive to RNase, and was partially inhibited by CAP and more strongly inhibited by puromycin or CXM. The translation products were associated with most synaptosomal subfractions except synaptic vesicles. Newly synthesized soluble and membrane proteins were selectively inhibited by CXM, while CAP only blocked the synthesis of mitochondrial proteins. On the whole, the data indicated that, in addition to a mitochondrial translation system, synaptosomes contained cytoplasmic polysomes and all the soluble factors and energy sources required for their protein synthetic activity. Comparable results were obtained with synaptosomes purified on a discontinuous Ficoll gradient (1, 196). They appeared to be better preserved and to exhibit an optimal rate of protein synthesis in the presence of 100 mM NaCl and 10 mM KCl (15). Because protein synthesis was not affected by addition of ATP, soluble factors, and RNase, it was attributed to membrane-enclosed synaptosomes rather than to contaminating microsomal polysomes. Likewise, a major contribution of bacterial and mitochondrial sources was excluded by the partial inhibition exerted by CAP. On the basis of EM analyses, the origin of most Ficoll synaptosomes (60%) was attributed to nerve terminals, while most other structures appeared to be postsynaptic elements rarely containing ribosomes.

Ultrastructural autoradiographic analyses further established the subcellular origin of Ficoll synaptosomes harboring newly synthesized proteins. According to Coleman and Taylor (54), almost half of the radiolabeled proteins were present in synaptosomes of presynaptic
origin, while most other newly synthesized proteins were localized in unidentified structures, some of which might be also derived from nerve endings. In addition, about one-tenth of the radiolabeled proteins were associated with membrane-bound particles containing endoplasmic reticulum. Comparable data were reported by Gambetti et al. (105). According to these authors, up to half of the radiolabeled proteins were present in synaptosomes originating from presynaptic endings. Of these proteins, one-fifth were associated with mitochondria and the rest were localized in the periphery of presynaptic synaptosomes. Of the remaining newly synthesized proteins, 5% was present in fragmented bodies, ~10% in nonpresynaptic structures without ribosomes, and about one-third in nonpresynaptic structures containing ribosomes. The latter particulates were attributed to fragmented glial cells or presynaptic structures containing ribosomes. The latter structures without ribosomes, and about one-third in nonpresynaptic structures containing ribosomes. The latter structures without ribosomes, and about one-third in nonpresynaptic structures containing ribosomes. Hence, the rat synaptosomal fraction contains other protein-synthesizing cell elements besides the predominant presynaptic nerve endings.

Inhibitors of glycolysis, tricarboxylic acid cycle, and oxidative phosphorylation strongly inhibited synaptosomal protein synthesis, which proved its dependence on ATP generation by intrasynaptosomal mitochondria (15, 236). Likewise, lowering the osmolarity of the incubation medium markedly decreased synaptosomal protein synthesis, due to synaptosome swelling and rupture of the plasma membrane, with the consequent release of synaptosomal contents into the medium. The dependence of synaptosomal protein synthesis on the appropriate concentrations of Na⁺ and K⁺ in the medium was traced to the activity of the synaptosomal membrane Na⁺-K⁺-ATPase, in agreement with the marked inhibition by ouabain, a specific inhibitor of the ion pump (10, 15, 308). Ouabain inhibited both CXM- and CAP-sensitive systems of synaptosomal protein synthesis, and its inhibition was mediated by lowering the level of intrasynaptosomal K⁺ (309).

The synaptosomal fraction contains extramitochondrial cytoplasmic RNA and other elements of the protein synthesis machinery. In early studies (14), cell body mitochondria and intrasynaptosomal mitochondria from rat brain were shown to contain similar concentrations of DNA (6.3 µg/mg protein) and RNA (12–13 µg/mg protein), but higher concentrations of RNA were present in synaptosomal membrane fractions (up to 21 µg/mg protein). In later studies, extramitochondrial synaptosomal RNA was identified as rRNAs and 4S RNA that were consistently attributed to contaminating elements (59, 69, 89, 211). Nonetheless, in addition to mitochondrial RNAs and typical cytoplasmic rRNA (18S, 28S) and small RNA (4S, 5S), the synaptosomal fraction of adult rat brain contained two RNA components that were absent in mitochondrial and microsomal fractions (59, 60). They were of medium size (8.9 kDa) and of large size (>60S). Upon electrophoretic fractionation, synaptosomal poly(A)⁺ RNA manifested a clearly distinctive distribution, with a marked prevalence of high-molecular-weight components. In addition, the 7S RNA component of the synaptosomal poly(A)⁺ RNA fraction was much less evident in the mitochondrial fraction, and altogether absent in microsomes. The selective synaptosomal localization of 7S RNA and of a larger RNA component was confirmed in adult rats (71). In that paper, intrasynaptosomal mitochondria were reported to contain cytoplasmic rRNAs and 4S RNA, while synaptic vesicles contained 5S RNA, 4S RNA, and cytoplasmic rRNAs. The cytosol fraction only contained 4S RNA. Hence, synaptosomes define a unique cytoplasmic microdomain whose RNA compositional profile is clearly distinguishable from that of other cell fractions.

It is of interest that in experiments on young rats, brain synaptosomes and mitochondria were reported to contain a much higher proportion of poly(A)⁺ RNA than polysomes (~18% compared with 7%), and to display a similar capacity to direct protein synthesis in a cell-free translation system (69). Synaptosomal membranes and synaptosomal mitochondria had a similar content of poly(A)⁺ RNA (15.5 and 13.5%, respectively), but the capacity to direct protein synthesis in a cell-free system was much lower for the RNA prepared from synaptosomal membranes. Lower concentrations of poly(A)⁺ RNA were reported in synaptosomes and mitochondria from adult rats, but their contents were still higher than in microsomes (60). Altogether, these observations may reflect that the synaptosomes generated by nerve endings may have higher activity of protein synthesis in the developing brain of younger animals.

The presence of particular RNA species in nerve endings was also documented in other systems. RNP particles known as “vaults” have been reported to be highly enriched in the cholinergic nerve terminals of the electric organ of Torpedo marmorata in which they are closely associated with synaptic vesicles (138). Vaults are transported along the axon in either distal or proximal directions, as indicated by their accumulation on either side of a nerve lesion (205). Vault RNA is a small RNA transcribed by RNA polymerase III.

1. Alternative interpretations: a commentary

The evidence described above supported the view that nerve endings contained a prevalent cytoplasmic system of protein synthesis and a less active mitochondrial system. Nonetheless, the validity of this concept was repeatedly challenged, and alternative interpretations were proposed to account for the data. To simplify our task, these interpretations were assigned to two groups according to whether 1) the existence of presynaptic protein synthesis was accepted but attributed to unusual
types of protein synthesizing machinery, or 2) the existence and role of presynaptic protein synthesis was marginalized in the belief that most synaptosomal protein synthesis derived from dendrites and/or glial processes.

Followers of the first alternative assumed that synaptosomal protein synthesis was due to either 1) a unique CAP-resistant, CXM-sensitive system selectively present in brain mitochondria and/or synaptosomes; 2) an aberrant CAP-sensitive extramitochondrial system; or 3) a nonribosomal incorporation of amino acids. However, these proposals were based on hypothetical speculations rather than on sound evidence, were mutually exclusive, or were quite limited in scope. In addition, they ignored the wealth of consistent data demonstrating the coexistence of cytoplasmic and mitochondrial systems of protein synthesis in synaptosomes. The detailed description of these data and the related criticism were provided in a comprehensive review (see Ref. 7).

If we now consider the second group of papers marginalizing the existence of presynaptic protein synthesis, they appear to be based on a misrepresentation of pivotal EM autoradiographic data which actually demonstrate the localization of a major share of newly synthesized proteins in presynaptic synaptosomes (54, 105; see also sect. IVB). Instead, their attention was focused on ribosome-containing synaptosomes assumed to derive from dendritic processes or glia (264, 291, 310). In the paper by Verity et al. (310), most protein synthesis was assumed not to be associated with presynaptic endings. The argument was based on the observation that the radiolabeled proteins of denser synaptosomal subfractions displayed higher specific activities. However, the substantial amount of total radioactive proteins recovered in the lighter fractions derived from the presynaptic endings was not taken into account. Hence, the negligible contribution of nerve endings to synaptosomal protein synthesis appears to be an overstatement. With that said, it should be made clear that the presence of postsynaptic and glial elements in mammalian synaptosomal preparations cannot be denied. We merely object to the conclusion that synaptosomes generated by those nonpresynaptic structures represent an overstatement. With that said, it should be made clear that the presence of postsynaptic and glial elements in mammalian synaptosomal preparations cannot be denied. We merely object to the conclusion that synaptosomes generated by those nonpresynaptic structures represent the main source of newly synthesized synaptosomal proteins. In a previous detailed review article (7), we fully acknowledged the experimental evidence supporting the presence of glial (136, 278) and dendritic elements (264, 323) in mammalian brain synaptosomes.

In summary, while there is general agreement that mammalian synaptosomes contain a cytoplasmic system of protein synthesis in addition to a mitochondrial system, the conclusion that protein synthesis is absent or negligible in nerve endings does not withstand critical analysis. Rather, this notion appears more based on the reiteration of the long-standing dogmatic view than on the available data. Despite the convincing demonstrations that the axon domain contains a local system of protein synthesis (see sects. III and IV), the opinion that mammalian nerve endings are substantially devoid of protein synthesis seems to be still lingering. The ongoing controversy on this issue has been reviewed elsewhere (7, 120, 183, 262).

2. Effects of stimulation on synaptosomal protein synthesis

Chemical and electrical stimulation of rat synaptosomes have been reported to induce marked increments in protein synthesis, but most effects have been ascribed to postsynaptic elements, often by default. Depolarizing treatments of the membrane potential elicited a marked enhancement of synaptosomal protein synthesis. Electrical stimulation of synaptosomes induced a fourfold increment of protein synthesis accompanied by increased O2 uptake (322). The largest increments regarded SDS-soluble and SDS-insoluble proteins of the “junctional complex,” while less conspicuous effects concerned Triton X100-soluble proteins. In addition, several proteins with molecular masses between 10–18 and 32–87 kDa became more intensely labeled. These effects were markedly inhibited by tetrodotoxin, a Na+ channel blocker. On the contrary, protein synthesis in C-6 glial cells was not modified by the same electrical stimulation, suggesting that the enhancement of synaptosomal protein synthesis was not mediated by the translational machinery of glial origin. As the increment of protein synthesis was reversible with CAP more than with CXM, the electric stimulation appears to affect the mitochondrial protein synthesis more than the cytoplasmic counterpart.

A different method was used to examine the effect of a depolarizing medium (40 mM KCl) on a synaptosomal fraction prepared by filtration of brain homogenates through pores of decreasing size (323). Under these conditions, the content of polysomes rapidly but transiently increased. Stimulation of protein synthesis in this mode persisted in the presence of CAP, thus excluding a mitochondrial contribution, but was strongly repressed by BAPTA-AM, a chelator of cytosolic calcium. The stimulating effect of depolarizing media was mimicked by glutamate, PKC-activating agent, and agonists of metabotropic glutamate receptors (mGluR). The PKC inhibitor calphostin C strongly inhibited the activation of mGluR and consequently repressed protein synthesis in rat synaptosomes, supporting the idea that Ca2+ signaling and mGluR are implicated in the synaptic protein synthesis.

A rather unusual approach was followed to distinguish neuronal and glial contributions to the changes in protein synthesis in the rat sensory-motor cortex induced by electrical stimulation of the brachial plexus in vivo. The approach was based on the assumption that glucose is the preferred substrate of neurons while acetate is the preferred substrate of glial cells (2). After an intraperito-
neal administration of $[^{14}\text{C}]$glucose, newly synthesized proteins increased significantly in the stimulated cortex, while administration of $[^{14}\text{C}]$acetate resulted in minor decrease. Interestingly, most enhanced protein synthesis took place in the synaptosomal fraction following $[^{14}\text{C}]$glucose administration (35). Despite the fact that glucose can also be used by glia, the lack of stimulating effect by acetate favors the interpretation that synaptosomal protein synthesis is stimulated by neuronal activity.

Local protein synthesis has been demonstrated to be crucial to the BDNF-induced long-term potentiation (LTP) of hippocampal synapses (168, 338), but no distinction was made as to the presynaptic or postsynaptic localization of the protein synthesis.

3. Mitochondrial protein synthesis in synaptosomal fractions

The mitochondrial synthesis of proteins examined in synaptosomal fractions of the rat cerebral cortex is most active in 10- to 13-day-old rats, but soon declines after weaning and remains essentially unmodified in 2-yr-old animals (211). Synthesis of mitochondrial proteins did not correlate with the content of corresponding mitochondrial mRNAs. Indeed, the ND3 and ND5 peptide subunits of NADH dehydrogenase were nearly absent among newly synthesized mitochondrial proteins, despite the normal levels of their mRNAs. Accordingly, gene expression in synaptosomal mitochondria appears to be largely regulated at the translation level. Gel electrophoretic fractionation of translation products of synaptosomal mitochondria demonstrated their strong inhibition by CAP. Surprisingly, CXM also inhibited the synthesis of a few proteins with molecular mass below 50 kDa that are generally considered mitochondrial proteins. Hence, it appears that, in the rat brain synaptosomal fraction, an extramitochondrial system of protein synthesis is at work utilizing a selective subset of mRNAs. This apparently paradoxical effect can be explained by recalling that, in squid presynaptic synaptosomes, nuclear-encoded mitochondrial proteins are synthesized by the local cytoplasmic system of protein synthesis (110, 164).

4. Nerve endings of the Mauthner neuron

Only a limited amount of work has concerned the short axon collaterals of the M-axon and their terminal projections. Nonetheless, these elements have been shown to contain polysomes using ribosome-specific antibodies (see Fig. 14 of Ref. 7), and more recently ESI methods (R. Martin and E. Koenig, unpublished observations). In these nerve terminals, most ribosomal aggregates display a peripheral localization.

V. SYNTHESIS OF AXONAL AND PRESYNAPTIC RNA: THE CELL BODY HYPOTHESIS

The identification of cytoplasmic RNA in the axon domain of vertebrate and invertebrate species raised the question of its cellular origin. Initial views held, almost by default, that axonal RNA derives from the neuron cell body, in agreement with the influential concept of axonal transport of proteins and organelles (125). However, the experimental demonstration of this hypothesis by radiolabeling perikaryal RNA and monitoring its displacement toward distal axonal sites was met by considerable difficulties in the interpretation of the data. Indeed, at variance with the axonal transport of radiolabeled somatic proteins, the wave of radiolabeled RNA invading the nerve was generally associated with, and often preceded by, a wave of radiolabeled RNA precursors (16, 34, 36, 106, 107, 160, 258, 263). Under these circumstances, the RNA precursors entered periaxial glia and were incorporated into radiolabeled RNA that was retained in the glial cells and partly transferred to the axon. Hence, axonal RNA might be produced locally as well as being transported from the cell body. Because of this experimental pitfall, axonal transport of RNA has been clearly demonstrated only in a few model systems, such as the transport of 4S RNA in regenerating goldfish axons (160).

Nonetheless, in more recent experiments with well-differentiated neurons, the microinjection of radiolabeled BC1 RNA into the cell body of the goldfish M-neuron was followed by its anterograde axonal transport (242). In addition, the microinjection of truncated fragments of this small RNA indicated that the 5’-segment specifically targeted to the axon. The experiment also showed that BC1 RNA colocalizes with PARPs (Fig. 6A). More recently, fish and mammalian PARPs have been shown to also contain β-actin mRNA and its binding protein ZBP-1 (287a). As the latter protein allows the association of mRNAs to the molecular motors of the kinesin family accountable for anterograde axonal transport, PARP’s mRNAs may be derived from the neuron cell body. The axonal transport of soma-derived small RNAs is likewise indicated by the accumulation of RNA-containing vault particles on the proximal side of a Torpedo nerve crush. As shown in Figure 6B, vault particles start to increase linearly a few hours after the crush, while the linear accumulation of synaptic vesicles does not display a lag period. This different behavior may be interpreted to indicate that the distal transport of vault particles in well-differentiated neurons only occurs after the lesion-induced neuronal dedifferentiation is under way. An analogous, albeit more tentative, explanation might hold for the axonal transport of BC1 RNA in the goldfish M-neuron, provided a comparable process of dedifferentiation is assumed to have followed the microinjection of this RNA into the neuron soma.
Neuronal mRNAs are largely associated with cytoskeletal elements, notably microtubules and actin filaments (139, 279, 290). As part of RNA granules also containing ribosomes and proteins, these mRNAs were later shown to move distally along microtubules in the neurites of cultured neurons (172, 197). Highly compacted RNA granules have been isolated and characterized from cultured neurons of embryonic rat forebrain (190) and from adult mouse brain (167, 141). These granules are prevented from engaging in translation activity during transport, but attain a less compact organization and give rise to active polysomes upon depolarization (190). RNA granules from mouse brain contain as many as 42 proteins and are translocated by kinesin exclusively into the dendritic domain (141, 167, 222, 283). Neuronal RNA granules have been distinguished into three classes on the basis of their macromolecular composition, i.e., transport RNPs, stress granules, and P (processing) bodies (171). Stress granules and P bodies are, respectively, involved in recruiting mRNAs to regulate their translation or to degrade them. So far, stress granules and P bodies have only been identified in dendrites, and transport RNPs have only been detected in growing neurites and in mature dendrites. To our knowledge, none of these three types of RNA granules has yet been described in the axons of well-differentiated neurons (287, 317; but see Ref. 205).

The necessary participation of somatic translation processes in plastic events occurring at synaptic sites has been initially reported in cultured Aplysia neurons (44, 220) and, more recently, in well-differentiated Aplysia neurons (128). In the latter preparation, the synapse-specific long-term facilitation induced at a distal synapse required active protein synthesis at the presynaptic site and at the neuron soma, as well as axoplasmic transport. The latter two requirements strongly suggest that the neuron soma is involved in the stabilization of the synaptic plastic change but fall short of suggesting that this event requires the delivery of soma-derived transcripts to the nerve terminal.

In conclusion, despite the clear demonstrations in cultured neurites and developing axons, only a few available data support the contribution of soma-derived neuronal transcripts to the process of gene expression in the axons and nerve endings of differentiated neurons. Hopefully, this putative intraneuronal pathway will be more extensively investigated in the future to elucidate the nature of the cell body’s participation to peripheral plastic events vis à vis with the concurrent local contribution of periaxonal and perisynaptic glial cells.

VI. LOCAL SYNTHESIS OF AXONAL RNA

A. Model Systems

1. The squid giant axon

Evidence for the local synthesis of axoplasmic RNA was first obtained when microinjected radiolabeled uridine was incorporated into RNA in the giant axon of the squid Dosidicus gigas (96). Experiments testing the possible origin of axoplasmic RNA from the neuron cell bodies or from periaxonal glia were later made on the giant axon from Loligo vulgaris (62). With the use of an intact stellate ganglion-nerve preparation placed into an elongated chamber, the ganglion was confined at one end.
sealed off from the nerve by silicone grease. To determine if axoplasmic RNA was synthesized in the neuronal cell bodies, radiolabeled uridine was added to the ganglion compartment, and incubation was allowed to proceed up to 24 h. Despite an extensive series of analyses, newly synthesized axoplasmic RNA did not appear to be derived from the neuron cell bodies, as it only reached the most proximal short segment of the axon that also contained large amounts of the radiolabeled precursor. Conversely, incubation of isolated nerves with $[^3H]$uridine gave unequivocal evidence of the local synthesis of axoplasmic RNA (62). Its amount was sizeable after 4 h and markedly increased after 8 h presumably because radiolabeled RNA synthesized in periaxonal glial cells needed additional time to reach the axoplasmic core. The glial origin of axoplasmic RNA was also confirmed in vivo. One day after $[^3H]$uridine was injected near the stellate nerve of live squid, radiolabeled RNA was recovered from the axoplasm of axon segments near the injection site, while the neuronal cell bodies manifested no evidence of incorporation of the injected RNA precursor.

Axoplasmic RNAs synthesized by isolated giant axons were eventually identified as tRNA and rRNAs by electrophoretic fractionation (Fig. 7), and as poly(A)$^+$ RNA by adsorption on oligo(dT)cellulose (265). rRNAs were identified by the unique features of squid rRNAs, whose larger species (30S) is formed by the noncovalent association of the smaller component (20S) with an rRNA of lower size (17S) (41). As these bonds are easily broken, in a significant number of analyses newly synthesized axoplasmic RNA displayed 20S and 17S species rather than 30S and 20S rRNAs. Newly synthesized axoplasmic poly(A)$^+$ RNA represented 1% of the total radiolabeled RNA. Axoplasmic RNA synthesized by isolated squid giant axons was also shown to be assembled in ribonucleoprotein particles. At short incubation time, newly synthesized axoplasmic RNA manifested a much larger proportion of low molecular weight components compared with the RNAs synthesized in the periaxonal glial sheath or the nerve cell bodies (229).

With periaxonal glia being the sole possible origin of the locally synthesized RNA in the isolated axon, the experiments with squid giant axons were eventually continued with internally perfused axons (Fig. 2B), which allowed more direct kinetic analyses of RNA synthesis. With the collection of perfusate samples at intervals, radiolabeled RNA was shown to enter the axon perfusate within minutes after the addition of $[^3H]$uridine to the incubation chamber. The radiolabeled RNA continued to accumulate in the perfusate at an approximately linear rate for several hours. This process was markedly inhibited by the transcription inhibitor actinomycin D. In sedimentation analyses on linear sucrose gradients, most newly synthesized perfusate RNAs were of low molecular weight, some of them colocalizing with a tRNA marker. In addition, sizable amounts cosedimented with marker subribosomal particles (92, 117). The prevalence of low molecular weight RNAs was in agreement with similar observations made with isolated squid giant axons (229).

The essentially linear accumulation of radiolabeled RNA in the perfusate was exploited to further examine the mechanism of glial RNA synthesis and transfer (62, 265). In a large set of experiments, the perfused axon was subjected to a number of manipulations after the basal rate of radiolabeled RNA delivery to the axon perfusate stabilized. Initial tests aimed at reproducing the modifications of glial membrane potential elicited by high-frequency stimulation of squid and crayfish giant axons that were attributed to the release of one or more neurotransmitters (91, 207, 312, 313). These experiments led to the identification of several receptors on the glial plasma membrane, including a cholinergic receptor and metabo-

![Fig. 7. Axoplasmic RNA synthesized by isolated squid giant axons includes tRNA and rRNAs.](http://physrev.physiology.org/)

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tropic and ionotropic glutamate receptors. Their activation was responsible for a long-lasting glial hyperpolarization (≈10 mV) from the resting value of about −40 mV. This condition was mimicked by exposing the perfused squid giant axon (and its surrounding glia) to 100 mM K+ that depolarized all plasma membranes. Interestingly, this treatment shifted the basal linear kinetics of radiolabeled RNA accumulation in the axon perfusate by creating a marked biphasic increase of the RNA transfer rate (Fig. 8A) (117). Essentially similar results were obtained by addition of glutamate, one of the products of ectopeptidase-mediated hydrolysis of the axon-released NAAG. On the contrary, no effect was yielded by N-acetylaspartate, the other product of NAAG hydrolysis that had been shown not to alter glial potentials. Both NAAG and glutamate bind to class II metabotropic glutamate receptors, but only glutamate binds to the glial NMDA receptor (102, 304). Addition of NAAG to the incubation medium also produced a dual wave of enhanced delivery of radiolabeled RNA to the axon perfusate, mimicking the effect of membrane depolarization (Fig. 8C). Comparable results were obtained by addition of glutamate, one of the agonists of either NMDA (MK801) or cholinergic receptors (d-tubocurarine) were used in combination with the corresponding agonists, the delivery of radiolabeled RNA to the axon perfusate remained at its basal level. Hence, the stimulating effect of the neurotransmitters on glial RNA transfer is specific to the receptors. Together with the previous data (62, 229, 265), the above results provide convincing evidence that the transfer of glia-synthesized RNA into the axoplasmic domain is modulated by axon-glia communication (92, 117). Comparable cell-to-cell RNA transfer takes place in different eukaryotic cells, such as the follicular cells and the oocytes of insects and reptiles (29, 217, 238).

A key problem raised by the intercellular transfer of glial RNAs and RNPs to the axons (see sect. VI, A2 and B) or other neuronal domains (see sects. VI B and VI B) regards the mechanism of such intercellular translocation process. Comparable problems are raised in all cases of transcellular exchange of macromolecules or organelles in eukaryotic organisms. Most likely, the underlying mechanisms may be different in different systems. Starting with the cytoplasmic bridges joining the follicular cells and the oocyte (217, 238) or glial cells and crayfish axons (253), a number of different mechanisms have been proposed. They include an invagination of glial cytoplasm into the axon followed by pinching off and engulfment of the glial invagination (103, 195), or a combined process of trans-endocytosis leading to the formation of spinules (288). More recently, a novel mechanism has been documented in different cell types including cultured PC12 nerve cells. The mechanism involves the intercellular formation of 50- to 200-nm diameter membrane-bound nanotubules containing F-actin and myosin Va. This specialized intercellular structure was postulated to mediate the

![Fig. 8. Glial modulation of [3H]RNA delivery to the axon perfusate. After the basal rate of RNA transfer was established, experimental manipulations were applied (vertical arrows). Marked increments in the content of perfusate [3H]RNA are induced by the following treatments. A: addition of 100 mM KCl to the incubation medium leading to massive depolarization of both axonal and glial plasma membranes. B: perfusion of the axon with a low concentration of K+ that induces depolarization of the axonal membrane only. C: addition of N-acetylaspartylglutamate (NAAG) to the incubation medium. D: addition of N-acetylaspartate (NMDA) to the incubation medium. NAAG activates a glial metabotropic glutamate receptor and generates glutamate and N-acetylaspartate by ectopeptidase hydrolysis. Glutamate (but not N-acetylaspartate) also activates a glial ionotropic glutamate receptor (NMDA receptor). [Modified from Eyman et al. (92).]]
translocation of organelles and membrane proteins (143, 273).

2. The Mauthner axon

Evidence of the local synthesis of axonal RNA was initially provided by microchemical analyses of axonal and myelin RNA in transected proximal stumps of the goldfish M-fiber (77). In specimens of 6–8 cm body length, the RNA content in M-axons and myelin sheaths remained essentially unchanged after axotomy. Nonetheless, their base composition underwent remarkable changes as early as 12 h after the lesion, with control values only reestablished after 20–30 days. The main variation concerned the A/G ratio that increased from 0.56 to 0.8 in the axon and from 0.6 to 0.9 in the myelin sheath. These changes indicated rapid RNA turnover inside the axon and the myelin sheath throughout the entire proximal segment of the M-fiber. Their rapid onset and similar direction of base composition changes suggested a common local origin of the newly synthesized RNA, most probably in the oligodendroglial nucleus.

Additional evidence for a local synthesis of axonal RNA was suggested by stimulation of the M-neuron in vivo, accomplished by rotating caged goldfish around their longitudinal axis at 60 cycles/min for 30 min (163). These conditions increased the sensory input to the M-axon. The content of axonal RNA initially dropped 40% by 30 min after the end of the stimulation, but started to increase in the following period. By 180 min, the content of axonal RNA fully recovered. The RNA content in the myelin sheath underwent similar changes, but with marked variability. Nonetheless, the simultaneous increments in axonal and myelin RNA occurring between 30 and 180 min after the stimulation suggested that they derived from the same source.

Incorporation of radiolabeled precursors into the RNA of isolated M-fibers lacking their glial nuclei was reported later (80). After incubation with radiolabeled uridine and cytidine lasting to 21.5 h, newly synthesized RNA was identified as 4S RNA by sedimentation on a continuous sucrose density gradient. Despite the absence of nuclei, the incorporation was strongly inhibited by actinomycin D, a finding that suggested its mitochondrial origin. Conversely, when the incorporation reaction was allowed in the spinal cord segments in which M-fibers still contained their glial cell bodies, newly synthesized RNA isolated after a 7-h incubation period comprised 16S and 28–30S rRNAs in addition to 4S RNA. Interestingly, most of this newly synthesized RNA was in the axon (almost 80%). The synthesis of cytoplasmic tRNAs in the axons of M-fibers containing glial nuclei, but not in M-fibers lacking these nuclei, strongly support their transcription on the oligodendrocyte nuclear DNA. The origin of 4S axonal RNA synthesized by isolated M-fibers remains a bit more puzzling even if a mitochondrial derivation appears likely.

B. Vertebrate Axons

Following the demonstration of low concentrations of axonal RNA in the cranial nerve roots of adult cats (175), evidence of the local synthesis of mammalian axonal RNA was provided in rabbit accessory nerve roots (177). In these experiments using 3-h incubation with radiolabeled RNA precursors, the synthesis of axonal RNA was markedly inhibited by actinomycin D, but most newly synthesized RNA was resistant to RNase, presumably because it was shielded by a dense cytoskeletal matrix (187). Pulse-chase experiments with cat nerve roots suggested that the axonal RNA originated from an exogenous source (178), presumably periaxonal cells.

The local synthesis of axonal RNA was also indirectly suggested by analyses of RNA content and base composition of spinal motoneurones, perineuronal glia and axonal balloons in rats treated with iminodipropionitrile for 10 wk (282). This compound induces behavioral excitement and the marked hypertrophy of nerve cell bodies and axons indicative of increased protein synthesis. While the RNA content and base composition of neuron somas and glial cells did not significantly differed from control values, the RNA base composition of axonal balloons became significantly different from that of neuronal cell bodies. The RNA of axonal balloons had a consistently lower adenine content (17.2 vs. 21.6%) but a higher content of cytosine (27.8 vs. 24.9%) and uracil (25.8 vs. 23.3%) compared with the RNA of the neuron soma.

Using light and EM autoradiographic methods, axons of the newt (Triturus viridescens) brachial nerves were shown to incorporate [3H]uridine from 1 h to 8 days after the intraperitoneal injection of this precursor (280). As noted for the incorporation of the protein precursor [3H]histidine in the same nerves (281), the decentralized nerve manifested the same extent and pattern of RNA precursor incorporation. As the silver grains were also distributed over the nucleus and cytoplasm of Schwann cells and over the myelin, these data supported the origin of axonal RNA from periaxonal glia, in agreement with similar data from other model systems (see sect. VI).

In the peripheral nervous system of rats, the local synthesis of axonal RNA (and protein) was demonstrated by quantitative autoradiography of proximal stumps of axotomized sciatic nerves in vivo (25). Following 30-min incubation with radiolabeled precursors, newly synthesized RNA was present in Schwann cells, myelin sheaths, and axons in order of decreasing concentrations. This finding suggests that the periaxonal glial cell is the origin of the axonal RNA in the sciatic nerve. Interestingly, the peak of incorporation occurred 1 day after axotomy, in
good agreement with the period of maximal increment of protein synthesis in transected hypoglossal nerves (298). As also noted in the newt experiments (280), the intercellular transfer of RNA might have occurred in Schmidt-Lantermans incisures and/or in paranodal regions, i.e., in loci where glial cytoplasm is abutting the axon (39, 192, 239).

Considerable support for the concept of intercellular transfer of ribonucleoproteins from myelinated Schwann cells to axons was obtained in peripheral nerves of the mouse W1d$ mutant in which the onset of Wallerian degeneration was obtained in peripheral nerves of the mouse W1d$ mutant in which the onset of Wallerian degeneration was markedly delayed (38, 123, 212). As shown by conventional EM methods, in distal stumps of sciatic nerves axotomized 1 wk earlier, the axons did not show signs of degeneration but contained large numbers of polysomes, some of which associated with membranes. Polysomes appeared to have been synthesized locally by surrounding glial cells, as they were associated with newly synthesized RNA (F. Court and J. Alvarez, unpublished data). More recent studies with fluorescent antibody probes selectively identifying ribosomes, axoplasm, and glial cytoplasm provided convincing evidence of a transcellular transport of ribosomes from Schwann cells to the axon (55). A comparable process also occurred in the wild-type C57BL strain, albeit with lesser intensity. With the use of conventional EM methods, double-walled vesicles containing ribosome-like particles have been described at the internode axon-myoelin interface of chicken spinal motoneurons. Some of these vesicles are continuous with the subjacent axon. They are thought to be involved in the transfer of glial ribosomes to the axon (206). Comparable ultrastructural data have recently been reported in the rat sciatic nerves examined with an antiribosomal polyclonal antibody (195).

VII. LOCAL SYNTHESIS OF PRESYNAPTIC RNA

A. Early Evidence

More than three decades ago, the local synthesis of a presynaptic enzyme in a tissue lacking the cognate nerve cell bodies suggested the local presence of the encoding mRNA. In this early experiment, the activity of the heart tyrosine hydroxylase (TH) was significantly increased a few days after the administration of reserpine, which depletes amine neurotransmitters from nerve terminals. TH activity in the heart is due to the innervating cathecolaminergic nerve terminals. Interestingly, this effect was markedly depressed by CXM, which was given when the heart TH activity was on a sharp rise (295). The latter observation and the substantial lack of involvement of axonal transport suggested that presynaptic TH protein in the heart may be locally synthesized and that the cathecolaminergic terminals contain TH mRNA.

More recently, RT-PCR analyses of the rat CNS extended this finding and demonstrated that TH mRNA is present in the cathecolaminergic axons and nerve terminals of cerebellum, striatum, and pituitary intermediate lobe. This conclusion is based on the absence of cathecolaminergic cell bodies from these brain regions (228). As observed for the heart enzyme, the content of TH mRNA in the axons and nerve terminals may be locally modulated. While unilateral damage to the cathecolaminergic pathway led to a significant decrease of the same mRNA in the ipsilateral striatum, reserpine induced a marked increase of TH mRNA in the cerebellum. Hence, the occurrence of protein synthesis and mRNA in the nerve endings raised a fundamental question on the cellular origin of the components of protein synthesis machinery in the presynaptic domain.

B. Model Systems

1. Squid nerve endings

As mentioned in section IV A1, the unusually large synaptosomes of squid optic lobes are exclusively generated by the nerve terminals of photoreceptor neurons. They constitute the most abundant component of the synaptosomal fraction and are essentially the only synaptosomes containing an active system of protein synthesis (57, 58, 226). These unique properties and the remote location of the cell bodies of these terminals suggested that they could be profitably used to test the hypothesis of the local origin of presynaptic RNA. Accordingly, the large synaptosomes isolated from optic lobe slices that had been incubated with $[^3H]$uridine were expected to contain radiolabeled RNA. If so, the result would have proven the local transcription of presynaptic RNA, in view of the anatomical absence of the parental photoreceptor cell bodies from the incubated slices.

When the optic lobe slices were incubated for periods longer than 30 min, $[^3H]$RNA was more abundant in the synaptosomal fraction than in the microsomal/cytosolic fraction. However, its content in the synaptosomal fraction more than doubled after 60 min. Hence, beyond 30 min, the newly synthesized RNA was largely prevalent in the synaptosomal fraction than in the microsomal/cytosolic compartment (92, 116). Such enrichment was all the more remarkable in view of the abundance of presynaptic nerve endings in squid optic lobe synaptosomes (Fig. 5B). The contribution of newly synthesized microsomal or cytosolic RNA was negligible. In fact, when the incubated optic lobe slices containing radiolabeled RNA were homogenized in a hypomotic buffer, the radioactive synaptosomal RNA was released into the medium and nearly completely lost from the synaptosomal fraction (92). The latter effect was expected from the swelling and bursting open of synap-
tosomes (110). Conclusive proof of the presence of newly synthesized RNA in the large synaptosomes was provided by autoradiographic analyses of the synaptosomal fraction. Essentially all radiolabeled RNA was localized in the presynaptic synaptosomes identified by their large size and their selective staining with a presynaptic antibody (Fig. 9A) (92, 116).

Similar to the experiments with the perfused squid giant axon (see sect. VI A1), sedimentation analyses of the locally synthesized presynaptic RNAs indicated that the largest share of radiolabeled RNA was of relatively low size, as it cosedimented with marker tRNA. In addition, sizable amounts of RNA cosedimented with marker subribosomal particles, raising the possibility that the newly synthesized RNA may include mRNA (Fig. 9B). The prev- alence of low-molecular-weight RNAs at shorter incubation times had previously been noted for axoplasmic RNA synthesized by isolated giant axons (229). On the whole, axonal and presynaptic RNAs of low molecular weight may undergo a remarkably high turnover. In principle, they may be held responsible for the rapid modification of the base composition of axonal RNA elicited by axotomy (77), or for the rapid metabolic changes following stimulation (162). While the physiological significance of the particular enrichment of small RNA species in axons and nerve terminals is still largely unknown, some of these small RNAs might be iRNAs involved in the modulation of axonal and presynaptic protein synthesis. This interesting possibility has been already observed and addressed in neurons (189), dendrites (276), developing axons, and growth cones (135), and more recently in axons of adult mouse sciatic nerves (240).

C. Vertebrate Nerve Endings

1. Synaptosomal mitochondrial RNA

Since rat synaptosomal fractions contain mitochondria, notably at nerve terminals, it was of interest to discern whether mitochondrial RNA synthesis occurred in synaptosomal fractions. As shown by Attardi and collaborators (89), the synthesis of mitochondrial rRNA is much more intense in synaptosomal preparations from 10-day-old rats than in rats of 30 days and is extremely low in adult rats. In rats of 10 days, the majority of radiolabeled RNAs included 16S, 12S, and 4S species. Trace amounts of newly synthesized RNAs larger than 18S were also observed. Interestingly, the synthesis of RNAs larger than 4S was markedly inhibited by ethidium bromide (EB; a compound that specifically inhibits mitochondrial RNA synthesis). On the other hand, the inhibition of 4S RNA synthesis by EB was much weaker. Likewise, RNAs larger than 4S satisfactorily annealed with mitochondrial DNA, but 4S RNA annealed less efficiently. These data established the occurrence of mitochondrial RNA synthesis in synaptosomal fractions, but implied that synaptosomal 4S RNA may partly comprise nonmitochondrial species. Comparable data were reported with regard to the synthesis of 4S RNA in the axons of isolated M-fibers (80). The latter findings were attributed to the synthesis of mitochondrial RNA without direct evidence (see sect. VI A2). Interestingly, EM autoradiographic data on the rat synaptosomes showed that 50% of newly synthesized EB-sensitive RNA was present in presynaptic synaptosomes.

![Presynaptic RNA of squid photoreceptor neurons is synthesized by local cells. A: colocalization of locally synthesized RNA (black dots) and anti-syntaxin antibody (purple) in large synaptosomes. Scale bar, 10 μm. Synaptosomes were prepared from squid optic lobe slices incubated 2 h with [3H]uridine and processed for immunostaining using an antibody against squid syntaxin, a presynaptic marker protein. B: sedimentation pattern of newly synthesized presynaptic RNA. Direction of sedimentation is to the right. Vertical arrows indicate the position of marker tRNA and of the small and large subribosomal particles (s-RNA and l-RNA). Inset: percent distribution of radiolabeled RNA (●; mean ± SE of 6 experiments) and marker subribosomal particles (○) in the gradient regions containing subribosomal particles. [Modified from Eyman et al. (92).]]
2. Synaptosomal extramitochondrial RNA

At variance with mitochondrial RNA synthesized in presynaptic synaptosomes, the cellular origin of extramitochondrial presynaptic RNA has not been directly investigated in vertebrates. As a result, only some data may be presumed to regard nerve terminals.

Synthesis of synaptosomal RNA was examined in young rats after the intracranial injection of $[^3H]$uridine and $[^3H]$adenosine (69). At the earliest time examined (1 h), the specific activity of synaptosomal RNA was approximately half that of mitochondrial RNA and one-fourth that of polysomal RNA. Nonetheless, in the following time period, the specific activity markedly increased in synaptosomes, but it increased to a lesser degree in mitochondria and polysomes. In addition, the specific activity of synaptosomal poly(A)$^+$ RNA remained higher than in polysomes for several hours (Fig. 10A), indicating that the turnover rate of poly(A)$^+$ RNA was much higher in synaptosomes than in polysomes. Interestingly, the poly(A) tail of newly synthesized synaptosomal poly(A)$^+$ RNA was only half as long as that in polysomes (Fig. 10B). This feature suggests that most newly synthesized synaptosomal poly(A)$^+$ RNA may have a presynaptic localization. Indeed, the poly(A) tail of axonal mRNAs in the mammalian hypothalamic-hypophysial tract is markedly shorter than that of perikaryal mRNAs (232, 233). As polyadenylation of mRNA is implicated in the regulation of the message (218, 269), this interesting feature of newly synthesized synaptosomal poly(A)$^+$ RNA may provide an important clue as to its role.

The subcellular distribution of newly synthesized brain RNA was also determined in adult rats 1 h after the intraventricular injection of $[^3H]$uridine (61). At this time, synaptosomes contained a lesser amount of newly synthesized RNA than mitochondria and microsomes. However, the proportion of their radiolabeled poly(A)$^+$ RNA was somewhat higher than in mitochondria and markedly higher than in microsomes and nuclei. These data confirmed the surprisingly high content and turnover of newly synthesized poly(A)$^+$ RNA in rat synaptosomes.

VIII. TRANSFER OF GLIAL RNA TO THE NEURON CELL BODY: THE GLIA-NEURON UNIT

A. A Brief Overview

Except in very primitive organisms and some tissue cultures, neuronal cell bodies, axons, nerve endings, and dendrites are always in close contact with glial cells. In oligodendrocytes and Schwann cells of myelinated axons, most cytoplasm resides in the perinuclear region surrounding the myelin sheath. Glial cytoplasm is also present in the spiral wrappings that abut the axon at the internodal Schmidt-Lantermans incisures and paranodal regions (39, 192, 239). Even in nonmyelinated axons, glial cells form a layer of variable thickness around axons. Morphological evidence of the intimate relationship of glial cells with neuronal cell bodies has been pointed out since the beginning of last century (140, 144). These observations suggested the name of “trophospongium” for the system of tiny channels presumed to mediate the exchange of cytoplasmic organelles from glial cells to neurons. By the
same token, glial cells were once named “trophocytes.” Comparable observations with better analytical methods were later made in a large number of vertebrate and invertebrate nervous systems, often leading to suggestions of underlying exchanges of macromolecules between glia and neurons (for reviews, see Refs. 68, 193, 230, 243, 292, 311, 320, 327). Glial cells have also been shown 1) to display resting membrane potentials of medium or high values that are liable to undergo slow and relatively long-lasting modifications (102, 193, 194, 312) and 2) to be endowed with a host of ion channels and membrane receptors for many types of neurotransmitters (20, 207, 241, 270).

These basic features suggest that glia and neurons are involved in mutually cooperative roles. In view of the widespread intercellular cooperation present in multicellular organisms, interactions of glial and nerve cells may be considered a more complex and specialized example of a general phenomenon. Cell-to-cell interactions are usually selective and dynamic, and the involved partner cells are likely to inform each other of their functional states before engaging in coordinated actions. Experimental evidence supporting intense exchange of signals among glial cells and neuronal domains has substantially increased in recent years, largely with regard to the mammalian perisynaptic glia (12, 28, 272, 314). Cooperation between glial cells and neurons is likely to have started in the early stages of phylogenetic evolution of the nervous system, presumably to maintain a proper ionic milieu in the extracellular space or to perform basic collaborative functions. In time, with the progressive increase in size and complexity of the nervous system in metazoan animals, the glia-neuron cooperation attained a wider scope and gave rise to functional specializations allowing more complex performances. An obvious example is the extensive myelin wrappings around vertebrate axons.

For a detailed basic knowledge on the classification, embryologic origin, and properties of glial cells, the readers are referred to the informative publications covering this field (19, 68, 193, 311, 320, 327).

B. Experiments With Single Cells

Microchemical methods of analyzing microdissected tissue samples (82) provided the first data on the biochemical composition of glial and nerve cells, notably in the experimental conditions of enhanced nervous activity or learning. An important issue raised in this field concerned the degree of mutual contamination of the glial and nerve cell samples and the contamination by extraneous structures (193). The purity of microdissected glial cells and neuron cell bodies has been largely assessed by visual inspection, and generally reported to be as high as 85–90%. On a first approximation, isolated neuronal nuclei and cell bodies, especially of large size, have been considered least contaminated, while clumps of glial cells are likely to be less pure.

1. Enhanced nervous activity

The identification of morphological changes induced by exposure of selected groups of nerve cells to functional loads (or to axotomy) was one of the main issues pursued in the later part of the 19th century, notably after the identification of Nissl bodies in neuronal perikarya. These studies were greatly stimulated by the development of quantitative cytospectrophotometric methods to determine nucleic acids in single cells (for a rigorous example, see Ref. 11; for a review, see Ref. 45). An even stronger impetus was provided by the later availability of micro-manipulators and microscale biochemical methods with which the RNA content and base composition of microdissected cell samples could be measured at the picogram level (82). The RNA base composition was determined with the standard errors of the mean typically within 1–2% of the average (84, 151). It was through this method that the existence of mRNA in single eukaryotic cells (initially termed “nonribosomal DNA-like RNA”) was first distinguished from rRNA or tRNA. A high value of the GC/AU ratio indicated the prevalence of rRNA, while lower values suggested the presence of mRNA.

In the early neurobiological investigations of this kind, the lateral vestibular nucleus of Deiters became an experimental system of choice, as its unusually large neurons could be hand-dissected with relative ease (Fig. 11). In rabbits, an average Deiters neuron was reported to have a volume of 93,000 μm³ and a surface area of 50,000 μm², most of which belonged to dendrites (60%) (158). Stained with a dilute solution of methylene blue, each neuron appeared to still harbor ~10,000 nerve terminals. The dry weights of its cytoplasm, nucleus, and nucleolus corresponded to 20,094, 800, and 100 pg, respectively. Each Deiters neuron was surrounded by 35–40 glial cells, mostly oligodendrocytes. Because of their distinct size, Deiters neurons could be analyzed singly, but the glial cells needed to be grouped in clumps of six to eight cells trimmed to about the same volume of the nerve cell. This constraint is likely to account for the relatively higher degree of contamination of the glial samples.

Although electrophysiological activity of Deiters neurons was never recorded, they were assumed to be stimulated through the vestibular input when rabbits were rotated 120° horizontally and 30° vertically at 30 rpm (with their head away from the axis of rotation). This treatment was routinely imposed 25 min each day for 7 consecutive days. At the end of this period, the dry weight of the neuron increased ~12%, mostly through an increment in density. A comparable increase also occurred after 1 day of stimulation. The content of protein in-
creased from 16,080 to 18,060 pg/cell, and that of RNA from 1,545 to 1,612 pg/cell. In contrast to these effects, the content of RNA in the same volume of glial cells underwent a marked decrease (from 123 to 85 pg). Comparable inverse changes in glia and nerve cells were also observed with regard to cytochrome oxidase, while succinoxidase increased in neurons (2.7-fold) but remained essentially unchanged in the glial sample. Under resting conditions, the activity of both enzymes was consistently higher in the glial sample. These data were interpreted to reflect a functional cooperation between neurons and glia (the neuron-glia supracellular unit) that was poised to give neurons "metabolic priority" in oxidative metabolism and to provide neurons with key glial components such as RNA under conditions of enhanced neuronal activity. Anaerobic glycolysis also showed inverse changes in neurons and glial cells, but in the opposite direction (130). Under a chronic work load, neurons enhanced their oxidative metabolism and reduced anaerobic glycolysis, while glial cells did the opposite. These responses were specific for perineuronal glia, as pericapillary glia was only barely affected (129). Hence, the physiological significance of this metabolic shift in glia was probably related to the metabolic need of adjacent neurons.

Comparable changes were induced by short-lasting treatments of live rabbits or rats with pharmacological compounds. In rabbits, administration of the malononitrile dimer (TRIAP: 1,1,3-tricyano-2-amino-1-propene) for 1 h elicited marked inverse changes in protein and RNA of Deiters neurons and glia (86). The neuronal RNA and protein increased by nearly 25%, while the glial RNA decreased by 45%. In addition, TRIAP modified the base composition of neuronal RNA by increasing G and decreasing C. Conversely, in glial cells close to the neuronal perikaryon, the RNA base composition changed in the opposite direction, in that G markedly decreased and C markedly increased. Comparable data were later reported in Deiters neurons and glia of rabbits treated for 1 h with tranylcyclopropylamine, an inhibitor of monoamine oxidase (155).

The Deiters neurons also provided insight into the intracellular transfer of RNA. In fact, when rabbits were treated with TRIAP for 1 h, the RNA content of the Deiters cell body increased (see above), but the RNA content of the cell nucleus decreased 16%. The nuclear RNA also underwent base composition changes, with a marked decrease in uracil (151). Hence, in addition to the RNA transfer from glia, the neuronal cytoplasm received a U-rich RNA fraction synthesized in the nucleus. These data may hardly be attributed to contamination by extraneous material, as assumed for the glial changes (193). In addition, they imply that some neuronal RNA responses may be attributed to the synthetic capacity of the neuron. When the RNA changes induced by TRIAP were calculated with regard to the fraction of RNA lost or gained by neurons or glia, striking similarities emerged (156, 157). The RNA gained by neurons had a base content of 22.5% A, 37.7% G, 21% C, and 18.8% U, which was strikingly similar to the base composition of the RNA lost by the glial sample (21.2% A, 37.8% G, 23% C, 17.6% U). Furthermore, the RNA gained by each neuron (570 pg) approximately corresponded to the RNA lost by its surrounding glia. These similarities were interpreted to support the hypothesis of an intercellular transfer of RNA. At variance with these data, chronic administration of TRIAP to mice and rats for 2–5 wk did not induce RNA changes in neurons and glial cells, although the treatment elicited marked increments in neuron size and neurofilament content, and in myelin thinning (282). These results supported the view that significant changes in the RNA of the neuron cell body and surrounding glia only occurred after acute stimulation, but were overshadowed by hypertrophic processes elicited by chronic stimulation (318–320).

**FIG. 11.** Single neurons dissected from the brain for microchemical analyses. A: micrograph of a large Deiters neuron obtained from an adult rabbit by freehand dissection. The neuron cell body and basal dendrites are densely covered with synaptic knobs. B: micrograph of a Deiters neuron whose synaptic knobs have been detached from the cell body and basal dendrites by a 30-min incubation in isotonic medium. The RNA content and base composition of single Deiters neurons were determined under conditions of enhanced vestibular activity or following training. [Modified from Hyden et al. (159).]
RNA responses to enhanced neuronal activity were also reported in neurons and glia cells examined by ultraviolet cytosectrophotometric methods (260). One advantage of these methods is that the measurement is made in situ, without microdissection of individual cells. In anesthetized cats, high-frequency stimulation of the preganglionic nerve to the superior cervical ganglia for 3 h induced a significant increase of RNA in neuronal cytoplasm (44%), but a 30% decrease of glial OD<sub>265</sub>, an index of its nucleic acid content. Since the cellular DNA content is not expected to change, these values were attributed to variations of the RNA content. The inverse pattern of changes in neurons and glia was similar to that obtained using microchemical methods (see above). Hence, this weakened the possibility that the comparable changes detected with the microchemical methods were due to the dendritic RNA entangled in the glial sample (see also sect. VIII B3). Comparable results were later obtained with different neurons and their corresponding glial cells analyzed with the same method (261). In agreement with previous data, these results suggested that an increment in neuronal RNA and protein was associated with the loss of glial RNA only if the stimulation was sufficiently intense and/or prolonged. Under conditions of greater functional load, neuronal and glial RNAs could both decrease. Conversely, in the following rest period, RNA recovery first occurred in glial cells and then, with delay, in neurons. Hence, glial cells synthesize RNA at a faster rate than neurons, in agreement with other experiments including direct analyses of microdissected hypoglossal neurons and glial cells (63).

The slowly adapting stretch receptor neurons of the lobster (*Homarus gammarus*) provided a considerable advantage as an experimental model system, as they allowed both microchemical RNA analyses and electrophysiological monitoring of neuronal activity. In one study, this neuron was stimulated up to 6 h by stretching the muscle. This created an average frequency of action potentials close to 5/s and a total number of spikes ranging from 24,000 to 132,000. Compared with the control cells, the RNA content was not modified by activity, but significant increments occurred in its A content, A/U ratio, and purine-to-pyrimidine ratio (126). This experiment is remarkable as it confirmed that the base composition of neuronal RNA could be significantly modified by a physiological work load imposed under rigorous experimental conditions. In the follow-up investigation, the same neuronal preparation was used to examine the effect of more intense stimulations (average spike frequency of 5–10/s) that lasted longer (up to 24 h) and yielded a higher total number of spikes (from 175,000 to 600,000) (85). The high turnover of neuronal and glial RNA was demonstrated by incubating for 8 h a nonstimulated preparation with the transcription inhibitor actinomycin D. Under these conditions of complete inhibition of RNA synthesis, marked losses occurred in neuronal and glial RNAs (~26 and ~19%, respectively). In addition, the RNA base composition of both neuron and glia showed marked parallel changes (~2.7% A, and +2.2% C), suggesting the loss of a short-lived RNA fraction rich in A and poor in C. On the other hand, when preparations incubated with actinomycin D were continuously stimulated for 8 h, the loss of neuronal RNA was conspicuously alleviated (~10% instead of ~26%), but the loss of glial RNA remained high (~15% instead of ~19%). In addition, when a control preparation not inhibited by actinomycin D was continuously stimulated for 8 h, the RNA content of the surrounding glia significantly decreased (~21%) while the RNA content and base composition of the stretch receptor neuron did not significantly change. These results imply that the stimulated neuron is able to maintain its RNA content through a route other than RNA synthesis, presumably through the uptake of glial RNA.

2. Learning

More intriguing results were reported with regard to the changes in the content and base composition of neuronal and glial RNA under learning conditions (152, 153). In the first experiment (152), adult rats were trained to climb a long wire placed at 45° from the cage floor to reach the food provided on a high platform. Rats trained in daily sessions of 45 min needed 4–5 days to perform a full walk. Thereafter, the number of daily visits to the platform increased from 3–4 to ~20 in the following 3 days. When food was removed from the platform, the frequency of trips started to decrease. In this experimental protocol (balancing task), the total content of cellular RNA in Deiters neurons slightly increased 8 days after the beginning of training, with no change in its base composition. However, the A/U ratio of the nuclear RNA increased from 1.06 to 1.35. Likewise, a marked increment in A and a decrease in C occurred in glial RNA. These changes suggested the prevalent synthesis of DNA-like RNA (mRNA) during the learning process. This shift in RNA base composition was specific to learning, as control rats subjected to vestibular stimulation did not display changes in A or U contents. The RNA change in the nucleus of experienced rats was not persistent, as the A and U contents returned to the normal level when rats were not trained for a day.

Comparable results were reported in cortical neurons of rats trained in a reversed handedness protocol (154). In this experiment, rats were initially selected according to the preferred paw they used to gather food from a glass cylinder placed 5 cm above the cage floor. Right-handed rats were then forced to use the left paw by an obstacle. Training sessions of 25 min were scheduled twice a day. After 200 forced reaches, memory was retained for at least 9 mo. Four days after the start of
training, analyses were made on groups of 10 pyramidal neurons of layers 5 and 6 that were shown to be required for learning the task. This procedure was followed because each nerve cell only contained 22 pg RNA. When cortical neurons of the right hemisphere were compared with the homologous neurons of the left hemisphere (assumed not to be involved in learning this task), their RNA content was ~23% higher and its base composition presented a marked decrease in C and small increments in A, G, and U. The lowered GC/AU ratio (from 1.72 to 1.51) suggested a significant increment of mRNA. This effect was also specific to learning, as in the experiments with control rats the RNA of pyramidal neurons from either cortical side was identical in content and base composition. The influence of contingent factors was excluded, as when rats used their preferred right paw, neurons of the left cortex only showed a minor increase in RNA and no change in its base composition.

3. Commentary

The interpretation of these microchemical data has been the target of a pointed criticism regarding technical and theoretical issues. Notably, in an alternative interpretation (193), the inverse changes reported in neurons and glia were attributed to the intracellular shift of RNA moving to the neuron soma from dendrites entrapped in the glial samples. Additional criticism regarded the general absence of electrophysiological monitoring of neuronal activity and the arbitrary selection of the analyzed cells. Forty years after such a negative assessment of this entire field, the growing body of evidence that the axon domain contains local systems of protein synthesis fed by glial transcripts calls for the reconsideration of those pioneering data. Indeed, their consistency and specificity support the view that those early studies opened more encompassing and intriguing perspectives in the understanding of the cellular biology of the nervous system.

True, most experiments on the effects of increased functional loads did not include monitoring by electrophysiological methods, but this was actually done in a few experiments (see Refs. 85, 126, 260, 261). True, it is surprising that randomly collected neurons of the same type displayed consistently similar biochemical changes when exposed to a certain experimental condition. Nonetheless, objections to an imperfect experimental design do not imply that neural activity did not increase, nor that a given type of neuron cannot respond in a similar fashion to a given challenge. These considerations convey doubts, not disproof, and doubts in science should lead to better designed experiments and appropriate controls, rather than to the neglect of a whole field of endeavor.

Furthermore, the alternative suggestion that the inverse changes detected in glial and nerve cells may reflect an intracellular translocation of RNA rather than an intercellular exchange is still lacking experimental support. Dendrites are known to import RNA from the neuron cell body rather than exporting it to the perikaryon, or even less likely to the neuronal nucleus. This early criticism appears to have been largely based on the lack of information on intercellular exchanges of materials, which is now well known to occur in eukaryotic cells, such as follicular cells and oocytes (217, 238). The data supporting intercellular transport of glial RNA to the neuron soma have also been corroborated by cytospectrophotometric methods that may hardly be blamed for confusing a glial cell with a dendrite (260, 261). As reviewed in this article, intercellular transport of newly synthesized RNA from periaxonal glia to the axon has been demonstrated in vertebrate (25, 80, 177, 280) and invertebrate species (9, 62, 92, 117, 229, 265), as well as between perisynaptic cells and squid nerve terminals (92, 116).

While the latter data make it more likely that comparable macromolecular exchanges may occur between perisomatic glia and neuron cell bodies, it is to be noted that the neuronal response to a chronic functional load consists in a long-lasting wave of enhanced RNA synthesis, as extensively documented by increments in the cytoplasm-to-nuclear ratio of neuronal radiolabeled RNA (318–320). Hence, at least some of the changes in neuronal RNA are due to the transcriptional capacity of neurons. These two modes of providing somatic RNA are not mutually exclusive. In neurons with a relatively limited cytoplasmic mass, deriving RNA from the cell nucleus may be sufficient, especially under conditions of moderate activity. On the other hand, neurons with an overwhelming cytoplasmic mass, notably if engaged in intense activity, might also require the additional supply of glial RNA (see sect. vi). Such a glial assistance is also suggested by the increased number of perineuronal glial cells concomitant with the progressive increment in the mass of neuronal cytoplasm. A clear example is provided by the striking linear correlation between the number of oligodendrocytes surrounding the neuron cell bodies of Clark’s column in the spinal cord and the length of their axons (100, 267). This correlation remains valid whether the axon length varies with the segmental position of its soma along the spinal cord or according to the body size of mammalian species (from mouse to whale). More recently, the number of perisomatic glial cells in spinal ganglia was also reported to increase with the volume of the neuron cell body (203, 227). Since cell body volume is related to axon length, the former variable may be more directly conditioning the ratio of glial number to axon length. Such a tightly paired association of neuron size with perineuronal glial number suggests that one of the major factors of this striking outcome regards the multiplication of glial genomes. A comparable multiplication at a genomic scale occurs in the follicular cells of developing...
local systems of gene export. It may be noted that the role played by multiple sets of cell genomes may not be substantially dissimilar from the amplification of more restricted DNA regions, except for the global extent of its trophic potential.

It may nonetheless be asked whether the RNA lost from perineuronal glia is exclusively targeted to the neuron cell body. An intriguing possibility is suggested by the alleged presence of a very large number of nerve terminals attached to the surface of dissected neuronal perikarya. In Deiters neurons, this number is estimated to be 10,000 (Fig. 11) (158). Hence, the increase in the cell body RNA could be attributed to its associated nerve endings, in analogy to the transfer of newly synthesized RNA from perisynaptic cells to squid presynaptic terminals (92, 116). According to this hypothesis, glial RNA would be transferred to a neuron domain incapable of transcribing but capable of translating cytoplasmic RNAs. A quick supply of transcripts to this domain would allow its quick response to rapidly changing cell commitments. Whatever the ultimate target of perisomatic glial RNA may be, it is worth noting that the rate of RNA synthesis in glial cells is at least twice as high as in neurons when calculated per unit amount of RNA (63). This observation implies that the turnover rate of glial RNA is twice as high as in neurons. Although the content of RNA in glial cells is generally much lower than in neurons, the large number of glial cells per neuron and the high turnover of glial RNA are likely to fulfill a role presumably related to its fleeting export function.

**IX. THE LOCAL SYSTEM OF GENE EXPRESSION IN AXONS AND NERVE TERMINALS**

The concept of a supracellular “glia-neuron unit” was proposed ante litteram at the beginning of last century (144), but gained momentum only thanks to the extensive microanalytical work on the transfer of RNA and protein from glia to the neuron cell body (for a review, see Ref. 157). While the latter data were severely criticized (193), and the possibility of glia-to-neuron transfer of RNA was largely forgotten, the concept of the glia-neuron unit was revived in studies of the local synthesis of proteins in isolated giant axons of crayfish (30, 147) and squid (104, 200, 201, 277, 303). The squid data were taken to support the idea that locally synthesized axonal proteins exclusively derive from periaxonal glia, although later experiments demonstrated this view to be incorrect (see sects. III and IV).

The demonstration that axons and nerve terminals possess autonomous systems of protein synthesis does not exclude the possibility that glial proteins may be transferred to these peripheral neuronal domains. Indeed, a number of experimental data demonstrate the transfer of glial proteins (and RNA) to the neuron cell body (see sect. VIII), to the squid giant axon (sect. A1) and nerve endings (sect. VII), and to the mouse Wld® axons (see sect. A2). Furthermore, axons, nerve terminals, and neuron cell bodies are capable of protein uptake (115, 145, 188, 191, 321). Hence, in each neuronal domain, it is reasonable to ask what proteins are locally synthesized and what others are derived from glia. Of the locally synthesized proteins, only some have been identified, but most of them are unknown and of doubtful origin. As exemplified in Figure 12 for the squid giant axon, the population of proteins synthesized by axoplasm may be quite different from those transferred from glia. In future studies, the comparison between locally synthesized and glia-transferred proteins may reveal interesting correlations, especially if basal data will be compared with those gathered under conditions of enhanced activity.

As outlined in the previous sections, the neuron soma cannot be considered the only source of axonal and presynaptic proteins. Rather, proteins of the axon domain also derive from local systems of gene expression that integrate glial transcription events with the translation machinery operating in axons and presynaptic terminals. These extrasomatic systems provide the neuronal periphery with the trophic support needed to implement responses that are largely independent from the neuron cell body. These local systems of gene expression are likely to be the phylogenetic consequence of the massive cytoplasmic growth of the axon domain that came to outpace the trophic capacities of the neuron soma (315). Clearly, this novel concept may only be accepted on the basis of the following evidence: 1) that glial cells transcribe RNA and deliver it to axons and nerve endings, 2) that glial transcripts are translated by the cytoplasmic systems of protein synthesis present in axons and presynaptic sites, and 3) that the latter systems are modulated by local transcription processes. The aforementioned conditions are all supported by the experimental evidence in axons, albeit to a different degree in different axons. On the other hand, some evidence is lacking for nerve terminals, notably for mammalian presynaptic endings, chiefly because of the lack of suitably designed experiments. Accordingly, the supportive data will be summarized separately for the two domains. Such a distinction is also justified by their different functional roles. The missing elements from this novel mosaic will be highlighted as they occur.

**A. Axons**

The presence of a cytoplasmic system of protein synthesis has been convincingly documented in all axons that have been investigated. The main features of this
system have been well characterized in model preparations of vertebrate and invertebrate species in terms of macromolecular components and translation products (see sect. III). Cogent data are likewise available with regard to the local synthesis of axonal RNAs. This information was initially obtained from axonal model systems of particularly large size that are more readily amenable to suitable analyses. Nonetheless, a number of other axons of normal size have provided supporting data from investigations with autoradiographic or microchemical methods (see sect. VI). In the isolated Mauthner fiber of the goldfish, the synthesis of axonal rRNAs only occurs in the presence of the glial nucleus. In the isolated squid giant axon, locally synthesized axonal RNAs have been identified as cytoplasmic tRNA, rRNAs, and poly(A) + RNA, which require nuclear DNA for transcription. Hence, the data imply transcription processes occurring in periaxonal glia, the only nucleus-containing cell element present in the isolated axon. Direct evidence of their glial origin has been recently provided by the involvement of glial glutamatergic and cholinergic receptors in the delivery of newly synthesized RNA to the perfusate of giant axons.

In mammalian peripheral nerves, the identification of the mRNAs coding for neuron-specific NF proteins in Schwann cells (95, 170, 271, 286) is consistent with a glial transcription of axonal mRNAs, and with their transcellular transfer to the axon. More recently, the latter process has been elegantly demonstrated in the myelinated axons of mouse Wld” mutants (55). In addition, axonal protein synthesis has been shown to depend on local transcription events. The first convincing observations of this modulatory process were made more than 30 years ago with regard to the resynthesis of AChE in intact and axotomized mammalian nerves. In these experiments, the local synthesis of axonal proteins was shown to be markedly inhibited by RNase or by inhibitors of RNA synthesis such as 5-fluororotate, and markedly stimulated by a paradoxical effect of actinomycin D (see sect. III). Hence, local protein synthesis in vertebrate axons is also largely dependent on the transcriptional contribution of periaxonal glial cells.

B. Nerve Terminals

Presynaptic protein synthesis has been convincingly demonstrated and characterized in a squid model preparation (see sect. IV A). As to mammalian nerve endings, a wealth of data supporting their contribution to synapticosomal protein synthesis has been presented and critically discussed in section IV B. Direct evidence is provided by EM autoradiographic data demonstrating that at least half of the newly synthesized synapticosomal proteins are localized in presynaptic synaptosomes (54, 105). A more detailed survey of the available evidence is reported in a previous review article (7).

The existence of cytoplasmic protein synthesis in mammalian nerve endings is also indirectly suggested by the existence of a translation system in growth cones (66, 190). In a number of experiments, this system has been shown to play an essential role in axon guidance during development (37, 42, 204, 231, 332, 333). Hence, the local system of protein synthesis is highly functional. The dramatic burst of protein synthesis that occurs at the very
end of the proximal stump of axotomized neurons (174, 177) demonstrates the participation of local protein synthesis in axon regeneration. Its physiological significance has been recently elucidated by investigations of regenerating axons in various model systems (75, 101, 133, 134, 146, 215, 254, 255, 302, 330, 339; for reviews, see Refs. 23, 331). There is hardly any reason to assume that growth cones would silence their protein synthesis soon after reaching their targets and maturing into nerve endings. In mature neurons, nerve endings are farthest away from the nerve cell body, and their proteins undergo continuous turnover to comply with rapid changes in synaptic plasticity. Experimental data demonstrate that the physiological need to replenish the constituent proteins is met by local protein synthesis (see sect. IV).

Direct evidence for local synthesis of presynaptic RNA has been recently documented in the nerve terminals of mammalian nerve endings, chiefly because of the lack of suitably designed experiments. Nonetheless, the following data provide some initial encouraging support: 1) the local modulation of TH mRNA in brain regions containing catecholaminergic nerve endings but not their cell bodies (228), and 2) the prevalence of poly(A)+ RNA and of newly synthesized poly(A)+ RNA in rat synaptosomal fractions (59–61, 69). The major presynaptic localization of this RNA is suggested by the markedly shorter length of its poly(A) tail compared with microsomal poly(A)+ RNA derived from cell bodies (Fig. 10B). A shorter poly(A) tail is a characteristic feature of axonal mRNAs (232).

A key link requiring experimental verification concerns the local transcriptional modulation of presynaptic protein synthesis. This may be considered the main missing tessera of the proposed novel mosaic. As the local transcription of presynaptic RNA has been reported only recently with regard to a squid model system (92, 116), it is not surprising that this tessera is still missing. In some respects, and with a due sense of proportions, the present state of the glia-neuron hypothesis may be reminiscent of the Metchinikoff’s map of chemical elements at the time of their compilation. Of course, we have good reasons to believe that even the presently missing links will be brought to light in the near future.

X. CONCLUSION AND NOVEL PERSPECTIVES

The original supracellular concept of the “glia-neuron unit” takes on a deeper and more stimulating meaning in the present proposal, as it leads to the view that the axon domain is served by a distinct local gene expression system based on the transcellular delivery of glial transcripts. This feature allows peripheral regions of the neuron to promptly respond to changing local challenges, thereby bypassing the slower communications with the cell body. Such a mechanism is of special euristic value for the nerve endings, as it also allows their highly selective plastic modifications (128, 220). From this point of view, a local gene expression system may also be assumed to exist in dendrites, notably those displaying an extensive arborization (e.g., Purkinje or pyramidal neurons). Nonetheless, macromolecular contributions from the neuron cell body are expected in all peripheral neuronal domains. A schematic representation of the view purporting the cell body origin of all neuronal RNAs is shown in Figure 13A. Conversely, the view proposed and discussed in the present article is presented in Figure 13B. The distinctive roles of the somatic and glial source of neuronal RNAs remain to be determined under basal conditions and during enhanced physiological activity.

As implied by our considerations, the main consequence of the novel version of the glia-neuron hypothesis regards the molecular basis of the substantial autonomy that axons and nerve endings implement in their plastic responses. Such autonomy is manifested in both physiological and pathological contexts. Several examples have been provided with regard to the long-term structural and functional changes of differentiated axons (for a review, see Ref. 7) and nerve terminals (94, 128, 168, 219). These changes require the activation of gene expression systems...
and are by far the most common and relevant modifications of the nervous system. Yet, it is still widely believed that maintenance and remodeling of axons and presynaptic terminals depend largely or exclusively on the neuron cell body. The recognition that these neuronal domains are actually endowed with local gene expression systems would substantially revise this prevailing belief and emphasize the essential and more prompt involvement of the local systems. This view does not only imply that the gene expression system of the neuron cell bodies is not relevant in promoting long-term local plasticity. Rather, it only emphasizes that axons and nerve terminals utilize local mechanisms that are integrated in a much more complex context than once thought. In the novel experimental perspective, the respective roles of the peripheral and somatic gene expression systems should be evaluated and integrated into a better understanding of the physiology and pathophysiology of the glia-neuron unit. In view of the overwhelming prevalence of brain plasticity in health (development, aging, learning, sleep, etc.) and disease (peripheral neuropathies, dementias, drug abuse, brain ischemic or hemorrhagic insults, mental disorders, etc.), the heuristic value of this perspective cannot be overemphasized.

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