Protein Synthesis and Processing in Cytoplasmic Microdomains Beneath Postsynaptic Sites on CNS Neurons

A Mechanism for Establishing and Maintaining a Mosaic Postsynaptic Receptive Surface

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Contents

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Abstract

Recent studies have shown that protein synthetic machinery consisting of polyribosomes and associated membranous cisterns is selectively localized beneath synaptic sites on neurons. In the present paper, the role of this machinery in neuronal function will be considered. We will:

1. Summarize the studies that characterize the polyribosomes and define their associations with membranous cisterns. Taken together, these observations suggest the existence of a system for the synthesis and posttranslational processing of proteins at individual synaptic sites;
2. Review the evidence that the protein synthetic machinery is particularly prominent during the initial formation of synaptic contacts (during early development), and during lesion-induced synaptogenesis in mature animals. These observations have led to the hypothesis that the polyribosomes produce proteins that play a role in the formation of the synaptic junction;
3. Review evidence that supports the hypothesis that there is a local synthesis of protein within dendrites, as well as local glycosylation;
4. Describe the evidence suggesting that at least some of the protein constituents of the synaptic junction itself are synthesized locally; and
5. Descibe our studies that reveal a mechanism for selective dendritic transport of RNA; this transport mechanism permits the delivery of RNA to postsynaptic sites throughout the dendritic arbor.

We will advance the hypothesis that neurons position protein synthetic machinery together with the mRNA's that are appropriate for particular synapses beneath synaptic contact regions. At the synaptic site, this machinery could then direct the synthesis of particular proteins that are critical for synapse formation or maintenance. The positioning of protein synthetic machinery at postsynaptic sites permits a rapid local regulation of the production of key proteins by events at individual synapses.

Index Entries: Polyribosomes; RNA, dendritic transport of; protein synthetic machinery; synaptogenesis; dendrites; synaptic junction.

Introduction

Neurons are unique among cells because they receive a large number and variety of synaptic contacts, and thus must construct and maintain an immensely complex mosaic surface membrane. This is particularly true of neurons of the CNS. Many CNS neurons elaborate dendritic arbors that are hundreds of micrometers long; these dendrites are contacted by tens of thousands of individual synapses. At each synaptic site is a postsynaptic membrane specialization that contains neurotransmitter receptors and their associated ion channels, molecules to define the chemical identity of the contact, adhesion molecules, regulatory proteins such as calmodulin, calmodulin-binding proteins, protein kinases, as well as proteins to anchor the intramembranous molecules at the junctional region.

It is thought that postsynaptic membrane specializations contain some molecules that are general, and some molecules that are unique to particular classes of synapses. For example, most postsynaptic membrane specializations apparently contain structural proteins including actin. On the other hand, receptors for particular neurotransmitters are presumably concentrated only at synaptic sites that are contacted by terminals that contain those transmitters. This has been established for at least some neurotransmitter receptors (for example, the glycine receptor, *see* Triller et al., 1985). Thus, synaptic specializations apposed to different types of presynaptic elements presumably contain a different complement of molecules. Since CNS neurons usually receive many different types of synaptic contacts, each requiring an appropriate complement of recognition molecules, receptors, channels, second messenger systems, and so forth, neurons must elaborate an immensely complex receptive surface composed of a large number of plasma membrane microdomains.

It is currently not known how the protein constituents of the neuronal receptive surface are synthesized and delivered to the different membrane domains. In other cell types, it is thought that different plasma membrane domains are constructed as a result of a targeted transport of proteins to particular destinations. For example, epithelial cells of the small intestine have two principle membrane domains (apical and basolateral) that contain different enzymes and permeases. These proteins are synthesized in the endoplasmic reticulum, and are found together in the Golgi vesicles, yet they somehow become segregated so that they can be inserted specifically into the appropriate plasma membrane domain (for a review, *see* Simons and Fuller, 1985). It is thought that this sorting is accomplished at the stage of formation of transport vesicles, and that the vesicles with different contents are then targeted to different cellular destinations on the basis of "address markers." The actual mechanism of the sorting and differential targeting are unknown, even in the relatively simple epithelial cell.

Obviously, the problem faced by even the simplest neuron in establishing its mosaic surface membrane is orders of magnitude more complex than for an epithelial cell, or indeed for almost any other cell type. Certainly, neurons possess elaborate mechanisms for protein sorting and transport (Grafstein, 1977; Hammerschlag, 1983; Kelly, 1985). However, if neurons construct their surface membranes through targeted transport of proteins, the transport machinery must be even more elaborate and specific than currently envisioned. It is especially difficult to envision how the delivery of proteins to particular sites could be regulated based on local need, for example, when individual synaptic sites undergo long-term plastic changes in response to local signals (activity), or when synaptic sites are rebuilt after injury.

In the present paper, we will review the evidence for an alternative mechanism for deliver-

ing different protein constituents to different intracellular destinations. Specifically, we will develop the hypothesis that neurons synthesize some of the key proteins of their receptive surface locally within cytoplasmic microdomains beneath the membrane surface that represents the final destination of the proteins. The hypothesis applies specifically to the synthesis of proteins for postsynaptic sites, and derives from our studies revealing a selective positioning of protein synthetic machinery comprised of polyribosomes and membranous cisterns beneath synaptic junctions (Steward and Levy, 1982; Steward, 1983b). We will review studies that:

1. Characterize the protein synthetic machinery positioned beneath synaptic sites;
2. Reveal that the protein synthetic machinery is particularly prominent during periods of synapse growth;
3. Demonstrate that protein synthesis as well as protein glycosylation occur throughout the dendritic tree of neurons, at a considerable distance from the cell body;
4. Provide evidence suggesting that some of the protein constituents of the postsynaptic membrane specialization are synthesized on-site; and
5. Define how the machinery itself, specifically RNA, is delivered as the result of a selective RNA transport system.

Selective Localization of Protein Synthetic Machinery at Postsynaptic Sites on CNS Neurons

The selective localization of polyribosomes beneath synaptic sites was initially discovered through electron microscopic studies of granule cells of the dentate gyrus (Steward and Levy, 1982). Although it had long been known that dendrites contain a few polyribosomes (Peters et al., 1976), the fact that polyribosomes were concentrated beneath postsynaptic sites had been noted only in passing (Bodian, 1965). Our quantitative analyses revealed that this localization was quite selective. For example, in granule cells of the dentate gyrus, about 80% of the polyribosomes in mid-proximodistal dendrites were precisely positioned just at the base of dendritic spines. The polyribosomes were seen under profiles that could unequivocally be identified as spines in single sections (Fig. 1) or under mounds in the dendrite; serial section analysis revealed that most of the mounds in fact represent the base of spines that extend out of the plane of the section (Steward and Levy, 1982). Quantitative evaluations of other brain regions revealed similar associations between polyribosomes and dendritic spines on all types of neurons (Steward, 1983b). Moreover, polyribosomes are also prominent beneath synaptic contacts on axon initial segments (Steward and Ribak, 1986). The presence of polyribosomes beneath synaptic sites on axon initial segments is of particular interest, since most of these synapses do not terminate on spines; moreover, many of the synapses are presumably inhibitory, since the terminals contacting the initial segment contain glutamic acid decarboxylase (Freund et al., 1983; Somogyi et al., 1983). These observations suggested that polyribosomes are a ubiquitous component of the subsynaptic cytoplasm of a variety of different types of synaptic junctions.

Polyribosomes are found in the subsynaptic cytoplasm at a wide variety of synaptic types, but they are not present beneath all spines in mature animals. Quantitative electron microscopic analyses of a variety of cell types in mature rats revealed that about 10–15% of the profiles that could be positively identified as spine bases in single sections have underlying polyribosomes (Steward and Levy, 1982; Steward, 1983b). Of course, the actual incidence of polyribosomes is higher than the relative incidence would suggest, because not all of the area comprising the spine base is visible in any

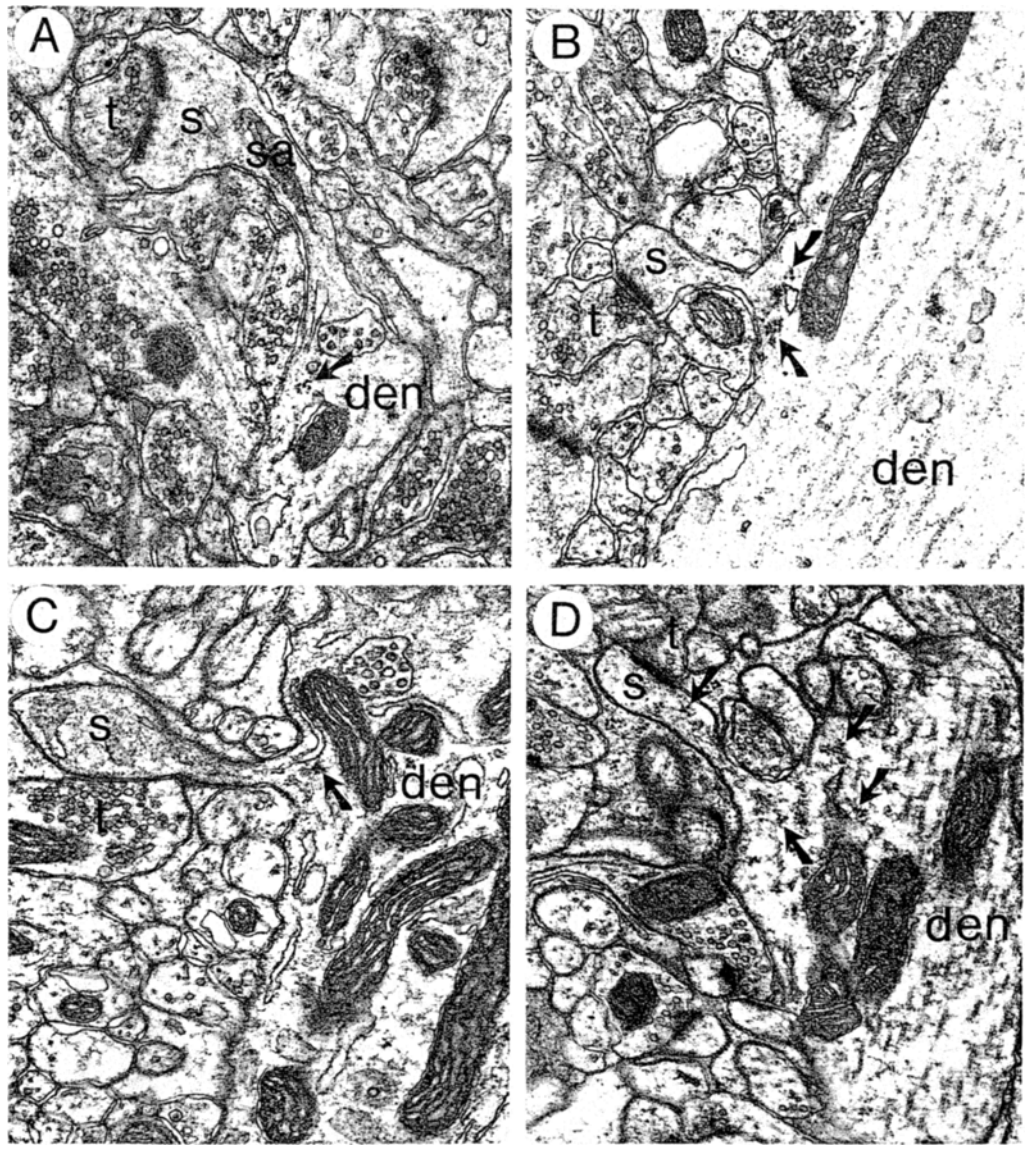

Fig. 1. Polyribosomes beneath dendritic spines in adult rats. A–C: Examples of polyribosomes (arrows) beneath dendritic spines on mid-proximodistal dendrites. A illustrates a spine on a granule neuron in the dentate gyrus; B illustrates a spine on a pyramidal neuron in hippocampus; C illustrates a spine on a Purkinje cell in the cerebellum. Note the proximity of the polyribosomes to membranous cisterns beneath the spine. D a spine on a proximal dendritic segment of a granule cell of the dentate gyrus. Polyribosomes are generally more numerous beneath spines on proximal dendrites. den = dendrite, s= spine, t = terminal, sa = spine apparatus.

single EM section. Serial section reconstruction of spines on dentate granule cells revealed that polyribosomes were actually present under about 25% of the spines on mid-proximodistal dendrites (Steward and Levy, 1982; Steward and Reeves, 1988). Similar values have been reported for the incidence of polyribosomes beneath synapses on Purkinje cells of the cerebel-

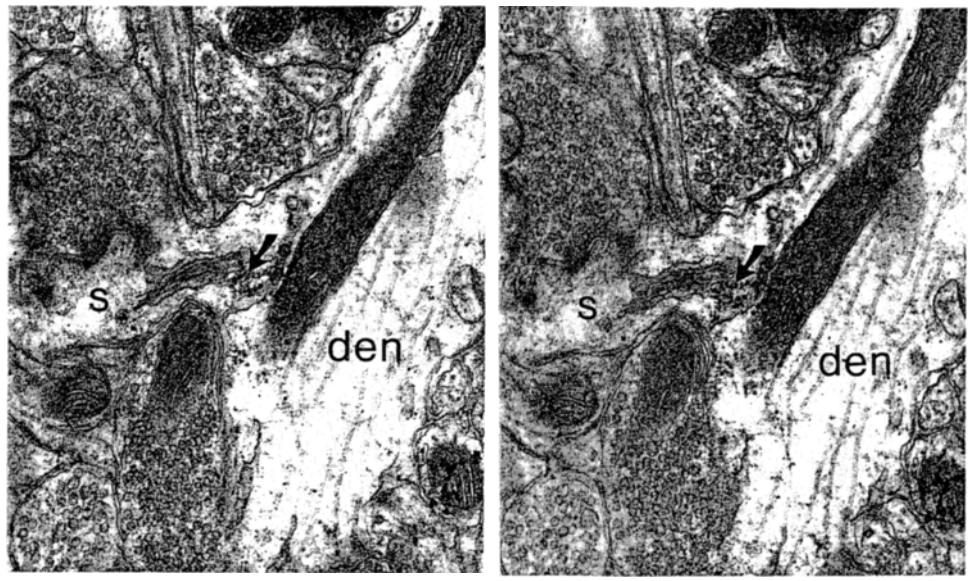

Fig. 2. A stereo photograph of a spine illustrating the association between polyribosomes (arrows) membranous cisterns beneath the spine base, and a spine apparatus. Adult rat; magnification, ×20,000. *From* Steward and Reeves, 1988.

lum (Spacek, 1985; Spacek and Hartman, 1983). In pyramidal neurons of the cerebral cortex, the incidence may be somewhat higher (Spacek, 1985). However, direct comparisons between studies are difficult, since the incidence of polyribosomes varies as a function of the proximodistal location of the spines (Steward, 1983b), and Spacek and his colleagues did not specify the proximodistal location of the spines that were analyzed.

Association Between Polyribosomes and Other Organelles in the Subsynaptic Cytoplasm

Electron microscopic studies of spine synapses reconstructed from serial sections together with analyses using relatively thick sections have revealed that the polyribosomes beneath synapses are often associated with membranous cisterns (Figs. 1 and 2). Quantitative evaluations of spines in the dentate gyrus and hippocampus revealed that about half of the clusters of polyribosomes lay close enough to a membranous cistern to suggest an association (Steward and Reeves, 1988). Polyribosomes in the spine head were almost never associated with cisterns, however. Many of the cisterns with polyribosomes appeared to be in continuity with a spine apparatus, a stack of membranes that is present in many but not all spines (for examples of spine apparatuses, *see* Figs. 1A and 2). Interestingly, the polyribosomes that were present beneath spines with a spine apparatus were more likely to be associated with membranous cisterns than the polyribosomes beneath spines without an apparatus.

The quantitative data suggest that there may be different classes of polyribosomes beneath synaptic sites, some that are associated with membranous cisterns and some that are free polyribosomes. These relationships are of interest because they provide some indication of the types of proteins that might be synthesized. In general, membrane proteins and proteins for

export from cells are synthesized on membrane-bound ribosomes (rough endoplasmic reticulum), whereas cytoplasmic and cytoskeletal proteins are synthesized on free polyribosomes (Palade, 1965). It is not known whether the association between polyribosomes and cisterns is the same type of selective association as between ribosomes and rough endoplasmic reticulum. If the polyribosomes and associated cisterns function as RER, then essentially any type of protein could be produced at the synapse.

The associations between polyribosomes, cisterns, and spine apparatuses suggest that these organelles may represent different components of a system for the synthesis and posttranslational processing of proteins (Steward and Reeves, 1988). On the basis of morphology alone, the polyribosomes on membranous cisterns are reminiscent of the rough endoplasmic reticulum, whereas the spine apparatus bears some similarity to the Golgi apparatus in the cell body. If these structural features do indicate function, the machinery would be present at the postsynaptic site not only for protein synthesis, but also for protein glycosylation. As will be further described below, studies of incorporation of radiolabeled sugars into glycoprotein provide evidence in support of the hypothesis that there may be local glycosylation of protein within dendrites.

The Association Between Polyribosomes and Growing Synapses

Studies of developing synapses have revealed that polyribosomes are particularly prominent during periods of synaptogenesis (*see* Figs. 3 and 4; Steward, 1983b; Steward and Falk, 1985,1986). In young animals, there are a greater number of polyribosomes at individual synapses, and a higher proportion of synapses with underlying polyribosomes than is the case in mature animals. Quantitative and qualitative observations of developing synapses suggest that the polyribosomes are most prominent at some time after the initial contact between pre- and postsynaptic elements. For example, in the dentate gyrus, the total number of synapses with polyribosomes is greatest at 7 d of age, even though synapse formation proceeds at a relatively constant pace for the first 10 postnatal days (Fig. 4). Moreover, there appears to be a greater number of polyribosome clusters per synapse at this stage. The peak in the number of polyribosomes appears to coincide with the period when postsynaptic membrane specializations thicken and assume their mature form.

Studies of synaptogenesis in the cerebellar cortex also suggest a special role for synapse-associated polyribosomes during synaptogenesis. Indeed, in the cerebellum, there appears to be an especially close association between the polyribosomes and the maturing postsynaptic membrane specialization of parallel fiber synapses (Palacios-Pru et al., 1981,1988). On the basis of these observations, Palacios-Pru et al. proposed that postsynaptic membrane specializations are actually synthesized by the polyribosomes.

Taken together, the data suggest the sequence of events illustrated in Fig. 5 (*see* p. 10). At the early stages of synaptogenesis, when the initial contact is made between the growing axon and the dendrite, polyribosomes are present under the primitive synaptic site, but the accumulations are not as dramatic as at later intervals. As the synapse begins to mature, polyribosomes accumulate, reaching a peak during the construction of the postsynaptic membrane specialization. The number of polyribosomes then declines. Because of this temporal sequence, the hypothesis that the polyribosomes synthesize protein constituents of the postsynaptic membrane specialization is attractive, although it is by no means the only possibility.

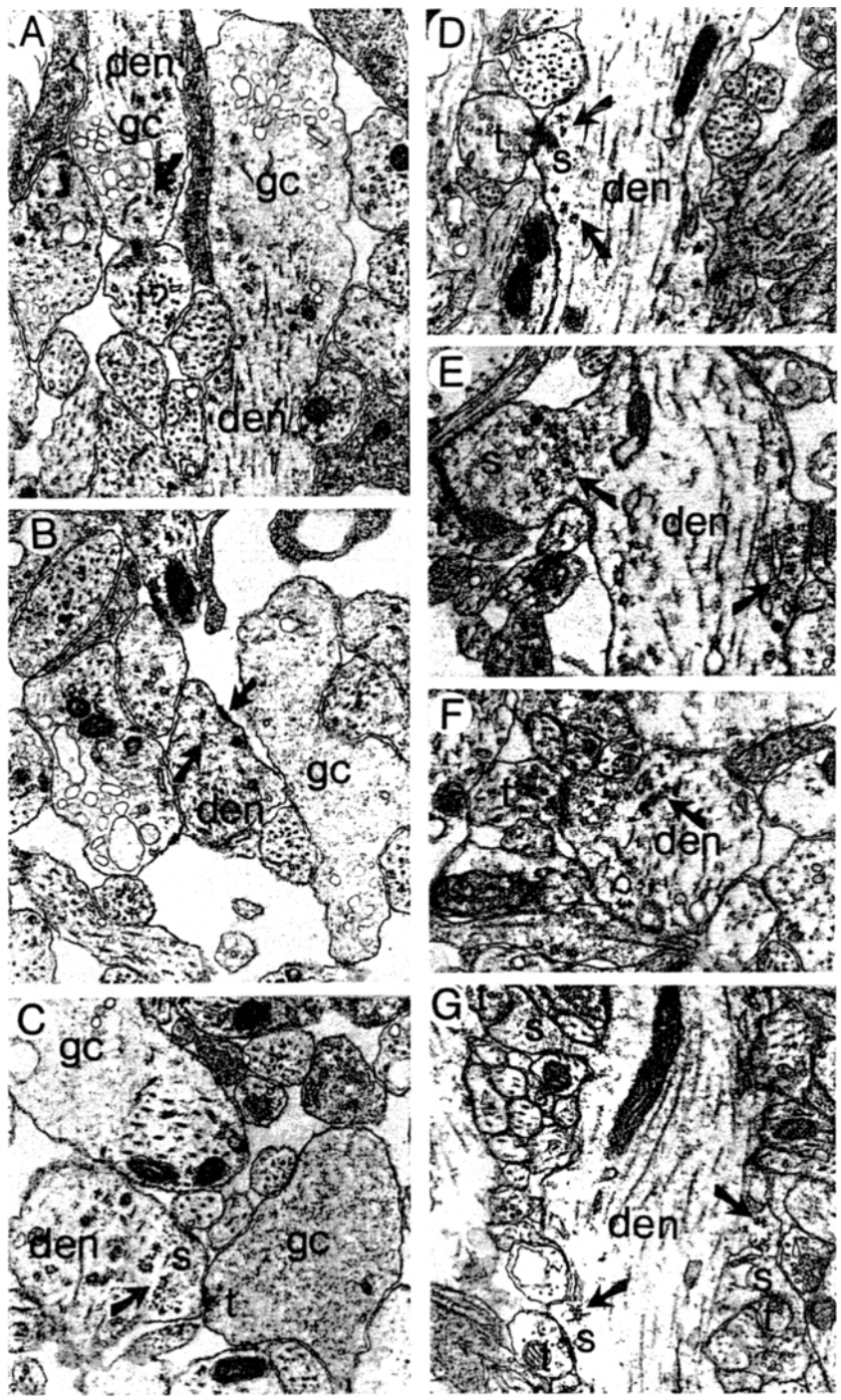

Fig. 3. Examples of polyribosomes beneath developing synapses from the molecular layer of the dentate gyrus. **A** and **B** illustrate examples of synapses from 1-d-old rats; **C** and **D** illustrate synapses from 4-d-old rats; **E** and **F** illustrate synapses from 7-d-old rats; and **G** illustrates examples of synapses from 10-d-old rats. gc = growth cone; other abbreviations are as in Fig. 1. *From* Steward and Falk, 1986b.

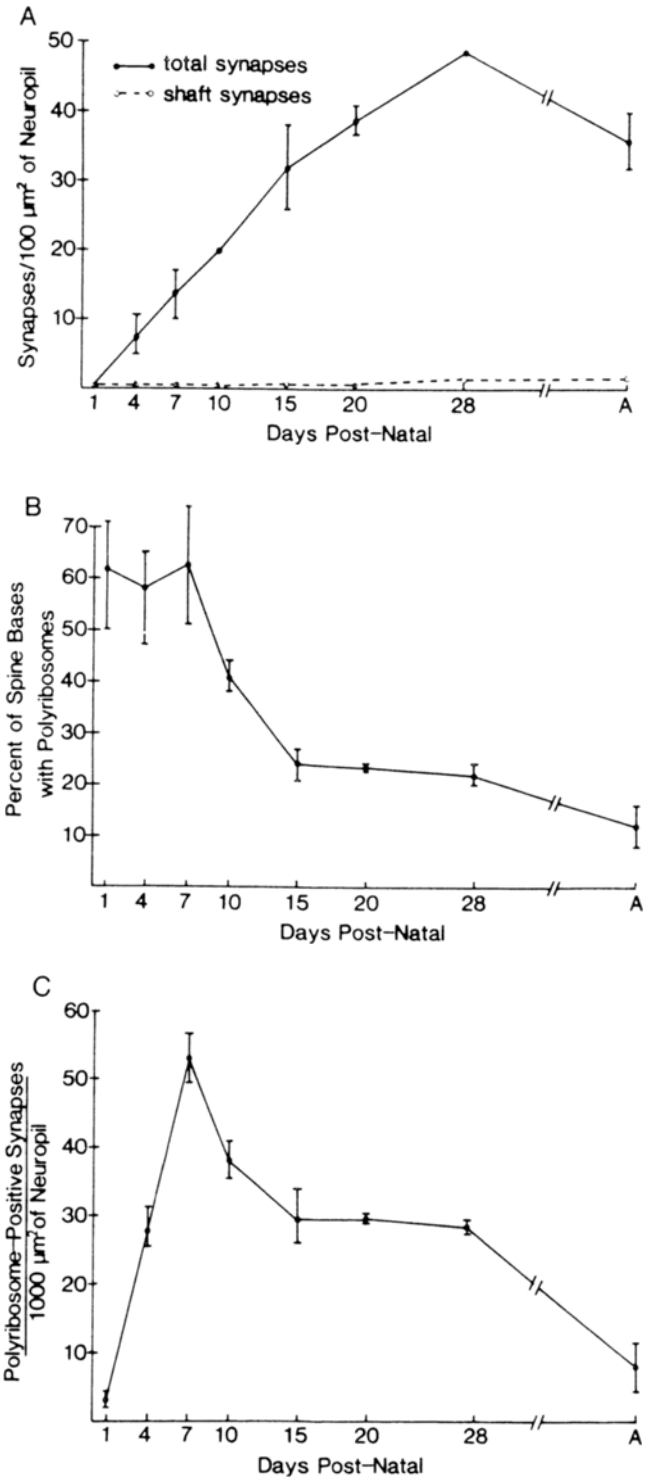

Fig. 4. Relationship between polyribosomes and developing synapses in the dentate gyrus. **A** illustrates the time course of synaptogenesis in the dentate gyrus of rats; the density of spine synapses (solid line) and shaft synapses (broken line) per 100 μm^2 of neuropile are indicated at various ages.

B illustrates the incidence of polyribosome-containing spine bases across the postnatal interval (the proportion of spines with underlying polyribosomes).

C illustrates the total number of polyribosome-positive synapses per area of neuropile across the postnatal interval (including spine synapses with polyribosomes at the base or in the head and shaft synapses with underlying polyribosomes). *From* Steward and Falk, 1986b.

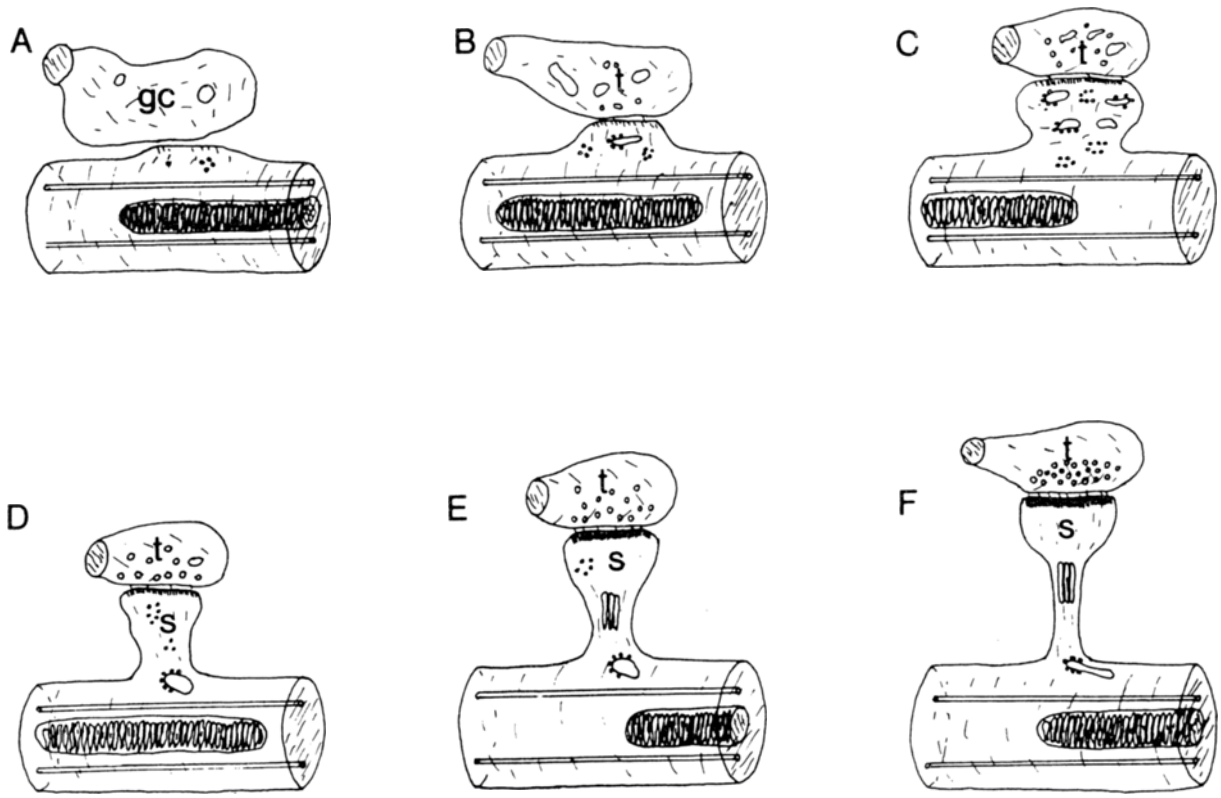

Fig. 5. A possible sequence of synaptic maturation indicating the relationship between polyribosomes and synapses at various stages of synapse maturation. A–F indicate the predominant type of synapse at 1, 4, 7, 10, 15, and 20 d of age, respectively, in the dentate gyrus. Note that polyribosomes are most prominent during the time that primitive contacts differentiate into mature appearing synapses.

Alterations in Polyribosomes Under Synaptic Sites During Lesion-Induced Synaptogenesis

Studies of synapse replacement following lesions in adult animals suggest that polyribosomes also play a role in a different form of synapse growth. One of the best characterized examples of lesion-induced synapse growth involves the reinnervation of granule cells of the dentate gyrus after removal of their principle input from the entorhinal cortex. Lesions of the entorhinal cortex lead to the degeneration of over 90% of the synapses on distal dendrites of dentate granule cells ipsilateral to the lesion (Steward and Vinsant, 1983). Between 6–12 d postlesion, there is an extensive proliferation of presynaptic processes within the denervated zone, and these terminals rapidly form new synapses with the denervated granule cells (Fig. 6A). Quantitative electron microscopic studies have revealed striking changes in the distribution of polyribosomes within granule cell dendrites during the period of postlesion growth; in particular, the proportion of spines with polyribosomes and the number of polyribosome-containing synapses increased about threefold during the period of synapse growth (Fig. 6B; and *see* Steward, 1983b).

The increases in synapse-associated polyribosomes suggest that there may be an increase

in protein synthesis at postsynaptic sites during reinnervation. Autoradiographic studies support this suggestion. When labeled protein precursors were injected intraveneously, and animals were prepared for autoradiography 30 min after the injection, there were substantial increases in labeling within denervated dendritic laminae during the period of synapse replacement (Fass and Steward, 1983; and *see* Fig. 6C). The increases were maximal during the same postlesion interval as the increases in spine-associated polyribosomes (*compare* Figs. 6B and 6C). Similar results were obtained when animals were prepared for autoradiography 10 min after the intraveneous injections. These results suggested that the labeling in the dendritic laminae reflected local protein synthesis within the neuropile rather than transport of proteins that had been synthesized in neuronal cell bodies into the dendritic laminae. Part of the increase in incorporation may be a result of protein synthesis within glial cells, but the parallels between the increases in synapse-associated polyribosomes and the increases in incorporation suggest that at least part of the increased labeling reflects an increased synthesis of protein within dendrites. Direct evidence for local protein synthesis within dendrites is discussed below.

Evidence for Synthesis and Glycosylation of Proteins Within Dendrites

Autoradiographic studies of protein synthesis in vivo suggest but do not prove that protein synthesis occurs in dendrites. More direct evidence in support of this hypothesis comes from studies of neurons grown in culture. Hippocampal neurons can be grown in low density culture, where they elaborate characteristic dendritic arbors and axonal plexuses that can be distinguished on the basis of a number of criteria (Bartlett and Banker, 1984a,b). As in hippocampal neurons in vivo, polyribosomes are present in the dendrites, extending throughout the dendritic tree (Bartlett and Banker, 1984b), and are positioned beneath the base of dendritic spines (Fig. 7) (*see*, p. 13). Most important for the present purposes, the dendrites of these neurons are not surrounded by glial or other elements, and can thus be visualized clearly.

When cultures of hippocampal neurons were pulse-labeled for 10 min with the protein precursor 3H-leucine, and were subsequently fixed and processed for autoradiography in a way that eliminates free amino acids but retains recently synthesized protein, there was heavy labeling of cell bodies as well as dendrites (Fig. 8) (*see*, p. 14). At the 10-min interval, there was no evidence of label over axons, yet dendrites appeared to be labeled throughout their proximodistal extent (Fig. 8A). After a 30-min pulse-labeling period, dendrites were even more heavily labeled (Fig. 8C). This incorporation was almost completely blocked by the protein synthesis inhibitor puromycin (Fig. 8E). In contrast to the pattern of labeling after a 10-min pulse, neurons that were pulsed for 60 or 90 min exhibited axonal labeling (data not shown). The absence of axonal labeling after 10- or 30-min exposures to 3H leucine provides direct evidence that these times are too short for there to be significant transport of recently synthesized protein from the neuronal cell body, even via the rapid transport system; thus, the presence of label in the dendrites after 10- and 30-min pulses is strong evidence that the protein synthetic machinery that is present there is actively synthesizing protein.

As noted above, ultrastructural observations suggest that polyribosomes and associated membranous organelles may represent a system for synthesis and posttranslational processing of protein at synaptic sites (Steward and Reeves, 1988). On the other hand, electron microscopic and immunocytochemical evidence indicates that the Golgi complex, presumably an obligatory site in the pathway for synthesis of

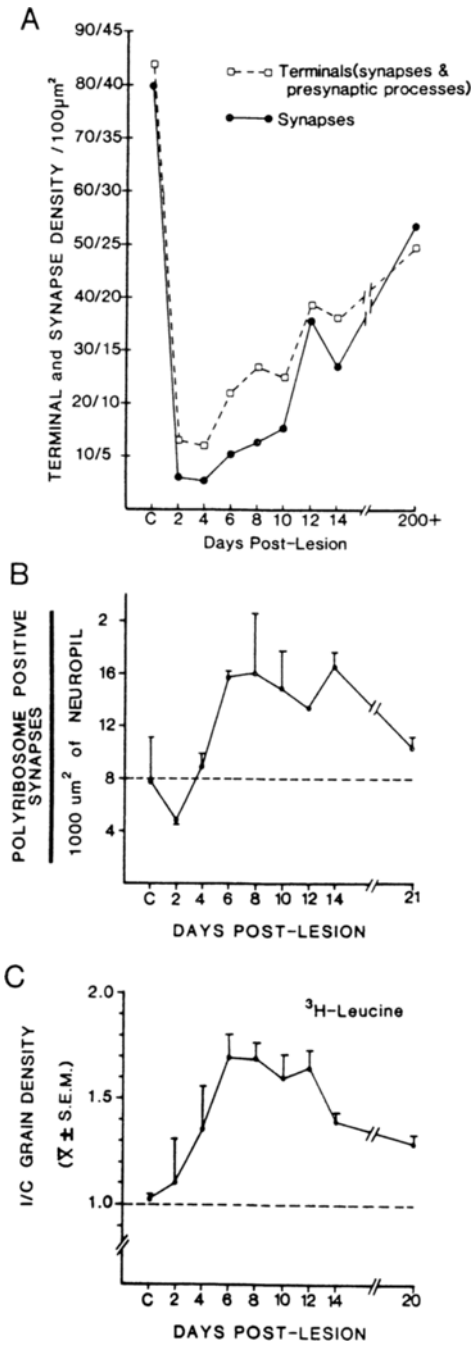

Fig. 6. Relationship between reactive growth in the denervated dentate gyrus, changes in polyribosomes under spines, and changes in incorporation of protein precursors in the denervated neuropile.

A illustrates the time course of the increase in terminals and synapses in the denervated neuropile of the dentate gyrus after unilateral entorhinal cortical lesions. The numbers on the ordinate refer to terminal density (numbers before slash) and synapse density (numbers after slash) per 100 μm^2 of neuropile. LT = long-term post-lesion (7 mo); (*redrawn from* Steward and Vinsant, 1983).

B. Time course of increases in polyribosome-containing synapses. The graph plots the total number of spines with polyribosomes either at the base or within the head of the spine at various times during the denervation–reinnervation cycle (a composite of two separate analyses *from* Steward, 1983a).

C. Time course of increases in incorporation of protein precursors (3H-leucine) in the denervated neuropile of the dentate gyrus after unilateral entorhinal cortical lesions (*from* Phillips et al., 1988). Note that the increase in incorporation and the increases in polyribosomes occur at about the same time (peaking at 6–8 d postlesion), corresponding to the initial period of terminal proliferation and reactive synaptogenesis.

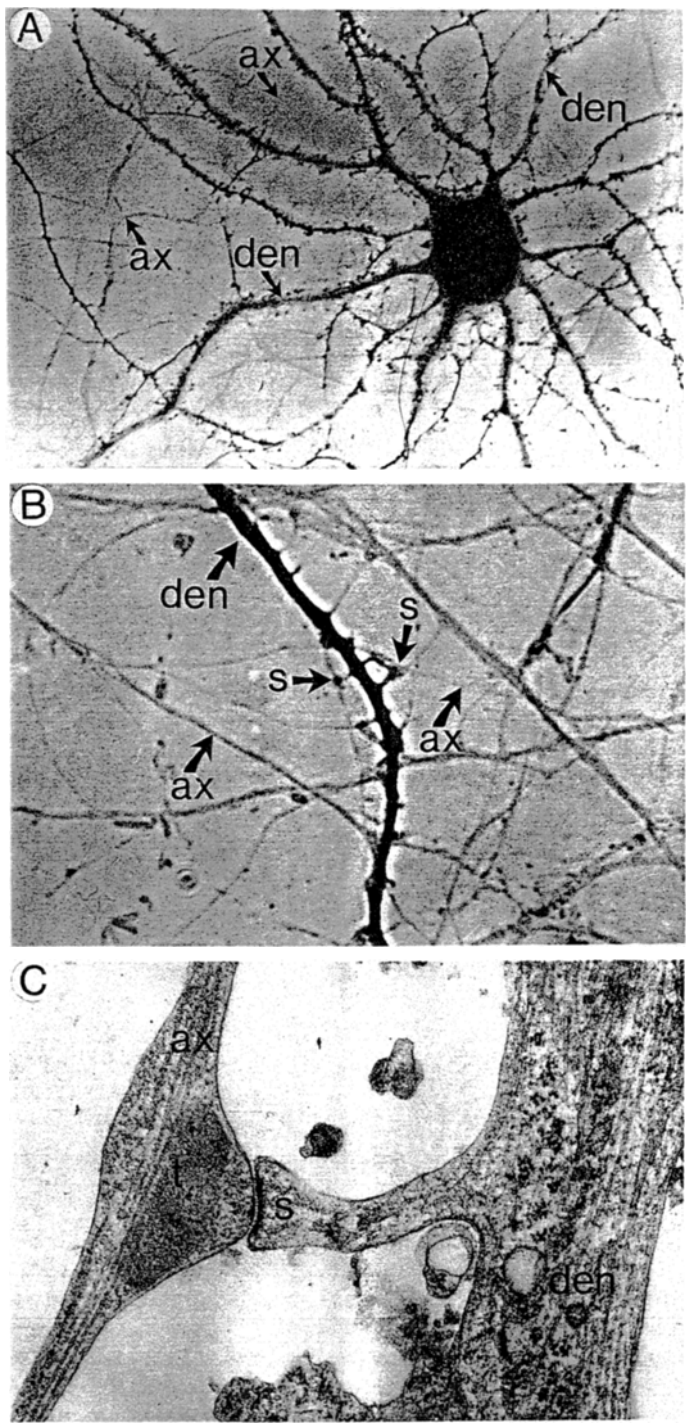

Fig. 7. Localization of polyribosomes at the base of dendritic spines on hippocampal neurons grown in culture.

A. Axons (ax) and dendrites (den) of hippocampal neurons in culture immunostained for MAP2. Dendrites can be identified by their heavy staining for MAP2, whereas axons are unstained.

B. Profiles of the size and shape of dendritic spines can be seen emerging from the dendrite (s), and these are often contacted by passing axons.

C. Electron microscopic evaluations reveal the presence of spines with apposed presynaptic terminal boutons (t). As is also true in vivo, polyribosomes are often localized beneath the spine base. *From* Steward et al., 1988.

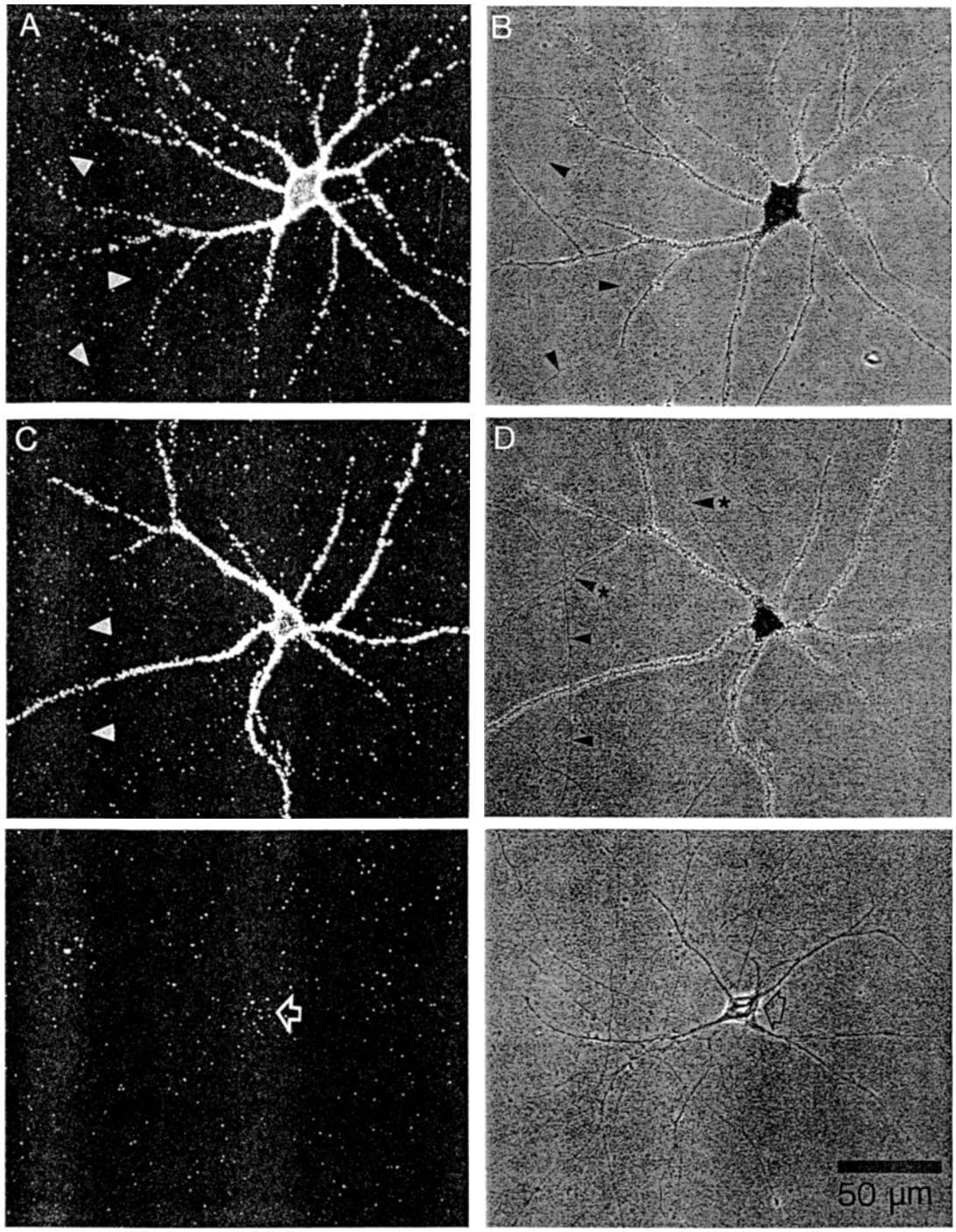

Fig. 8. Autoradiographic analysis of sites of protein synthesis in neurons in culture. Neurons in low density cultures were incubated for 10 or 30 min in a medium containing 125 µCi/mL 3H-leucine (60 Ci/mmol) prior to fixation and processing for autoradiography. Autoradiographs were exposed for 5 d in the case of **A** and **C**, and 7 d in the case of **E**. **A** and **B** = Dark field and phase contrast photomicrographs of a neuron fixed after a 10-min incubation. Dendrites and somata were heavily labeled. Axons visible in the phase contrast photomicrograph (arrowheads) were unlabeled. **C** and **D** = Darkfield and phase contrast photomicrographs of a neuron fixed after a 30-min incubation. Occasionally, axons appear to emerge from the tips of dendrites (arrowheads with asterisks). Usually, these are axons from other neurons that contact, and then grow along the dendrite. **E** and **F** = Darkfield and phase contrast photomicrographs of neurons that were exposed to 3H-leucine in the presence of puromycin ($10^{-4}M$), and fixed after a 30-min incubation. An unlabeled neuronal somat is indicated by the open arrow.

membrane glycoproteins is confined to the cell body, although the Golgi complex may extend for a short distance into proximal dendrites (De Camilli et al., 1987). Thus, the question arises whether there may be a local glycosylation of proteins within dendrites. The low density cultures of hippocampal neurons provide an opportunity to evaluate this question directly.

To evaluate the possibility that glycosylation might occur in dendrites, cultures of hippocampal neurons were exposed for 10 min to 3H-sugars such as galactose or fucose. The cells were then either fixed immediately, or they were returned to a medium containing an excess of unlabeled galactose or fucose and maintained for an additional 15–60 min. Neurons were fixed so as to retain glycoproteins but not the free sugars, and then prepared for autoradiography (Dotti et al., in preparation). Cells that were prepared for autoradiography immediately after a 10-min pulse with galactose or fucose exhibited heavy labeling associated with the Golgi apparatus in the cell body; there was also light labeling throughout the dendritic tree (Figs. 9 and 10). There was no evidence of axonal labeling, however. When cells were pulse-labeled for 10 min, and then maintained in a medium with excess unlabeled sugars for an additional 15–60 min, the extent of dendritic labeling increased, and axons also became labeled. To confirm that the processes that were labeled after a 10-min pulse were dendrites, the autoradiographic preparations were also immunostained for MAP2, which is present in cell bodies and dendrites, but not axons (Caceres et al., 1984); in such preparations, the restriction of the autoradiographic labeling to the somato-dendritic domain is evident (Figs. 10B and 10C).

Two lines of evidence indicate that the labeling seen in these experiments represents *N*-linked glycosylation of proteins. First, the labeling was completely inhibited by tunicamycin (Fig. 10D), an agent that inhibits protein glycosylation specifically by inhibiting *N*-acetylglucosaminyl pyrophosphoryl dolicol transferase, the enzyme responsible for transferring core oligosaccharides to asparagine residues (Tkacz et al., 1975; Heifetz et al., 1979). Second, the labeling persisted following extraction of the unfixed cells with chloroform: methanol (Fig. 9). This treatment quantitatively extracts glycolipids, but does not remove glycoproteins (Suzuki, 1973).

Since rapid transport could theoretically move materials from the soma to the tips of the dendrites within minutes, might some of the dendritic labeling reflect the transport of proteins that were glycosylated in the cell body? This possibility seems unlikely because it is generally accepted that about 20 min are required for a protein to be processed through the Golgi complex (Lodish et al., 1983). Moreover, if dendritic labeling arose from somatic incorporation followed by transport, one would expect comparable labeling of the proximal portions of the axon, since glycoproteins are a major constituent of the material transported via rapid axonal transport. However, axonal labeling was not observed at the 10-min interval. Finally, as a direct experimental evaluation of this issue, some cells were exposed to 3H-fucose in the presence of monensin, a drug that blocks the export of recently synthesized glycoproteins from the Golgi complex (Griffiths et al., 1983). In neurons, monensin prevents newly synthesized proteins from entering the axonal transport system (Hammerschlag et al., 1982). As illustrated in Fig. 10G, treatment of cells in culture with monensin did not prevent the appearance of dendritic labeling after a 10-min pulse with 3H-fucose. However, as expected, monensin did inhibit the increase in labeling of axons and dendrites seen in cells prepared for autoradiography 60 min after the pulse.

These results leave little doubt that the labeling of dendrites at short intervals reflects a local incorporation of labeled sugars within the dendrites, presumably into glycoproteins. Taken together, these observations strongly suggest that a mechanism for protein glycosylation ex-

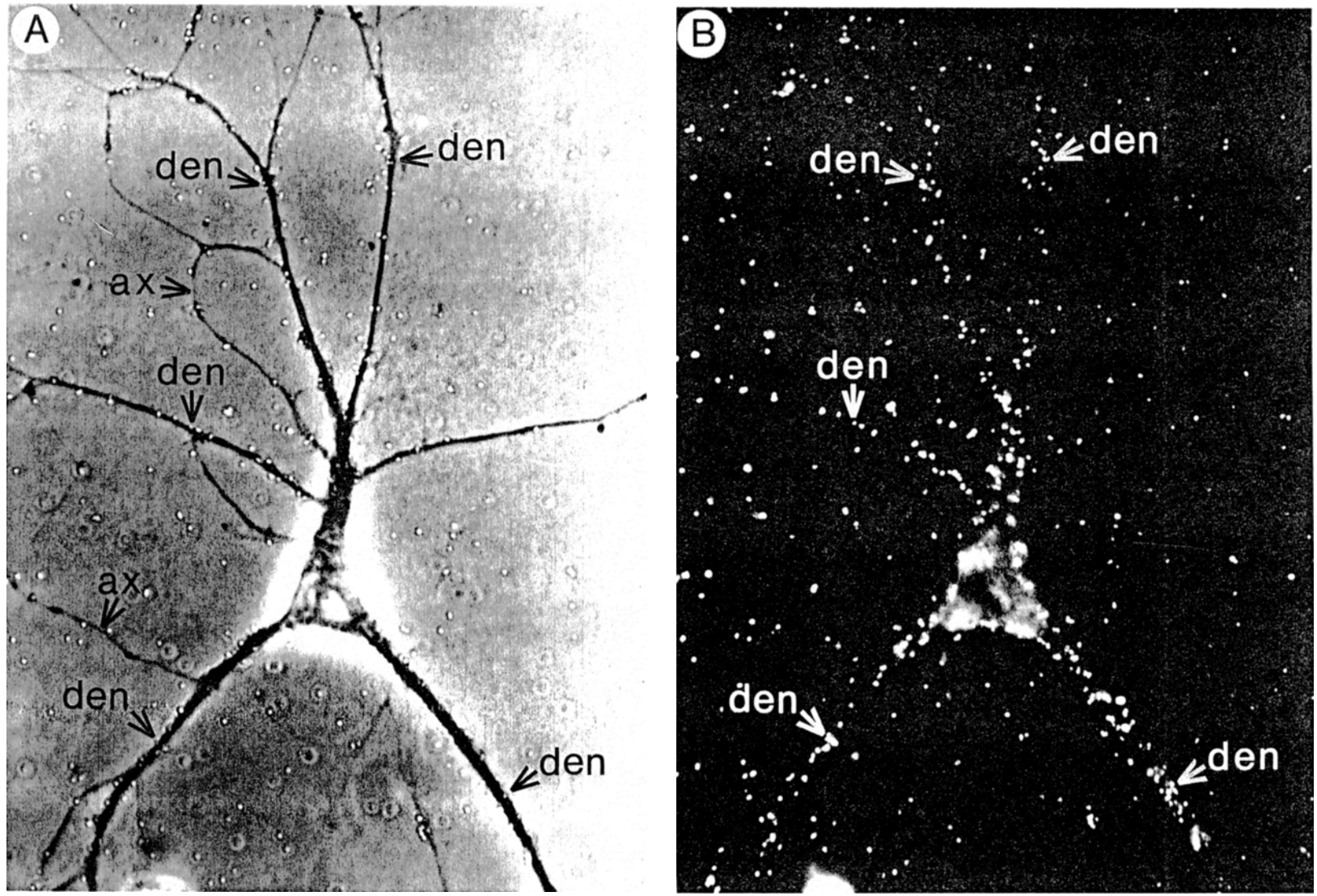

Fig. 9. Autoradiographic analysis of sites of glycosylation in neurons in culture. Neurons in low density cultures were incubated for 10 min in a medium with 1 mCi/mL D-[4,5-3H-galactose, 45.1 Ci/mmol]. Lipids were extracted with chloroform: methanol, and the cells were then prepared for autoradiography. A = Phase contrast photomicrograph indicating the dendritic arbor of a neuron, with several axons contacting the dendrites. B = Darkfield photomicrograph illustrating labeling over the cell body and dendrites (den), and the lack of label over axons (ax).

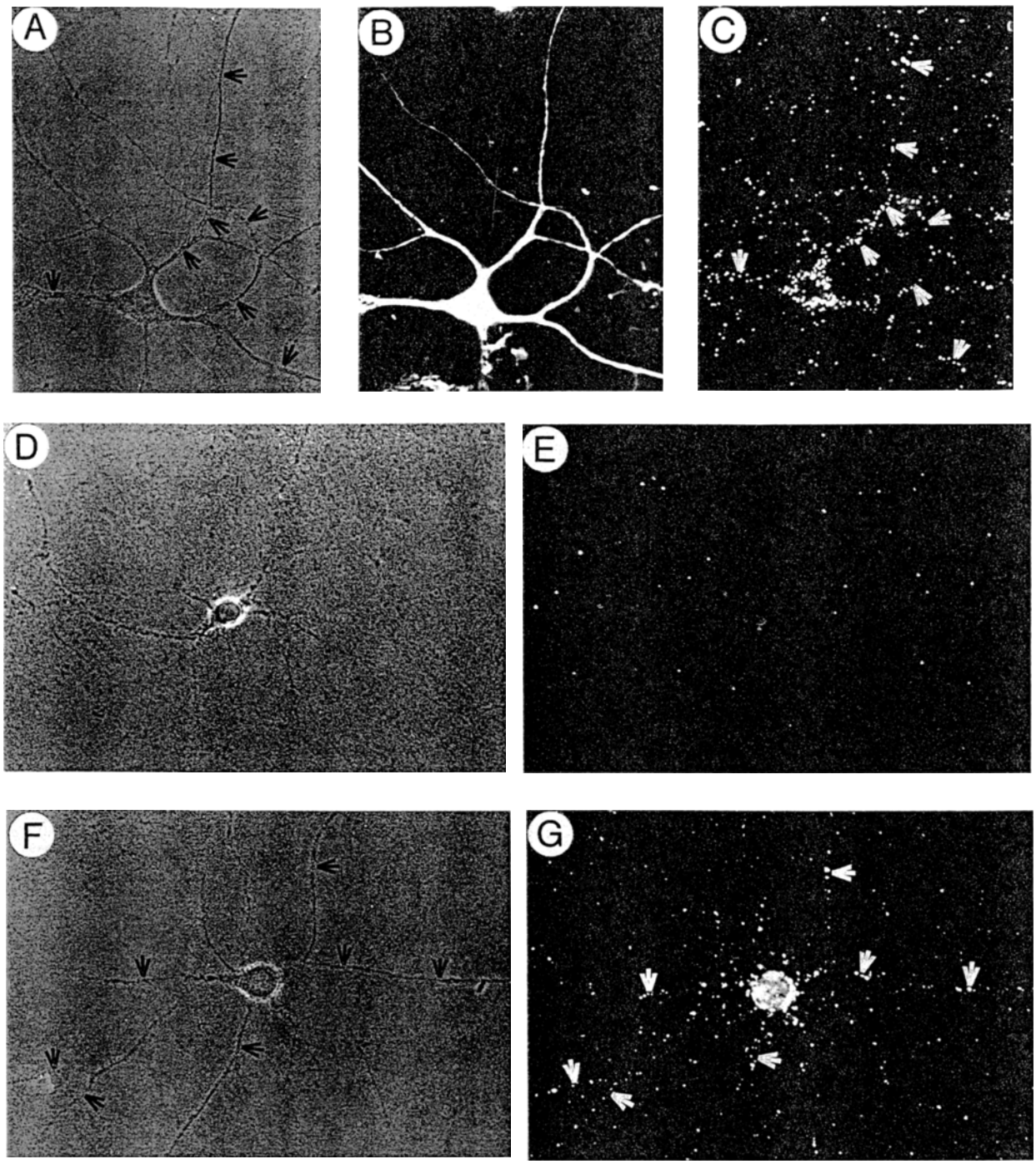

Fig. 10. Autoradiographic analysis of sites of glycosylation in neurons in culture. Neurons that had been pulse-labeled with 1 μCi/mL L- [6-3H-fucose, 86. 3 Ci/mmol] for 10 min also exhibit labeling over dendrites. **A** = phase contrast photomicrograph illustrating axons and dendrites of neurons in culture; **B** = the same neurons were immunostained for MAP2 to distinguish dendrites from axons. MAP2 is a microtubule-associated protein found within dendrites and cell bodies, but absent from axons; **C** = darkfield photograph indicating the pattern of labeling. Note that label is found over dendrites, but not over axons crisscrossing the field. **D** and **E**= neurons that were exposed to 3H-fucose in the presence of tunicamycin (an inhibitor of glycosylation) were unlabeled. **F** and **G**= dendritic labeling is still present, when neurons were pulse-labeled with 3H-fucose in the presence of monensin, which blocks the export of recently synthesized proteins from the Golgi apparatus. This indicates that the label in dendrites reflects local glycosylation.

ists in dendrites that would permit a posttranslational glycosylation of proteins that were locally synthesized.

Evidence for Local Protein Synthesis in Synaptosome Preparations

As noted above, the selective positioning of polyribosomes and associated organelles at individual synaptic sites has suggested the hypothesis that neurons synthesize at least some of the protein constituents of their synapses on-site. Of course the polyribosomes could also produce other types of proteins; nevertheless, their proximity to the synaptic membrane specialization makes the hypothesis that they produce synaptic constituents particularly attractive. One way to test this hypothesis would be to isolate the postsynaptic compartments containing the polyribosomes, and identify proteins that are synthesized in these isolates. Indeed, experiments of this sort were undertaken almost two decades ago, but for a very different reason.

An issue that received considerable attention in the late 1960s and early 1970s concerned the possibility of local synthesis of proteins within synaptosomes (subcellular fractions containing pinched-off nerve endings). Of particular interest was the possibility that proteins were synthesized within presynaptic terminals. A number of studies indicated that isolated synaptosome fractions were capable of synthesizing proteins from labeled amino acids (*see*, for example, Morgan and Austin, 1968; Autilio et al., 1968). Moreover, studies using selective inhibitors of mitochondrial and eukaryotic protein synthesis (chloramphenicol vs cycloheximide or puromycin) revealed that only a portion of the synthesis could be accounted for by the mitochondria that represent a significant contaminant of the fractions.

These early studies were initially interpreted as indicating a local synthesis of protein by presynaptic elements. However, electron microscopic autoradiographic studies revealed that labeling of presynaptic elements was light, whereas ribosome-containing profiles that resembled postsynaptic elements (presumably pinched-off dendrites) along with other unidentified ribosome-containing profiles were much more heavily labeled (Cotman and Taylor, 1972; Gambetti et al., 1972). Indeed, the relative specific activity of the profiles with ribosomes was about 10-fold higher than the relative specific activity of presynapic terminals (Gambetti et al. 1972). Thus, much of the local cycloheximide-sensitive incorporation was not a result of protein synthesis within presynaptic terminals, but rather indicated synthesis within contaminating elements such as pinched-off dendrites with ribosomes or resealed membranes containing cytoplasm from neuronal cell bodies or glial cells.

More recent studies of synaptosomes isolated from developing animals have shown that the greatest cycloheximide-sensitive incorporation occurred in ribosome-containing structures having morphologies consistent with postsynaptic elements (Verity et al., 1979,1980). Comparisons of synaptosome fractions isolated from developing and mature animals revealed that the protein synthetic activity, as well as the number of ribosome-containing postsynaptic profiles, were much greater in synaptosomes isolated from developing animals. It was also possible to isolate different subpopulations of synaptosomes. A "heavy" subfraction was more active in synthesizing protein, and contained more ribosomes than a "light" subfraction. Thus, both developmental age and the methods of isolation influence the degree to which the ribosome-containing postsynaptic compartments are preserved in the synaptosome fractions. This fact has important implications for evaluating the sometimes contradictory results

from studies using synaptosomes from adult animals, where separation methods may be different, and where the overall level of synthesis may be relatively low.

Studies of the classes of proteins synthesized within synaptosomes using gel electrophoresis, in combination with fluorography, have revealed that chloramphenicol and cycloheximide selectively reduce labeling of different protein species (Irwin, 1985). Chloramphenicol reduced the labeling of a number of bands in the low mol wt range (below 50kD). Cycloheximide, on the other hand, reduced the labeling of a larger number of protein bands distributed across the mol wt range, most of which are not affected by chloramphenicol. We have obtained very similar results in our own studies using synaptosomes isolated from young animals (Fig. 11). These results leave little doubt that the cycloheximide-sensitive incorporation reflects something other than mitochondrial protein synthesis.

Evidence that Some of the Proteins Synthesized by Synaptosomes Are Constituents of the Synaptic Plasma Membrane

Although the studies discussed above indicate that the proteins synthesized by synaptosomes are not mitochondrial, there are problems in identifying the elements responsible for the protein synthesis in the synaptosomal fractions. In particular, synaptosomal preparations may be contaminated with membrane-bound particles that contain cytoplasm from the neuronal cell body, which could account for a substantial proportion of the incorporation in the synaptosomal fraction. To evaluate the hypothesis that some of the local incorporation reflects the synthesis of components of the synaptic junction, we have allowed synaptosomes to synthesize proteins from labeled precursors (35S-methionine), and then prepared subcellular fractions of the junctional region. If the incorporation is occurring exclusively in the contaminants of the synaptosomal fractions (membrane-bound particles containing cytoplasm from the cell body), then the more pure fractions of the synaptic junction should have a lower specific activity than fractions of other cellular membranes that might be contaminants. Alternatively, if some of the local incorporation within the synaptosomes reflects the synthesis of proteins of the synaptic region, then proteins in the fractions of the synaptic junction should be labeled.

When synaptic plasma membranes and other cellular membranes (myelin and "light" membranes that band at the 0.8–1.0*M* sucrose interface) were isolated from synaptosomes that had been allowed to incorporate 35S-methionine into protein, as described above, we found that the specific activity (cpm/mg protein) of the SPM fraction was as high or higher than the other membrane fractions (data not shown). The synaptic plasma membranes were further fractionated by isolating synaptic junctional complexes (SJC) according to the protocols of Cotman and Taylor (1972b). These SJC fractions were prepared by treating SPM fractions with Triton X-100, which dissolves most of the membrane. The Triton-insoluble residue is highly enriched in postsynaptic membrane specializations that are associated with a small amount of membrane immediately surrounding the junctional region (Fig. 12). These fractions also exhibited a specific activity that was as high or higher than the other cellular membrane fractions. As illustrated in Fig. 13, fluorographic analyses revealed that a number of the protein constituents of the SJC fractions were heavily labeled. This labeling was almost completely eliminated when the synaptosomes were pulse-labeled in the presence of cycloheximide, indicating that the labeled bands are not likely to be

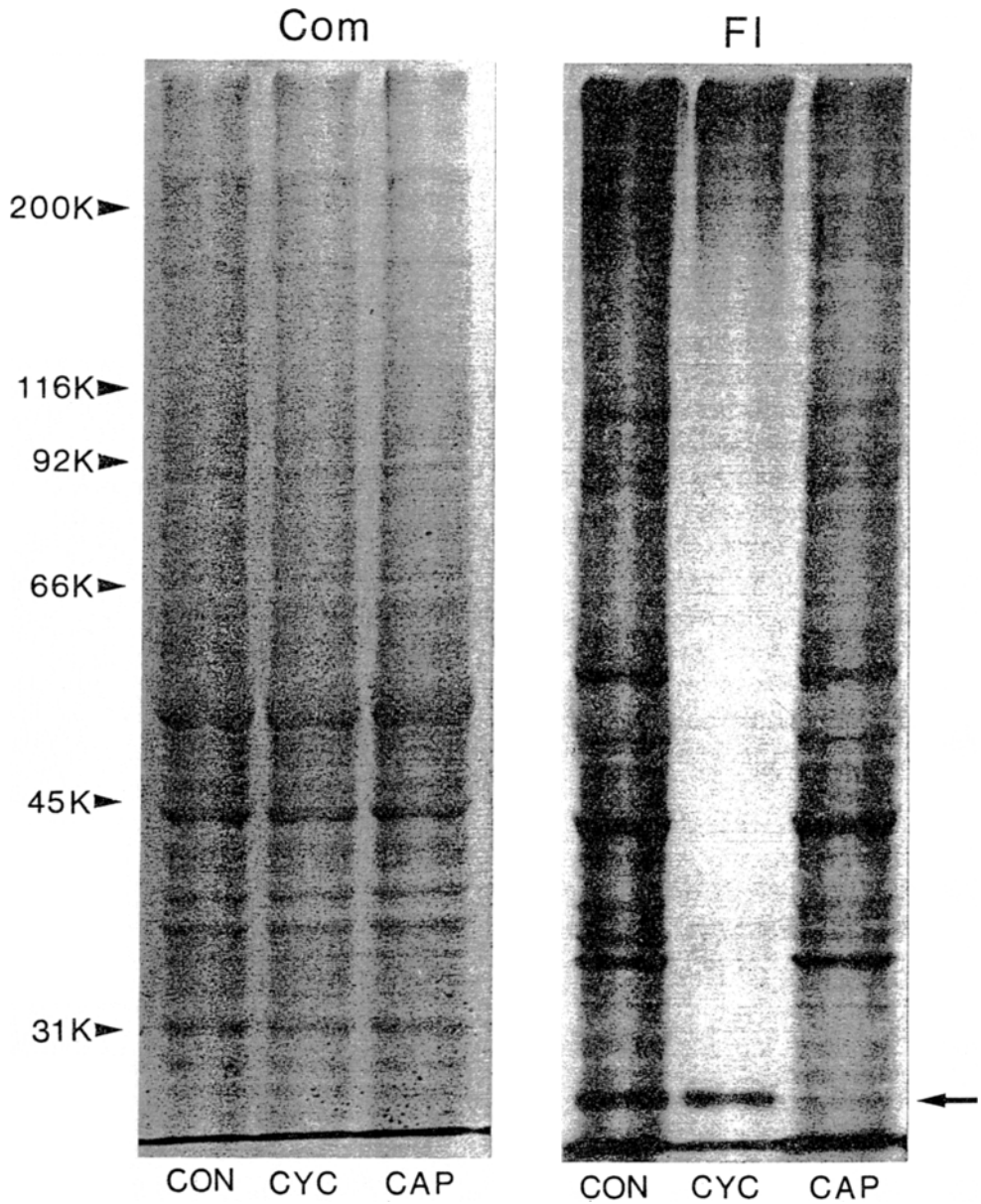

Fig. 11. SDS-PAGE analysis of protein synthesis in synaptosomes. Synaptosomes were prepared from 32-d-old rat forebrains, and incubated for 30 min in a Tris buffered medium containing 35S-methionine (200 μCi/mL). Parallel preparations were incubated with chloramphenicol (50 μg/mL) or cycloheximide (500 μg/mL) to inhibit mitochondrial or general cellular protein synthesis, respectively. At the end of the incubation, synaptosomal proteins were precipitated by adding an equal volume of 10% trichloroacetic acid, the protein pellets were washed in acetone, and the dried pellet was dissolved in Laemmli buffer for SDS-PAGE. 15 μg of protein (about 700,000 cpm for the control) was loaded onto each lane of a 10% gel. The gel was stained with Coomassie blue, photographed, and then dried onto paper. Fluorographs were prepared by apposing the gel to Kodak film (4 d exposure). The Coomassie stained gel is indicated on the left (COM); the corresponding fluorograph is on the right (F1). CON = control; CYC = cycloheximide; CAP = chloramphenicol. The arrow indicates one of the low molecular weight proteins that is labeled in the CYC-treated sample, and unlabeled in the CAP-treated sample (presumably a protein synthesized by mitochondria). Most of the other mitochondrial proteins are in a lower molecular weight range that has run off this gel.

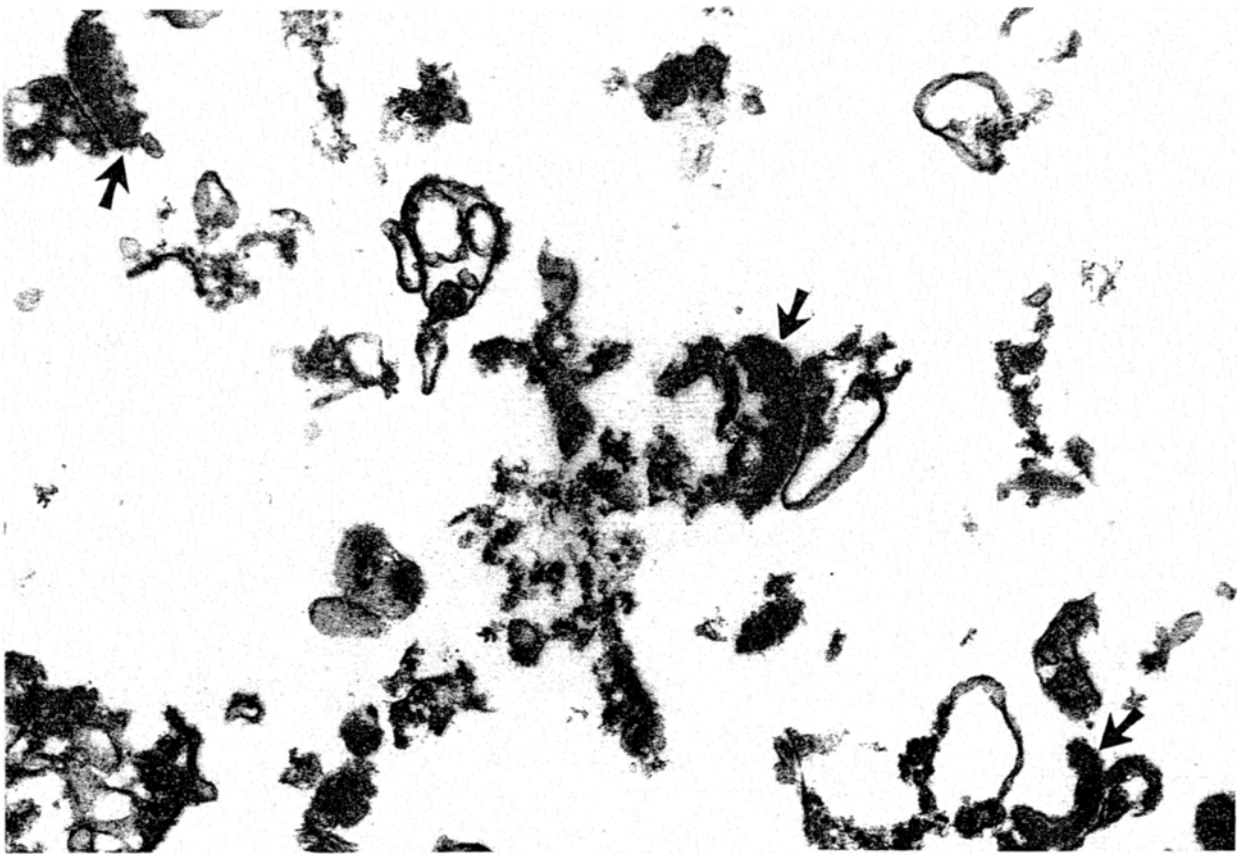

Fig. 12. Synaptic junctional complexes (SJC). The figure illustrates the composition of the SJC fraction isolated according to the protocol of Cotman and Taylor (1972b). Arrows indicate electron dense material of the size and shape of postsynaptic densities (psds). The other electron dense material in the field presumably represents psd fragments, on the basis of cytochemical studies (Cotman and Taylor, 1972a).

of mitochondrial origin. The proteins that are labeled have not yet been identified with certainty. These results are thus consistent with the hypothesis that some of the protein species of the synaptic junctional complex are synthesized locally, with local being defined in this case as within the isolated particles that provide the source of the SPM and SJC fractions. One possibility that cannot be excluded, however, is that the incorporation is occurring in only a small subfraction of the synaptosomes that derive from positions near the cell body, and which contain "cell body" cytoplasm.

Time Course of Appearance of Recently Synthesized Proteins in Subcellular Fractions of the Synaptic Junction

Another way to test the hypothesis that synaptic membrane proteins are synthesized locally is to evaluate the time course of appearance of recently synthesized proteins in synaptic junctional fractions. If some of the protein constituents of the synaptic junction are synthesized locally, one would predict that they could

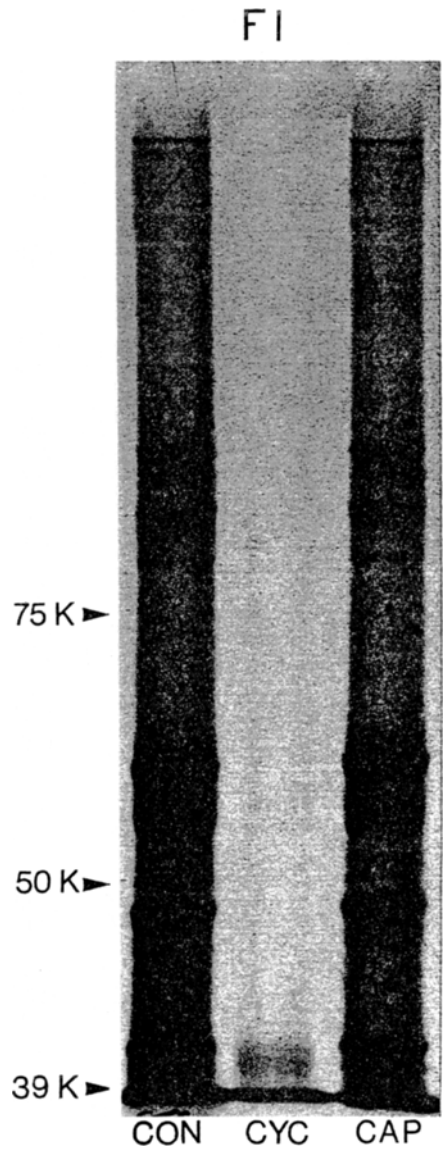

Fig. 13. Evidence that proteins synthesized by synaptosomes are constituents of the synaptic junctional complex. Synaptosomes from 17-d-old animals were incubated for 45 min in 35S-methionine containing media with or without cycloheximide or chloramphenicol, as described in Fig. 11. The synaptosomes were then subfractionated in order to obtain synaptic junctional complexes, and the protein constituents of these fractions were prepared for SDS-PAGE and fluorography, as described in Fig. 11. The resulting fluorograph is illustrated. Note that many protein species are labeled, although it is difficult to resolve individual protein bands in this deliberately overexposed fluorograph. The labeling is almost completely eliminated when the incubation medium included cycloheximide.

become assembled into the junction very soon after their synthesis. On the other hand, proteins that were synthesized in and transported from the cell body would become assembled into the junction only after a delay. Even assuming rapid transport, a delay of 20-30 minutes would seem to be a reasonable expectation because most rapidly transported proteins must first be glycosylated.

To evaluate this question, we have again used pulse-labeling techniques in conjunction with subcellular fractionation. These experiments were also carried out in semi-intact tissue (hippocampal slices), to be sure that we were not

inadvertantly eliminating some of the polyribosomes beneath the synaptic site, as might be the case with the synaptosome fractions. Hippocampal slices were used rather than carrying out the studies in intact animals in order to obtain sufficiently high levels of labeling of recently synthesized protein constituents of SJC fractions. The slices were pulse exposed to 3H-protein precursors for varying periods of time, and synaptic junctional complexes were isolated in order to define the time course of appearance of the recently synthesized proteins in the fractions.

We found that there was essentially no delay in the accumulation of recently synthesized proteins in SJC fractions, as would be expected if the protein constituents in those fractions were transported from a distant cell body (Fig. 14). Instead, there was a steady increase in label in the fractions over time. These results are consistent with the hypothesis that some of the proteins of the synaptic junctional complex are synthesized on-site. Studies of the turnover of identified protein constituents of the synaptic junction have suggested a similar conclusion (Sedman et al., 1986). Of course, the results do not prove that the proteins of the SJC are synthesized by the polyribosomes positioned beneath the synaptic sites, although the polyribosomes certainly seem the most likely site of synthesis. One cannot exclude the possibility that the labeled proteins derive from synaptic junctions that are very close to the neuronal cell body. If this were true, one could argue that there would be little delay in the appearance of recently synthesized protein in SJC fractions even if the synthesis occurred in the neuronal cell body.

Isolation of mRNA from Synaptosome Preparations

In pursuing the story of local synthesis of protein within synaptosomes, a number of authors have also attempted to isolate mRNA from synaptosomal preparations. For example, Cupello and Hyden (1975) compared RNA from synaptosomes and cytoplasmic subcellular fractions; they report that two synaptosome-specific RNA bands were found, and that these did not correspond to mitochondrial RNA species. However, DeLarco et al. (1975), reported that the RNA isolated from synaptosomes was quite similar to that of mitochondria. In these studies, even if the issue of mitochondrial vs nonmitochondrial RNA could be resolved, there is the problem of contamination by membrane-bound particles containing "cell body" cytoplasm, or glial cytoplasm. Indeed, the ways of evaluating the nature of the proteins produced (i.e., by evaluating whether proteins of the SPM or SJC are among the proteins that are locally synthesized) are not available in the case of the RNA isolations.

More recent studies have focused on developing tissue, where the number of polyribosomes beneath synapses is much greater. In an interesting study, Palacios-Pru et al. (1988) dealt with the problem of contamination by membrane particles containing "cell body" cytoplasm by first isolating a dendritic fraction by physical dissection, thus removing most of the neuronal cell bodies. This was accomplished by using cerebellar cortex, where the laminated organization makes it possible to dissect neuropile layers containing dendrites from the cell body laminae. Synaptosomes that were purified from these dendritic laminae contained mRNA; moreover, the concentration of the mRNA was highest during the period of synapse formation. These results strongly suggest that mRNA is present in dendrites, and that the levels of mRNA are highest during the same period that the polyribosomes are most prominent. The two stage approach combining physical separation with subcellular fractionation should provide a means of obtaining relatively enriched fractions of dendritic mRNA for further characterization in future studies.

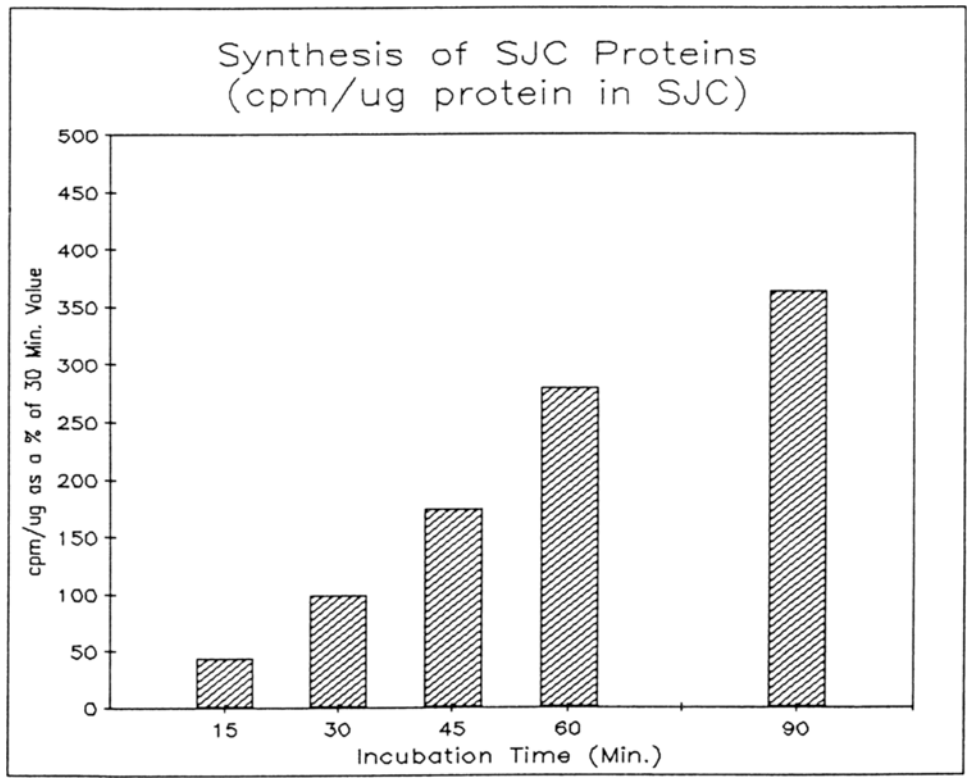

Fig. 14. Time course of appearance of recently synthesized protein in SJC fractions. Hippocampal slices were pulse-labeled with 3H-leucine for 15, 30, 45, 60, and 90 min, and SJC fractions were prepared. The graph indicates the time course of the increase in labeling of the SJC fractions. Each experiment analyzed three time points including the 30 min point, and the data from different experiments are expressed as a percentage of the 30 min value.

Selective Transport of RNA into Dendrites

The selective positioning of polyribosomes beneath synaptic sites raises the question of how this localization comes about. There are really two issues: how ribosomes and mRNA are translocated into the dendrite from their site of synthesis in the nucleus, and how the machinery comes to be positioned precisely beneath synapses. To date, information is available on only the first of these two questions.

Studies of the transport of recently synthesized RNA in hippocampal neurons grown in culture have revealed a selective transport system that moves RNA into dendrites but not axons. As noted above, hippocampal neurons in low density culture elaborate dendrites and axons that can be distinguished on the basis of a number of criteria. One of the distinguishing features of dendrites is the presence of polyribosomes (Bartlett and Banker, 1984b; and *see* Fig. 7).

The presence of polyribosomes within dendrites of neurons in culture provides an opportunity to study how the protein synthetic machinery is delivered to dendrites. In neurons, as in other cells, RNA and ribosomes are synthesized exclusively within the nucleus; thus, one can trace the migration of labeled RNA from its site of synthesis in the nucleus following pulse-labeling with an RNA precursor such as 3H-uridine. When hippocampal neurons in low density cultures were exposed to 3H-uridine for 1 h, and were fixed immediately, label was found

only over the nucleus and to a lesser extent over the cytoplasm of the cell body (*see* Fig. 15 and Davis et al., 1987). When the neurons were pulse-labeled, and then returned to a medium with an excess of unlabeled uridine for a chase period to allow for transport of labeled RNA, the label migrated progressively into the cytoplasm and then the dendrites. The label reached the most distal dendritic processes of most neurons by about 12 h. At no time was there any evidence of labeling within axons, even when neurons were continuously exposed to 3H-uridine for 24 h.

To obtain a quantitative measure of the rate of transport, we measured how far out the label extended at different times after pulse labeling. Calculations based on measurements from all dendrites revealed an apparent rate of about 0.2 mm/d (Fig. 16A). However, label reaches the tips of the shortest dendrites relatively quickly; as a result, estimates of the rate of transport based on all labeled dendrites are low because of the inclusion of the shortest dendrites. In an attempt to provide a more accurate measurement of transport rate, we analyzed only the longest dendrites of each neuron. As illustrated in Fig. 16B, the apparent rate of migration in the longest dendrites is about 0.4–0.5 mm/d, a value that is similar to that reported for slow axonal transport.

To begin to explore the cellular mechanisms of the transport, we evaluated whether the dendritic transport system for recently synthesized RNA exhibited a sensitivity to metabolic poisons similar to that of the rapid axonal transport system. Neurons were pulse-labeled with 3H-uridine as above, and then transferred into the chase medium with an excess of cold uridine for 1.5 h. During the 1.5-h chase, recently synthesized RNA appeared in the cytoplasm and in the initial portion of the dendrites. Then, the neurons were transferred into a chase medium containing either sodium azide or dinitrophenol (DNP) at concentrations sufficient to block oxidative phosphorylation (10 m*M* in both cases). Cultures were fixed 4.5 h after transfer to the medium with DNP or azide, which was 6 h after the end of the pulse. The extent of dendritic transport in these cells was then compared with the extent of transport in cells maintained in normal media during a 6 h chase. As illustrated in Fig. 17 (*see* p. 28), treatment with either of the metabolic poisons effectively blocked the dendritic transport of recently synthesized RNA. The extent of dendritic labeling 4.5 h after transfer to the media containing DNP or azide was substantially less than a control group of neurons that were untreated. Indeed, the distance of labeling in treated neurons was approximately comparable to neurons harvested 1.5 h after the end of the pulse, at the time that the treated cells were transferred to the DNP or azide-containing media. Control studies demonstrated that neurons that failed to exhibit dendritic transport were still capable of protein synthesis, and were thus viable, indicating that the metabolic poisons were not simply killing the neurons (Davis et al., 1987b; and in preparation).

At this time, the composition of the labeled RNA that is transported is not known. If it is mRNA that is transported at the rate of slow transport, the question of mRNA stability arises. At the rate of 0.5 mm/d, it would take hours for the mRNA to reach the tips of long dendrites. However, most mRNA molecules have relatively short half-lives (on the order of a few hours). Thus, it would seem that the mRNA that is transported must have a longer half-life than the average mRNA. It is possible that the mRNAs that are transported are of a type that normally has a short-half-life, but are somehow protected from normal turnover. Alternatively, there may be separate classes of mRNA that are inherently longer-lived. In this regard, it is of interest that mRNAs with half-lives on the order of 12 h have been described in brain (Appel, 1967). It also remains possible that the bulk of the material that is transported is not mRNA, and that the message itself is transported via a

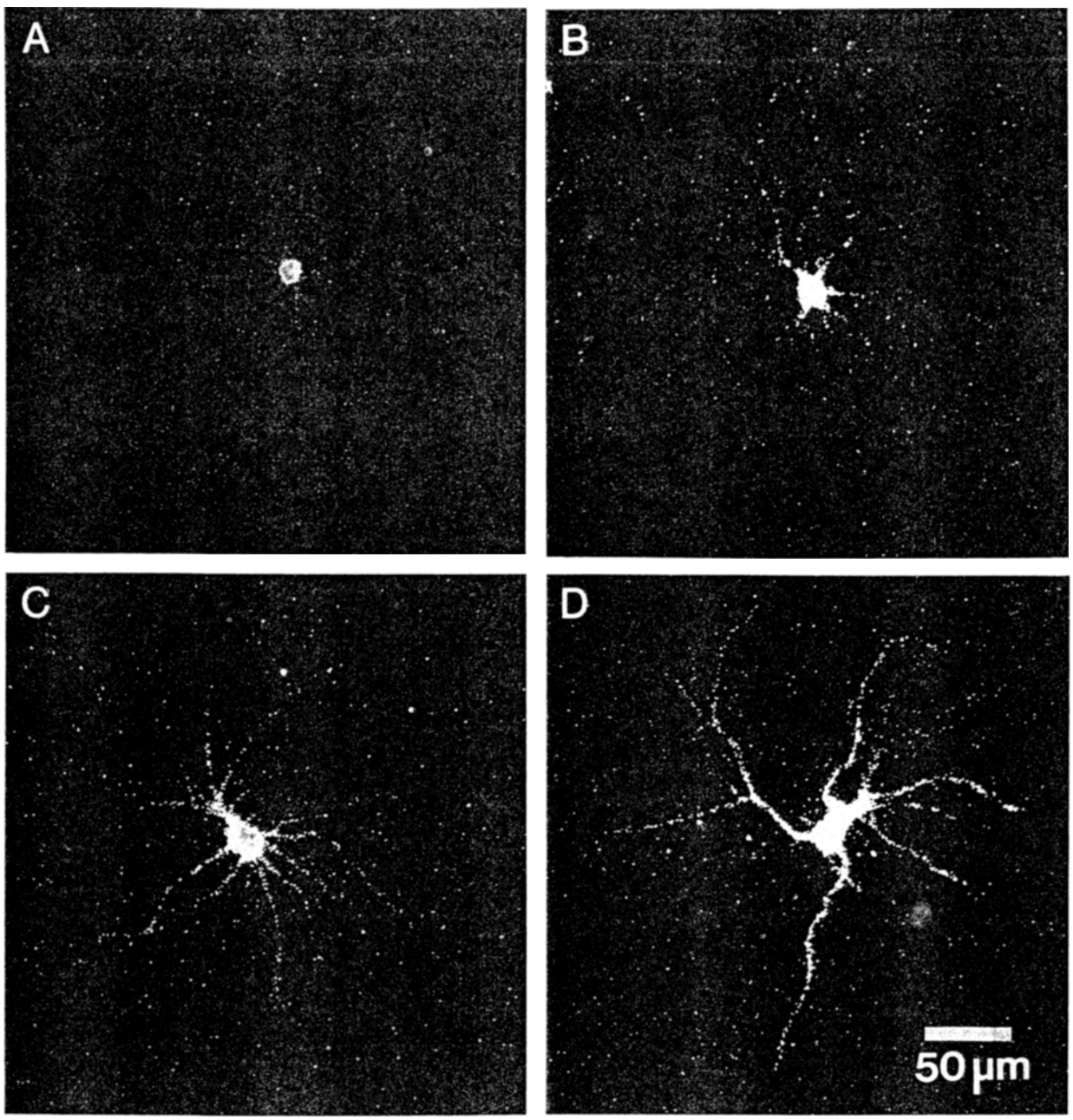

Fig. 15. Selective transport of 3H-labeled RNA into the dendrites of hippocampal neurons grown in culture after pulse-labeling with 3H-uridine. A illustrates the pattern of labeling at the end of a 1 h pulse. B–D illustrates the migration of label into dendrites at 3, 6, and 12 h after the pulse. Note that at 1 h, labeling is found only over the nucleus. At 6 h, proximal dendrites are labeled, and by 12 h, the label extends nearly to the tips of the dendrites. *After* Davis et al., 1987.

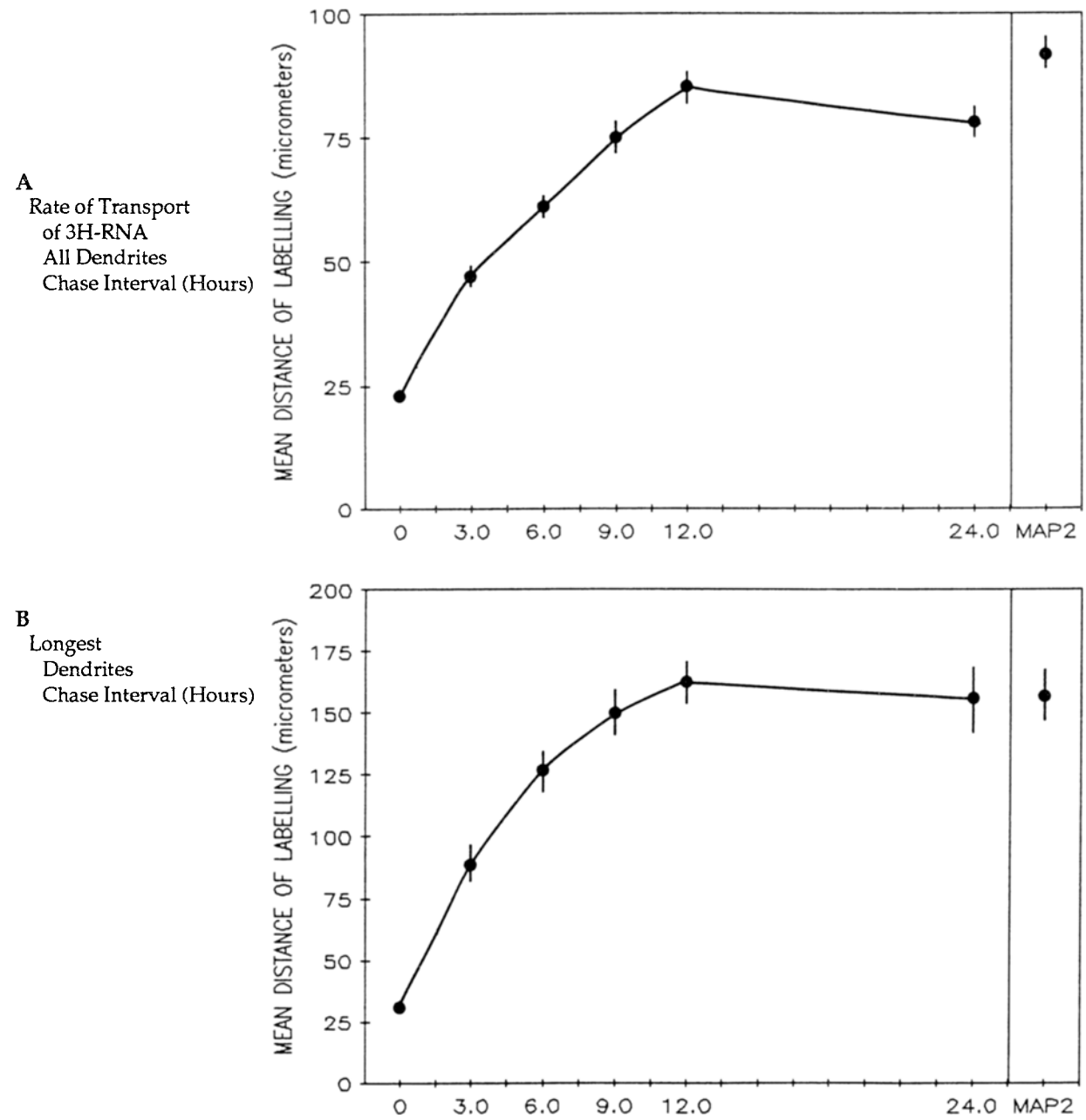

Fig. 16. Rate of dendritic transport of recently synthesized RNA. Neurons in culture were pulse-labeled with 3H-uridine for 1 h. One group of cells was fixed immediately, and prepared for autoradiography (0 h chase). Other groups of cells were placed in a chase medium containing unlabeled uridine for 3, 6, 9, 12, or 24 h to permit the transport of recently synthesized RNA from the nucleus and throughout the cell. At the end of the chase, cells were fixed and prepared for autoradiography. To calculate the average distance of transport at each interval, 25 neurons were randomly chosen for analysis. The average distance of dendritic labeling was defined by determining the point along each dendrite where grain density decreased to fewer than 3 grains in a template circle 10 μm in diameter. The average length of the dendrites themselves was determined in sister cultures that were immunostained for the selective dendritic marker MAP2. **A** illustrates the average distance of labeling on the basis of all dendrites. **B** illustrates the average distance of labeling when measuring only the longest dendrite of each cell (to correct for the presence of very short dendrites that would skew the average). Note that the label migrates out the dendrite at a rate that is similar to the rate of slow axonal transport. *From* Davis et al., 1987.

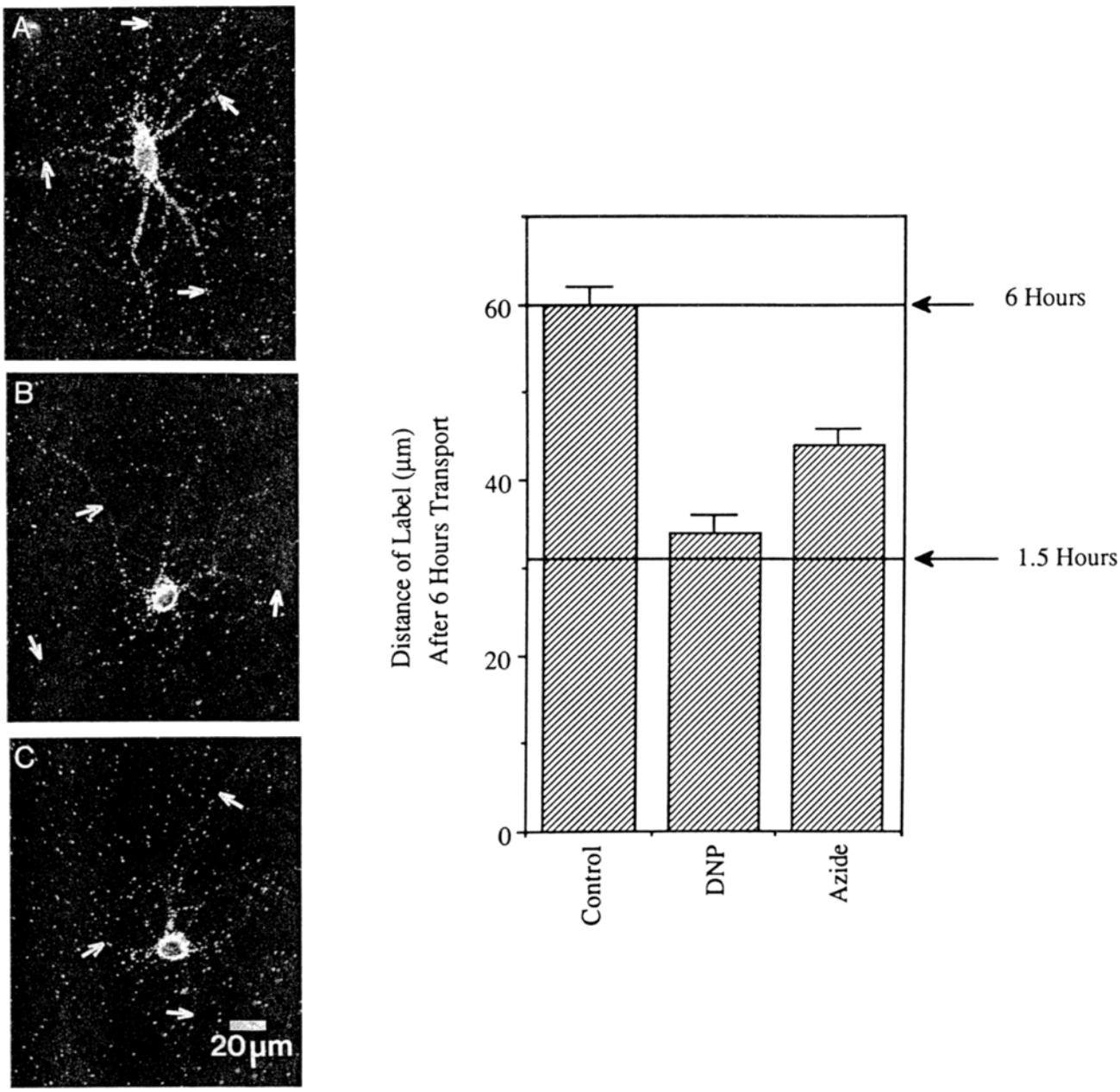

Fig. 17. Dendritic transport of recently synthesized RNA is blocked by metabolic poisons. Cultures were pulse-labeled with 3H-uridine (40 µCi/mL) for 1 h, washed twice in unlabeled medium to remove excess radioactivity, and then transferred to media with an excess of unlabeled uridine for 1.5 h to allow transport to be initiated. The neurons were then transferred into chase media with: 1) sodium azide (at 10 m*M*); or 2) 2,4,-dinitrophenol (DNP) (at 10 m*M*) and allowed to transport for an additional 4.5 h. Control neurons were left in the chase medium without metabolic inhibitors. At the end of the chase period, the neurons were fixed and processed for autoradiographty. **A** = in control neurons, labeling extended about halfway out the dendritic tree. In neurons treated with either DNP **(B)** or sodium azide **(C)** only the most proximal dendrites were labeled. Quantitative analyses revealed that the distance of labeling in the treated neurons was significantly less than the control, and in fact was no greater than in neurons that were prepared for autoradiography 1.5 h after the pulse (when the treated neurons were transferred into the media with the metabolic inhibitors). For the quantitative analyses, fifteen neurons from each condition were photographed in darkfield illumination (final magnification, 475X) and the photographs were scored for the distance of labeling in each dendrite, as described in Fig. 16.

rapid transport system. Until the composition of the material that is transported at the slow rate is defined, this question will remain open.

Is There a Differential Distribution of Different mRNAs in Neurons?

If our hypothesis is correct that different types of proteins are synthesized at different intracellular locations, then there should be a differential distribution of mRNAs within the neurons. Indeed, one would expect that certain mRNAs will be selectively transported into dendrites, whereas others will remain in the neuronal cell body. At present, there is only limited evidence bearing on this question. As would be expected, most of the mRNAs that have been evaluated are most abundant in cell bodies. For example, in our own *in situ* hybridization studies, labeling following exposure to probes for actin and tubulin mRNA is certainly most dense over cell bodies. This is true when evaluating the distribution of mRNAs in vivo in laminated structures where cell body and dendritic laminae can be distinguished, or in vitro, where the distribution of mRNA can be evaluated within the processes of individual cells (Trimmer et al., 1987). Interestingly, in the case of the probes for actin mRNA, there is a low level of labeling in dendritic laminae, and the level of labeling increases during times of synapse growth (Phillips et al., 1987). Thus, actin may be one of the molecules synthesized within dendrites, but if so, it is also synthesized to a much greater extent within the cell body. This is certainly not a surprise, since actin is found throughout the neuron as a major component of the cytoskeleton.

There has been one report, however, of an mRNA that appears to be relatively abundant in dendritic laminae in the cerebellar cortex (Levinthal et al., 1987). This mRNA codes for a protein (termed L7) that appears to be specific to Purkinje cells, and which is thought to be a marker for the terminal differentiation of these cells. *In situ* hybridization studies have revealed that the mRNA for this protein is present in Purkinje cell bodies and in the molecular layer that contains the Purkinje cell dendrites. Indeed, at certain ages, the mRNA for L7 is almost as abundant in the molecular layer as in the cell body laminae. Since this protein is apparently specific to Purkinje cells, it is not likely that the mRNA in the molecular layer is present in cells other than Purkinje cells (including interneurons and glia). Thus, the label that is present in the molecular layer presumably reflects the presence of mRNA for L7 in the dendrites of Purkinje cells. It will be important to be vigilant for other mRNAs that may be relatively more abundant in dendrites.

Discussion

Elegant studies of transport phenomena within neurons have led to the conception that virtually all neuronal protein synthesis takes place in the cell body; it has been presumed that such transport systems deliver the molecules required for the structural specializations of axons, dendrites, and their synapses from the cell body to their final destination. It is relatively easy to envision how selective transport mechanisms could be sufficient for relatively simple cell types with only a few different types of membrane surfaces. However, the construction and maintenance of synaptic junctions on CNS neurons is more problematic because of the complexity of neuronal form, the multiplicity of different membrane domains that neurons must construct and maintain, and the fact that these domains are presumably modifiable throughout the life of the organism. The modifiability of individual synaptic connections presents a particular problem for spatially complex cells; selective growth of synapses would presumably require that new protein constituents be

made available at the individual synaptic sites undergoing modification.

The present observations suggest a mechanism that would permit local synthesis and posttranslational processing of key protein constituents of the postsynaptic junctional region. Local synthesis and processing of proteins would provide an opportunity for local regulation, including regulation by synaptic activity. Indeed, it is possible to conceive of a richness and specificity of regulation that would be completely impossible if all of the protein synthesis of the neuron took place in the cell body.

Such a mechanism fits well with prevailing notions of the nature of synapse maintenance and modification. There is a growing body of evidence that dramatic modifications of synaptic structure and function occur as a consequence of certain types of physiological activation. The establishment of synaptic connections during development often involves a selective stabilization of certain synaptic connections coupled with a selective elimination of others. In mature animals, patterns of physiological activity can induce very long-lasting changes in synapse function (long-term potentiation, *see* Bliss and Lomo, 1973). The increases are restricted to the synapses that are active during the stimulation and do not generalize to other synapses on the postsynaptic cell (Andersen et al., 1977; Lynch et al., 1977; McNaughton et al., 1978; Levy and Steward, 1979). This suggests a modification at the level of the individual synaptic contact. These changes in efficacy are accompanied by changes in spine shape (Fifkova and Van Harreveld, 1977; Desmond and Levy, 1983), increases in the number of certain types of synapses (Lee et al., 1977; Greenough et al., 1985), and increases in the size and configuration of the junctional specialization (Desmond and Levy, 1983, 1988). Moreover, long-term potentiation may be accompanied by changes in protein metabolism (Duffy et al., 1981), and under certain circumstances, protein synthesis inhibitors block the structural changes that accompany LTP (Fifkova et al., 1982), and the increases in synaptic efficacy (Stanton and Sarvey, 1983; and Deadwyler et al., 1987). All of these observations suggest that synapses can be individually modified as a result of functional activity, and that the modifications depend upon ongoing protein synthesis. If this is so, it would certainly not be surprising if the polyribosomes under the postsynaptic site played some role in the modification.

Local synthesis could also help to govern whether particular synapses would be retained or eliminated during early development. It is well known that there are adjustments in synapses during the developmental period. Often these adjustments involve eliminating some types of synapses, and stabilizing others. The elimination or selective stabilization of synapses obviously must occur on a synapse by synapse basis, since these processes apparently depend upon differential activity. Local regulation of the production of proteins of the junctional region could contribute to this selection process. In this regard, it is of considerable interest that there is an increase in the number of synapses with polyribosomes in the visual cortex in animals that had been exposed to an enriched environment during the stage in development when such exposure leads to increases in synapses on cortical neurons (Greenough et al., 1985).

The prominence of polyribosomes at postsynaptic sites during developmental and lesion-induced synaptogenesis strongly suggests that local synthesis is particularly important during periods of synapse growth. Because polyribosomes are most prominent sometime after the formation of the initial contact, it seems most likely that the proteins that are produced are important for the maturation of the junction. Indeed, as noted above, the biochemical evidence suggests that the polyribosomes beneath synapses may synthesize some of the protein constituents of the junctional specialization. If this is true, local synthesis may actually contri-

bute to the construction of different types of synaptic junctions at different locations. By regulating the positioning of different mRNAs at particular sites, the postsynaptic cell could synthesize proteins that are unique to particular types of junctions on site. Such differential localization of mRNAs could come about in response to the presence or activity of particular types of terminals, or could be regulated independently by the postsynaptic cell so that the site is defined prior to the arrival of the terminal, and is only receptive to particular types of terminals.

The notion that there might be a differential distribution of different types of mRNA in neurons is in accord with a growing body of evidence from studies of other cell types. For example, in myoblasts and fibroblasts, mRNA for actin is concentrated near the cell periphery, especially within lamellipodia, whereas mRNA for vimentin is localized near the nucleus (Lawrence and Singer, 1986). In oligodendrocytes, there is evidence that mRNA for myelin basic protein is concentrated in the processes of the cell that are involved in myelin formation, whereas mRNA for proteolipid protein is largely confined to the oligodendrocyte cell body (Colman et al., 1982). Finally, in muscles, mRNAs for the different subunits of the ACh receptor are concentrated beneath the synaptic junction. Indeed, the concentration of ACh receptor mRNA is 50-fold higher in the junctional portion of the muscle fiber than in nonjunctional regions, whereas mRNA for actin is uniformly distributed (Merlie and Sanes, 1985). Thus, there is ample precedent from differential distribution of different types of mRNA in a variety of cell types.

If there is an intracellular sorting of mRNA within neurons, it would seem that the routing would require some sort of molecular address marker (for a discussion of molecular address markers, *see* Hammerschlag, 1983). One could imagine molecular address markers that mediate the binding of mRNAs to the dendritic transport machinery, which would then determine whether the mRNA would enter the dendrite or be restricted to the cell body. Similarly, the selective docking of mRNAs beneath particular types of synapses could be mediated by different markers. We can envision three possible types of molecular address marker:

1. A signal contained within the mRNA sequence itself may serve as the molecular address marker for intracellular routing;
2. The mRNA may direct the synthesis of a recognition sequence that is part of the nascent polypeptide, which would then serve as a molecular address marker; or
3. A signal within the mRNA sequence may bind a protein intermediate (not directly synthesized by the RNA) that would then serve as a molecular address marker.

The first mechanism discussed above is appealing because many mRNAs have sequences at either the 3' end, the 5' end , or both, which are not part of the coding region. The functional significance of these noncoding regions is not known, but these sequences could well code for intracellular sorting. The second mechanism is appealing because it is already known to operate in directing particular mRNAs to the endoplasmic reticulum (for reviews, *see* Walter and Lingappa, 1986; Colman and Robinson, 1986; Dingwall and Laskey, 1986). Thus, mRNAs that encode intrinsic membrane proteins exit the nucleus, engage a ribosome, and begin translation. A "signal sequence" present near the *N*-terminal end of the nascent polypeptide binds to a signal recognition particle (SRP), which freezes translation until the SRP encounters and then binds to an appropriate receptor on the endoplasmic reticulum. One could imagine analogous "signal recognition particles" that mediate the binding of mRNAs and their nascent peptides to the dendritic transport machinery. Finally, the third mechanism is of interest because there are a number of RNA binding proteins, and again, the function of most of these is not known.

The above speculations make it clear that selective intracellular routing of mRNA requires mechanisms that are certainly no less complex than would be required for selective transport of protein. Indeed, two of the possible sorting mechanisms discussed above postulate that mRNA sorting actually occurs via protein sorting. Thus, the advantage provided to the neuron by a mechanism for intracellular sorting of mRNA and local synthesis of proteins is not simplicity. Instead, it is the opportunity for the local regulation of the levels of particular proteins, the timing and site of synthesis of proteins, and the blend of different types of proteins within specific cytoplasmic microdomains.

Conclusion

The selective positioning of polyribosomes under postsynaptic sites reveals a microcompartmentation of the protein synthetic machinery of neurons that has not been observed in other cells. The existence of such microcompartmentation will necessitate new approaches to the molecular biology of neurons. It may be impossible to understand gene expression in neurons without understanding the interactions between the machinery for gene expression and the complex form of the neuron. It may be that neurons will be especially useful for studying intracellular sorting because the mechanisms may be more highly developed and thus more easily recognized in comparison with other less complex cell types.

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References

Andersen, P., Sundberg, S. H., Sveen, O., and Wigstrom, H. (1977) Specific long-lasting potentiation of synaptic transmission in hippocampal slices. *Nature* **266**, 736,737.

Appel. S. H. (1967) Turnover of brain messenger RNA. *Nature*, **213**, 1253,1254.

Autilio, L. A., Appel, S. H., Pettis, P., and Gambetti, P. L. (1968) Biochemical studies of synapses in vitro. I. Protein synthesis. *Biochemistry* **7**, 2615–2622.

Bartlett, W. P. and Banker, G. A. (1984a) An electron microscopic study of the development of axons and dendrites by hippocampal neurons in culture: I. Cells which develop without intercellular contacts. *J. Neurosci.* **4**, 1944–1953.

Bartlett, W. P. and Banker, G. A. (1984b) An electron microscopic study of the development of axons and dendrites by hippocampal neurons in culture: II. Synaptic relationships. *J. Neurosci.* **4**, 1944–1953.

Bliss, T. V. P. and Lomo, T. (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anesthetized rabbit following stimulation of the perforant path. *J. Physiol.* **232**, 331–356.

Bodian, D. (1965) A suggestive relationship of nerve cell RNA with specific synaptic sites. *Proc. Natl. Acad. Sci.* **53**, 418–425.

Caceres, A. O., Banker, G. A., and Binder, L. (1986) Immunocytochemical localization of tubulin and microtubule-associated protein 2 during the development of hippocampal neurons in culture. *J. Neurosci.* **6**, 714–722.

Colman, D., Kreibich, G., Frey, A. B., and Sabatini, D. (1982) Synthesis and incorporation of myelin polypeptides into CNS myelin. *J. Cell Biol.* **95**, 598–608.

Colman, A. and Robinson, C. (1986) Protein import in organelles: Hierarchical targeting signals. *Cell* **46**, 321,322.

Cotman, C. W. and Taylor, D. (1972a) Isolation and ultrastructural studies on synaptic complexes from rat brain. *J. Cell Biol.* **55**, 696–711.

Cotman, C. W. and Taylor, D. (1972) Autoradiographic analysis of protein synthesis in synaptosomal fractions. *Brain Res.* **29**, 366–372.

Cupello, A. and Hyden, H. (1975) Fractionation of RNA from brain synaptosomes and cytoplasmic subcellular fractions. *J. Neurochem.* **25**, 399–406.

Davis, L., Banker, G. A., and Steward, O. (1987a) Selective dendritic transport of RNA by hippocampal neurons in culture. *Nature* **330**, 477–479.

Davis, L., Dotti, C., Banker, G., and Steward, O.

(1987b) Dendritic transport of RNA: An energy-dependent process. *Neurosci. Abs.* **13,** 121.

Deadwyler, S. A., Dunwiddie, T., and Lynch, G. (1987) A critical level of protein synthesis is required for long-term potentiation. *Synapse* **1,** 90–95.

DeCamilli, P., Moretti, M., Donini, S. D., Walter, U., and Lohmann, S. M. (1986) Heterogeneous distribution of the cAMP receptor protein RII in the nervous system: Evidence for its intracellular accumulation on microtubules, microtubule-organizing center, and in the area of the Golgi complex. *J. Cell Biol.* **103,** 189–203.

DeLarco, J., Nakagawa, S., Abramowitz, A., Bromwell, K., and Guroff, G. (1975) Polyadenylic acid-containing RNA from rat brain synaptosomes. *J. Neurochem.* **25,** 131–137.

Desmond, N. L. and Levy, W. B. (1983) Synaptic correlates of associative potentiation/depression: An ultrastructural study in the hippocampus. *Brain Res.* **265,** 21–30.

Desmond, N. L. and Levy, W. B. (1988) Anatomy of associative long-term synaptic modification. *Long-term Potentiation: From Biophysics to Behavior* (Lanfield, P. W. and Deadwyler, S. A., eds.), Liss, NY, pp. 265–305.

Dingwall, C. and Laskey, R. (1986) Protein import into the cell nucleus. *Ann. Rev. Cell Biol.* **2,** 367–390.

Duffy, C., Teyler, T. J., and Shashoua, E. (1981) Long-term potentiation in the hippocampal slice: Evidence for stimulated secretion of newly synthesized proteins. *Science* **212,** 1148–1151.

Fass, B. and Steward, O. (1983) Increases in protein precursor incorporation in the denervated neuropil of the dentate gyrus during reinnervation. *Neurosci.* **9,** 633–664.

Fifkova, E., Anderson, C. A., Young, S. J., and Van Harreveld, A. (1982) Effect of anisomycin on stimulation-induced changes in dendritic spines of the dentate granule cells. *J. Neurocytol.* **11,** 183–210.

Fifkova, E. and Van Harreveld, A. (1977) Long-lasting morphological changes in dendritic spines of dentate granule cells following sitmulation of entorhinal area. *J. Neurocytol.* **6,** 211–230.

Freund, T. F., Martin, K. A. C., Smith, A. D., and Somogyi, P. (1983) Glutamate decarboxylase-immunoreactive terminals of Golgi-impregnated axoaxonic cells and of presumed basket cells in synaptic contact with pyramidal neurons of the cat's visual cortex. *J. Comp. Neurol.* **221,** 263–278.

Gambetti, P., Autilio-Gambetti, L. A., Gonatas, N. K., and Shafer, B. (1972) Protein synthesis in synaptosomal fractions: Ultrastructural radioautographic study. *J. Cell. Biol.* **52,** 526–535.

Greenough, W. T., Hwang, H. -M. F., and Gorman, C. (1985) Evidence for active synapse formation or altered postsynaptic metabolism in visual cortex of rats reared in complex environments. *Proc. Natl. Acad. Sci.* **82,** 4549–4552.

Griffiths, G., Quinn, P., and Warren, G. (1983) Dissection of the Golgi complex 1. Monensin inhibits the transport of viral membrane proteins from medial to trans Golgi cisternae in baby hamster kidney cells infected with semliki forest virus. *J. Cell Biol.* **96,** 835–850.

Hammerschlag, R. (1983) How do neuronal proteins know where they are going? Speculations on the role of molecular address markers. *Dev. Neurosci.* **6,** 2–17.

Hammerschlag, R., Stone, G. C., Bolen, F. A., Lindsey, J., and Ellisman, M. (1982) Evidence that all newly synthesized proteins destined for fast axonal transport pass through the Golgi apparatus. *J. Cell Biol.* **93,** 568–575.

Heifetz, A., Kienan, R. W., and Elbein, A. D. (1979) Mechanisms of action of tunicamycin on the UDP-Glc Nac: Dolichyl-phosphate GlcNAc-1-phosphate transferase. *Biochem.* **18,** 2186–2192.

Irwin, C. C. (1985) Comparison of protein synthesis in mitochondria synaptosomes, and intact brain cells. *J. Neurochem.* **44,** 433–438.

Kelly, R. B. (1985) Pathways of protein secretion in eukaryotes. *Science* **230,** 25–32.

Lawrence, J. B. and Singer, R. H. (1986) Intracellular localization of messenger RNAs for cytoskeletal proteins. *Cell* **45,** 407–415.

Lee, K. S., Schottler, F., Oliver, M., and Lynch, G. (1980) Brief bursts of high frequency stimulation produce two types of structural change in rat hippocampus. *J. Neurophys.* **44,** 247–258.

Levinthal, F., Oberdick, J., Yang, S. M., and Levinthal, C. (1987) Specific mRNA identified during development in mouse Purkinje cells and their dendrites. *Neurosci. Abs.* **13,** 1708.

Levy, W. B. and Steward, O. (1979) Synapses as associative memory elements in the hippocampal formation. *Brain Res.* **175,** 233–245.

Lodish, H. V., Kong, N., Snider, M., and Strous, G. J.

A. M. (1983) Hepatoma secretory proteins migrate from rough endoplasmic reticulum to Golgi at characteristic rates. *Nature* **304**, 80–83.

Lynch, G. S., Dunwiddie, T., and Gribkoff, V. (1977) Heterosynaptic depression: a postynaptic correlate of long-term potentiation. *Nature* **266**, 737–739.

Matus, A., Bernhardt, R., and Jones, T. H. (1981) High molecular weight microtubule-associated proteins are preferentially associated with dendritic microtubules in brain. *Proc. Natl. Acad. Sci.* **78**, 3010–3014.

McNaughton, B. L., Douglas, R. M., and Goddard, G. V. (1978) Synaptic enhancement in fascia dentata: Cooperativity among coactive afferents. *Brain Res.* **157**, 277–293.

Merlie, J. P. and Sanes, J. R. (1986) Concentration of acetylcholine receptor mRNA in synaptic regions of adult muscle fibers. *Nature* **317**, 66–68.

Morgan, I. G. and Austin, L. (1968) Synaptosomal protein synthesis in a cell-free system. *J. Neurochem.* **41**, 41–51.

Palacios-Pru, E. L., Palacios, L., and Mendoza, R. V. (1981) Synaptogenetic mechanisms during chick cerebellar cortex development. *J. Submicrosc. Cytol.* **13**, 145–167.

Palacios-Pru, E. L., Miranda-Contreras, L., Mendoza, R. V., and Zambrano, E. (1988) Dendritic RNA and postsynaptic density formation in chick cerebellar synaptogenesis. *Neurosci.* **24**, 111–118.

Palade, G. (1965) Intracellular aspects of the process of protein synthesis. *Science* **189**, 347–358.

Peters, A., Palay, S. L., and Webster, de. F. (1976) *The Fine Structure of the Nervous System: The Neurons and Supporting Cells.* Saunders, Philadelphia, PA.

Phillips, L. L., Chikaraishi, D. M., and Steward, O. (1987) Increases in messenger RNA for actin and tubulin within the denervated neuropil of the dentate gyrus during lesion-induced synaptogenesis. *Neurosci. Abs.* **13**, 1428.

Sedman, G. L., Jeffrey, P. L., Austin, L., and Rostas, J. A. P. (1986) The metabolic turnover of the major proteins of the postsynaptic density. *Mol. Bain Res.* **1**, 221–230.

Simons, K. and Fuller, S. D. (1985), Cell surface polarity in epithelia. *Ann. Rev. Cell. Biol.* **1**, 243–288.

Somogyi, P., Smith, A. D., Nunzi, M. G., Gorio, A., Takagi, H., and Wu, J. Y. (1983), Glutamate decarboxylase immunoreactivity in the hippocampus of the cat. Distribution of immunoreactive terminals with special reference to the axon initial segment of pyrimidal neurons. *J. Neurosci.* **3**, 1450–1468.

Spacek, J. (1985) Three-demensional analysis of dendritic spines. II. Spine apparatus and other cytoplasmic components. *Anat. Embryol.* **171**, 235–243.

Spacek, J. and Hartmann, M. (1983) Three-dimensional analysis of dendritic spines. I. Quantitative observations related to dendritic and synaptic morphology in cerebral and cerebellar cortices. *Anat. Embroyl.* **167**, 289–310.

Stanton, P. K. and Sarvey, J. M. (1983) Blockade of long-term potentiation in rat hippocampal CA1 region by inhibitors of protein synthesis. *J. Neurosci.* **4**, 3080–3088.

Steward, O. (1983a) Alterations in polyribosomes associated with dendritic spines during the reinnervation of the dentate gyrus of the adult rat. *J. Neurosci.* **3**, 177–188.

Steward, O. (1983b) Polyribosomes at the base of dendritic spines of central nervous system neurons: Their possible role in synapse construction and modification. *Cold Spring Harbor Symposia on Quantitiative Biology* **48**, 745–759.

Steward, O., Davis, L., Reeves, T. M., and Banker, G. (1988) Microcompartmentation of the protein synthetic machinery of neurons: polyribosome localization under postsynaptic sites. *Intrinsic Determinants of Neuronal Form and Function* (Lasek, R. J., ed.), Liss, NY, pp. 521–544.

Steward, O. and Falk, P. M. (1985) Polyribosomes under developing spine synapses: Growth specializations of dendrites at sites of synaptogenesis. *J. Neurosci. Res.* **13**, 75–88.

Steward, O. and Falk, P. M. (1986) Protein synthetic machinery at postsynaptic sites during synaptogenesis: A quantitative study of the association between polyribosomes and developing synapses. *J. Neurosci.* **6**, 412–423.

Steward, O. and Levy, W. B. (1982) Preferential localization of polyribosomes under the base of dentritic spines in granule cells of the dentate gyrus. *J. Neurosci.* **2**, 284–291.

Steward, O. and Reeves, T. M. (1988) Protein synthetic machinery beneath postsynaptic sites on CNS neurons: Association between polyribosomes and other organelles at the synaptic site. *J. Neurosci.* **8**, 176–184.

Steward, O. and Ribak, C. E. (1986) Polyribosomes associated with synaptic specializations on axon initial segments: Localization of protein synthetic machinery at inhibitory synapses. *J. Neurosci.* **6,** 3079–3085.

Steward, O. and Vinsant, S. V. (1983) The process of reinnervation in the dentate gyrus of the adult rat: A quantitative electron microscopic analysis of terminal proliferation and reactive synaptogenesis. *J. Comp. Neurol.* **214,** 370–386.

Suzuki, K. (1965) The pattern of mammalian brain gangliosides-II. Evaluation of the extraction procedures, postmortem changes, and the effect of formalin preservation. *J. Neurochem.* **12,** 629–638.

Tkacz, J. S. and Lampen, J. O. (1975) Tunicamycin inhibition of polyisoprenyl *N*-acetylglucosaminyl pyrophosphate formation in calf-liver microsomes. *Biochem. Biophys. Res. Commun.* **65,** 248–257.

Triller, A., Cluzeaud, F., Pfeiffer, F., Betz, H., and Korn, H. (1985) Distribution of glycine receptors at central synapses: An immunoelectron microscopy study. *J. Cell Biol.* **101,** 683–688.

Trimmer, P. A., Phillips, L. L., and Steward, O. (1987) Use of *in situ* hybridization and immunocytochemistry to examine the distribution of actin and tubulin and their messenger RNA in CNS neurons. *Neurosci. Abs.* **13,** 1428.

Verity, M. A. Brown, W. J., and Cheung, M. (1980) Isolation of ribosome containing synaptosome subpopulation with active in vitro protein synthesis. *J. Neurosci. Res.* **5,** 143–153.

Verity, M. A., Brown, W. J., Cheung, M., Huntsman, H., and Smith, R. (1979) Effects of neonatal hypothyroidism on cerebral and cerebellar synaptosome development. *J. Neurosci. Res.* **2,** 323–335.

Walter, P. and Lingappa, V. (1986) Mechanism of protein translocation across the endoplasmic membrane. *Ann. Rev. Cell Biol.* **2,** 499–516.