The Cell Biology of the Nerve Terminal

Review

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As more is known about membrane trafficking within cells, it becomes increasingly attractive to consider neurotransmitter release as a specialized instance of exocytotic secretion followed by endocytotic recycling. Cell biological experiments have taught us, for instance, that there are at least two types of secretion, regulated and constitutive, and that different secretory proteins are targeted to different plasma membrane domains of polarized cells. We know that in many types of nonneuronal cells, organelles move along microtubules by mechanisms that parallel axonal transport in neurons. We have learned that some plasma membrane proteins are selectively internalized into early endosomes, from which some proceed to the late endosome, while others return to the plasma membrane. From the late endosome, another choice can be made, either to go to the lysosome or to the Golgi apparatus. It may be equally attractive to consider synapse formation as a specialized example of cell-cell or cell-matrix contact.

In this review, we ask whether such recent findings in cell biology are pertinent to our understanding of the nerve terminal. In the first section, our limited knowledge of the molecular components of the nerve terminal is summarized. I have focused on proteins associated with the synaptic vesicle, the synaptic vesicle anchoring machinery, and the synaptic junction, all of which might have functional equivalents in nonneuronal secretory cells or in cell-cell contacts. The second part considers the biogenesis of both synaptic vesicles and the vesicles with electron-dense cores that are frequently present in nerve terminals. The third and final section considers how synapse formation may be considered in light of current cell biological thinking.

Molecular Architecture of the Active Zone

The functional unit responsible for the exocytotic release of neurotransmitter from the presynaptic terminal is the active zone. Anatomical and deep-etch studies, such as those of Hirokawa and Heuser (1982), demonstrate that there are three elements of the active zone: a cluster of synaptic vesicles anchored in the vicinity of the release site, a release site at the plasma membrane, at which synaptic vesicles are thought to dock, and extracellular elements holding the release site in apposition to the postsynaptic receptors. I consider each of these three elements in turn.

The Proteins of the Synaptic Vesicle

Monoclonal antibodies have allowed conclusive identification of five integral membrane proteins apparently present in all synaptic vesicles (Matthew et al., 1981; Buckley and Kelly, 1985; Wiedenmann and Franke, 1985; Jahn et al., 1985; Obata et al., 1987). The presence of these five proteins in all vesicles suggests that they play a fundamental role in vesicle function. The remarkable structural conservation of some of these proteins, over a wide range of species, suggests that the functions they serve are important. All five antigens are also present in endocrine cells, a point to which I return later. Of the five proteins, we have functional information for only two: p38 (synaptophysin) and p65; synaptophysin has the ability to bind calcium, while antibodies to p65 bind a synaptic vesicle protein that elutes from a calmodulin-affinity column (Fournier and Trifaro, 1988). In the case of synaptophysin, sequence data have been obtained (Buckley et al., 1987; Sudhof et al., 1987; Leube et al., 1987).

Current views of the mechanisms of exocytosis predict at least two universal functions (Figure 1): certain proteins must be capable of anchoring vesicles to the cytoskeleton, and others must serve as docking/fusing proteins. Calcium or calcium-calmodulin could regulate either or both of these functions.

Association with Release Sites

Synaptic vesicles are held in close proximity to the active zone, presumably by cytoskeletal elements. Cytoskeletal elements that interact with vesicles to prevent movement are believed to be quite different from the microtubule-based components, which generate movement. What cytoskeletal elements are present at the active zone? Although the high quality immunoelectron microscopy that would help answer this question is not yet available, a role for actin seems plausible. Immunofluorescence and immunoelectron microscopy have provided some evidence for F-actin, ankyrin, and fodrin in electroplax nerve terminals (Walker et al., 1985; Kordeli et al., 1986) but have not provided information about their exact location. Rare F-actin filaments can be seen in quick-freeze, deep-etch preparations of neuromuscular junctions (Hirokawa and Heuser, 1982). Sparse actin labeling can be seen by immunoelectron microscopy of nerve terminals on dendritic spines (Cohen et al., 1985). One protein that may be important in vesicle anchoring is synapsin I. Since synapsin I is known to have an actin-bundling capacity (Bahler and Greengard, 1987; Petrucci and Morrow, 1987), it may link the synaptic vesicle to the cytoskeleton by binding to vesicle proteins or lipid (Benfenati et al., 1987, Soc. Neurosci., abstract). In endocrine cells, secetory granules are anchored to the plasma membrane prior to release by an interaction thought to involve an actin-fodrin subplasmalemmal cytoskeleton (for review see Burgess and Kelly, 1987).

A freeze-etch study of ribbon synapses in the frog retina (Usukura nd Yamada, 1987) identifies one type of vesicle-cytoskeleton interaction. The ribbon in a ribbon synapse is a specialized and perhaps efficient way of
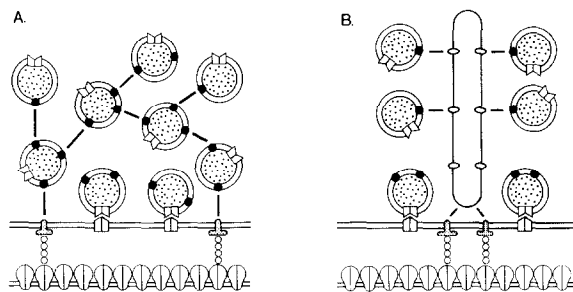
Figure 1. Model Comparing the Release Mechanisms of Conventional and Ribbon Synapses

Both models contain an anchorage zone where synaptic vesicles are kept close to the release site. The cytoskeletal elements holding the vesicles in place may include synapsin I. The anchoring mechanism must itself be attached to membrane components near the release site. Vesicles ready for release are docked at specialized sites on the nerve terminal plasma membrane. Both the proteins that attach the anchoring cytoskeleton and the docking sites must be kept in register with the postsynaptic receptor-rich regions.
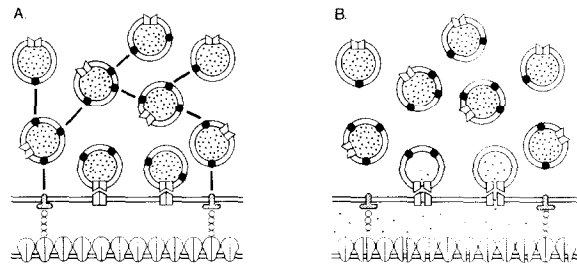

Figure 2. Changes in the Active Zone on Stimulation

The figure shows that when exocytosis of neurotransmitter is stimulated to occur in a terminal such as that shown in (A), the first exit of neurotransmitter might be through a channel-like structure. In addition to stimulating fusion, the incoming calcium ions may cause dissolution of the cytoskeletal network to allow fresh vesicles move up to the docking zones.

clustering synaptic vesicles in close proximity to the release site. Vesicles are linked to the ribbon structure via several filamentous arms, 8–10 nm in diameter and 30–50 nm long. The other ends of the arms are attached to the ribbons, which are attached by filaments to the presynaptic membrane. The ribbon itself has parallel arrays of 4–6 nm particles, a periodicity reminiscent of actin filaments. The model illustrated in Figure 2 compares these three functions—anchoring, docking, and alignment—for both normal and ribbon synapses.

The Docking and Release Site
While the majority of synaptic vesicles are anchored near the release site by cytoskeletal elements, a smaller number dock at specific sites on the nerve terminal plasma membrane (Figure 1). It is these latter vesicles that are thought to lose their content on nerve stimulation. An anatomical correlate of the docking machinery may be the presynaptic grid, a regular array of dense projections that stains with phosphotungstic acid and lies just inside the nerve terminal plasma membrane. It is premature, however, to conclude that these dense structures on the cytoplasmic face of the nerve terminal plasma membrane actually cause docking. The molecular composition of the presynaptic grid structures in still completely unknown, and there is no obvious parallel in nonneural tissue from which we can readily extrapolate.

Extracellular Alignment
The filamentous proteins that hold the vesicle clusters together must themselves be tied to the plasma membrane. Furthermore, the attachments to the nerve terminal plasma membrane must be confined to the region of the nerve terminal plasma membrane facing the postsynaptic membrane. To do this, the attachment proteins of the presynaptic terminal must be connected via some form of molecular bridge to proteins of the postsynaptic

membrane (see Figure 1). The docking proteins must be constrained similarly. Molecular bridges of the type predicted here have been described in cell–cell contacts between nonneuronal cells. The crucial finding in nonneuronal cells is that when cell–cell contact occurs, molecular bridges linking the cytoskeleton of one cell to that of another form (zonulae adherens and desmosomes). Such contacts and the analogous specialized attachments to the extracellular matrix (focal contacts and hemi-desmosomes) are relatively well described. A characteristic of focal contacts, but not zonulae adherens, is the presence of the cytoskeletal protein talin. The finding of talin at nerve muscle synapses (Sealock et al., 1985) strengthens the parallel between neuronal and nonneuronal cell contacts and suggests that the neuromuscular junction has homologies to focal junctions rather than zonulae adherens. At focal contacts, actin cytoskeletons are anchored to extracellular matrix. A homology between focal contacts and the neuromuscular junction makes sense in light of the considerable evidence that regenerating nerve terminals recognize the extracellular matrix of muscle (for review see Burden, 1987).

At the zonulae adherens and focal contacts, an actin-based cytoskeleton is attached to the extracellular world via transmembrane proteins of the cadherin (Hirano et al., 1987) and integrin (Hynes, 1987) type. An alternative way of anchoring actin-based cytoskeleton, in particular the subplasmalemmal actin-fodrin network, is to the $(Na^+,K^+)ATPase$ via the intermediary protein ankyrin (Nelson and Veshnock, 1987a, 1987b). Although the nerve terminal may anchor its synaptic vesicles to the nerve terminal plasma membrane by an entirely novel molecular mechanism, it is wise to look first for some parallels to these known anchoring systems.

Extracellular matrix proteins present in synaptic regions are also candidates for proteins involved in holding release sites and receptors in register. The extracellular matrix protein agrin, enriched in the synaptic cleft, causes the aggregation of acetylcholine receptors (Nitkin et al., 1987). In addition, terminal anchorage proteins, which appear to be proteoglycans, are enriched in

the synaptic cleft region (Carlson and Wight, 1987) and believed to link the nerve terminal to the extracellular matrix in peripheral synapses (Buckley et al., 1983; Caroni et al., 1985; Carlson et al., 1986; Stadler and Kiene, 1987; Carlson and Wight, 1987).

Returning to the speculative model of the nerve terminal (Figure 1), we can make some predictions about the properties expected of the three major components. The alignment and opposition of the active zone to the postsynaptic receptor surface resemble focal contacts in having the protein talin link the actin cytoskeleton to the extracellular matrix. In turn, cytoskeletal anchoring elements may resemble the cadherins and integrins by having conserved cytoplasmic domains to which cytoskeletal elements are anchored. The cytoskeletal elements could resemble the erythrocyte cytoskeleton or the basolateral cytoskeleton of epithelial cells and contain molecules of the ankyrin, actin, and spectrin families. Three of the functions of synaptic vesicles — anchoring to the cytoskeleton, binding Ca^{2+}, and docking to the nerve terminal plasma membrane — may be performed by some of the five identified integral membrane proteins. One element about which no prediction can be made is the nerve terminal plasma membrane docking site. In fact, the way that two intracellular membranous organelles dock and fuse has yet to be identified in any cell (for review see White et al., 1983).

Function of the Nerve Terminal

The static view of the nerve terminal generated from anatomical and molecular descriptions can be developed into a more dynamic one by incorporating recent findings from cell biology. Release of neurotransmitter involves the exocytosis of synaptic vesicle contents followed by the endocytosis of the vesicle membrane for reutilization. Since nonneuronal cells have much better characterized mechanisms for exocytosis and endocytosis, we can learn a great deal from them, depending on the extent to which these processes are the same in neurons and nonneuronal cells. In this section, transmitter release is viewed from the standpoint of membrane trafficking. Outside the scope of this review are the intriguing new findings on regulation of exocytosis by second messengers.

Exocytotic Release of Neurotransmitter
All cells are thought to transport their plasma membrane proteins to the surface in small secretory vesicles. In simple cells such as yeast, the same secretory vesicle also carries proteins destined for extracellular secretion (Holcomb et al., 1988). Since the secretory vesicles appear to have a short half-life, there are relatively few of them in the cytoplasm. Fusion, which must be efficient, is not regulated in any known way by variation in the levels of a cytoplasmic second messenger. Such secretion is called nonregulated (Tartakoff et al., 1987) or constitutive secretion (Gumbiner and Kelly, 1982). In addition to constitutive secretion, there is an alternative mode of exocytosis called regulated secretion. In regulated secre-

tion, secretory vesicles fuse poorly with the plasma membrane and so accumulate in the cytoplasm. Variation in second messenger level activates the fusion event. Neurons fall into the regulated secretory class as do exocrine and endocrine cells and some cells of the hematopoietic lineage, such as basophils, neutrophils, mast cells, and cytotoxic killer cells. The regulated class should perhaps also include cases in which intracellular vesicles provide a reservoir of a plasma membrane protein, for example, a glucose transporter or a proton pump (see Schwartz and Al-Awqati, 1985). Stimulation of such cells causes insertion of the transporters into the plasma membrane. In endocrine and exocrine cells there is evidence that cells with the regulated pathway also have a constitutive pathway; one function of this pathway appears to be the transport of membrane proteins to the surface (Gumbiner and Kelly, 1982; Arvan and Castle, 1987; Rhodes and Halban, 1987). Neurons presumably have regulated pathways for release of neuropeptide and neurotransmitters and constitutive pathways for turnover of membrane proteins.

The mechanism for secretion of neuropeptides by neurons is likely to be highly homologous to mechanism for secretion of peptide hormones by endocrine cells (for a recent review see Burgess and Kelly, 1987). The contents of dense core secretory vesicles separate from elements of the constitutive pathway in the trans-Golgi network (Tooze and Burke, 1987; Orci et al., 1987), and the vesicles move along microtubles to the cell periphery (Matsuuchi et al., 1988; Tooze et al., 1987). In chromaffin cells the dense core vesicles accumulate round the periphery of the cell in association with the subplasmalemmal cytoskeleton. Stimulation of exocytosis involves a disassembly of the subplasmalemmal cytoskeleton (Cheek and Burgoyne, 1987; for review see Linstedt and Kelly, 1987), which may allow the vesicle access to the plasma membranes. A key question is whether stimulation of such cells is activating a fusion mechanism, dissolving a cytoskeletal barrier to fusion, or both.

Two key insights into the mechanism of regulated secretion come from the study of mast cells. First, it appears that swelling of dense core vesicles comes after the fusion of granule and plasma membranes (Zimmerberg et al., 1987; Breckenridge and Almers, 1987a). There seems little support, therefore, for the once popular idea that osmotic swelling of the vesicle causes the fusion event. The second exciting development is the use of conductance and admittance measurements to estimate the size of the channel that forms when the mast cell granule membrane first fuses with the plasma membrane. The channel is estimated to have the conductance of a gap junction and may show flickering (Breckenridge and Almers, 1987b). Although both mast cells and neurons have regulated secretion, the fusion events may not have identical molecular mechanisms.

Relationship between Synaptic Vesicle and Dense Core Granule Secretion
While dense core vesicles can be found in many nerve terminals, by far the more common secretory vesicle is
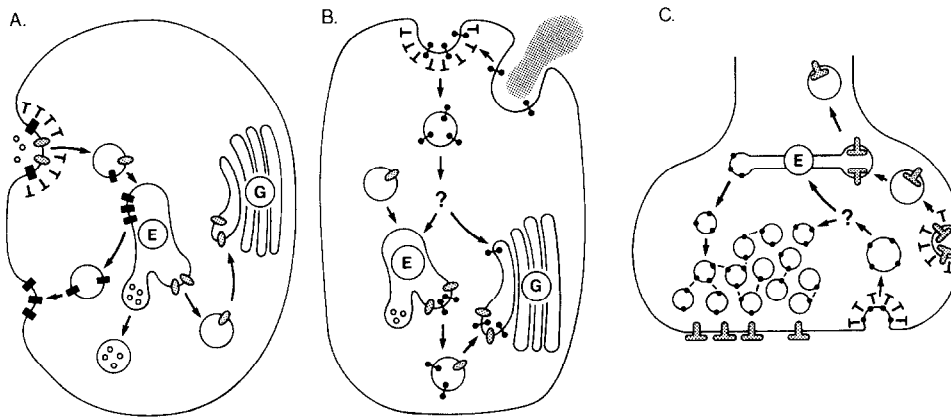
Figure 3. Endocytotic Pathways

(A) In a nonneuronal cell, there are three routes taken by endocytosed material. After internalization in a coated pit, the coat is lost and the vesicle fuses with an endosome structure. From the endosome three destinations are possible: a return to the plasma membrane, delivery to the lysosome, or transfer to the Golgi region. In nonneuronal cells, the latter route is thought to be a minor one.

(B) In endocrine cells, recycling of the secretory granule membrane requires a massive membrane flow back to the Golgi region. It is not known whether this is a separate pathway or whether the granule membrane components take the route that is a minor one in nonsecretory cells.

(C) In nerve terminals, there is recycling of membrane proteins and of receptors, presumably through a conventional endosomal compartment. It is not resolved whether synaptic vesicle membranes recycle through an endosome or bypass the endosome by a neuron-specific endocytotic route.

the synaptic vesicle, the contents of which are characteristically electron-lucent. It is reasonable to assume that dense core vesicles in vesicle terminals behave like dense core granules in endocrine and exocrine cells. But how appropriate is it to assume that information on dense core granule fusion is pertinent to synaptic vesicle fusion? The dense core granule is formed in the cell body and releases protein at sites that need not coincide with the sites of synaptic vesicle fusion (e.g., Zhu et al., 1986). However, the presence of three synaptic vesicle membrane proteins in endocrine secretory granules (Lowe et al., 1988) argues in favor of some similarity in mechanism, as does the similar membrane composition of dense and light vesicles of sympathetic nerves (for review see Winkler et al., 1987). The similarity in the two vesicle types has led to the frequent speculation that synaptic vesicles arise by endocytosis of dense core granule membranes (Winkler et al., 1987; Lowe et al., 1988). If this is true, the remarkable enrichment of vesicle proteins in small vesicles of endocrine and neuroendocrine cells (Navone et al., 1986) implies that the endocytotic mechanism must concentrate proteins of the secretory granule membrane (Lowe et al., 1988). The evidence for different sites of exocytosis (Zhu et al., 1986) and different responses of dense core and low density synaptic vesicles to stimulation (for review see Burgess and Kelly, 1987) is also consistent with an alternative hypothesis (Navone et al., 1986) — that there are two separate and independent regulatory pathways in neurons. At present, the idea that synaptic vesicles are generated independently of dense core vesicles is as tenable as the idea that they are generated from them. Even comparison with nonneuronal cells does not help. For instance, the correct analogy in nonneuronal cells to neurotrans-

mitter release could be the regulated recycling of the proton pump (Schwartz and Al-Awqati, 1985) and not the regulated secretion hormones.

A recent advance in cellular neurobiology has been the cloning and sequencing of one of the integral membrane proteins of synaptic vesicles. The protein, p38 or synatophysin, forms a dimer or even tetramer in detergent and may be a calcium-binding protein (Rehm et al., 1986). Since each monomer crosses the membrane four times (Buckley et al. 1987; Leube et al., 1984; Sudhof et al., 1987), the molecule itself may have 8–16 transmembrane regions and so would be comparable to the family of proteins usually associated with transmembrane transfer of information. One appealing speculation is that calcium binding to the cytoplasmic tail exposes hydrophobic domains in the way that low pH activates the fusion capacity of influenza hemagglutinin (White et al., 1983). The hydrophobic domains could facilitate association with the nerve terminal plasma membrane, leading perhaps to the formation of gap junction–sized channel of the type reported for mast cell granule fusion. Alternatively, calcium binding could help cause dissociation from cytoskeletal elements (Figure 2). Fortunately, with cloned DNA available, speculations of this type can be addressed experimentally.

Recycling of Synaptic Vesicle Membrane

After exocytotic release of neurotransmitter, synaptic vesicle membranes are recycled, refilled with neurotransmitter, and used again. Is the vesicle membrane incorporated into the nerve terminal plasma membrane or, is it only transiently associated with it? It has been proposed that transmitter is released through a transient pore which forms between vesicle and plasma membrane (for review

see Meldolesi and Ceccarelli, 1981). Although the data for conductance channel flickering at the point of contact between mast cell granule and plasma membrane (Breckenridge and Almers, 1987b) suggest that a pore can exist, direct experimental evidence for such a pore at the nerve terminal is scarce. There is little debate, however, that some vesicle membrane does add to the plasma membrane as a result of exocytosis, increasing the surface area of the nerve terminal membrane (for recent examples see Wiley et al., 1987; Torri-Tarelli et al., 1987). The question we need to address is whether this excess membrane is removed from the membrane by conventional endocytotic mechanisms or by a mechanism specific for neuronal cells.

Neurons clearly do perform endocytosis in their resting state. For example, considerable uptake of horseradish peroxidase (HRP) into cytoplasmic organelles has been demonstrated in the absence of stimulation in cochlear hair cells (Siegel and Brownell, 1986). In non-neuronal cells, it is now well established that endocytosed plasma membrane is delivered to endosomes (Figure 3a). From the endosomes, several destinations are possible. Proteins such as the transferrin receptor can return to the plasma membrane; some occupied receptors (e.g., the insulin receptor) and some cross-linked proteins go to the lysosome; other receptors, such as the mannose-6-phosphate receptors, go back to the Golgi region (Duncan and Kornfeld, 1988). Neurons must have conventional endocytosis to internalize, for example, NGF receptors from nerve terminals and for turnover of the proteins of the nerve terminal plasma membrane (Figure 3C).

Since neurons frequently contain dense core secretory vesicles, it is likely that they also have the endocytotic pathways characteristic of endocrine cells. Endocrine secretory granule membranes are reutilized, but unlike synaptic vesicle membranes, they must go back to the Golgi apparatus to be refilled. Endocrine cells must therefore have a major pathway from the plasma membrane to the Golgi region. The route taken by one granule membrane component, the gpIII protein, is now well characterized by immunoelectron microscopy of chromaffin cells (Patzak and Winkler, 1986). Internalization of gpIII involves the conventional coated pit step followed by fusion of the small vesicle membranes to form an endosome-like structure. With time, gpIII is found back in the Golgi region where it is packaged in newly synthesized dense core secretory granules. We do not yet know whether the return path to the Golgi apparatus taken by gpIII is identical to or different from that taken by other proteins returning to the Golgi (Figure 3B).

There is suggestive evidence that an endocytic pathway from the plasma membrane or nerve terminal plasma membrane to the Golgi exists in neuronal cells and that it is the explanation for trans-synaptic marker transfer of exogenous markers. In most cases, HRP will mark the organelles that deliver material to the lysosome; HRP itself does not show trans-synaptic transfer (Trojanowski and Schmidt, 1984). However, when HRP is coupled to wheat germ agglutinin (Gonatas et al.,

1984; Broadwell and Balin, 1985) or when wheat germ agglutinin is used alone (Rhodes et al., 1986), the Golgi apparatus becomes labeled. It is reasonable to conclude, therefore, that neurons resemble endocrine cells in having a major endocytotic pathway to the Golgi apparatus. Endocytosis of wheat germ agglutinin is a convenient label for such a pathway in neurons, while HRP identifies and routes to the lysosome.

Synaptic vesicle recycling occurs against a background of other endocytotic activities. For example, the uptake of HRP into endosomal elements of the cochlear hair cell in the absence of stimulation (Siegel and Brownell, 1986) is presumably due to basal endocytosis, while the appearance of HRP in synaptic vesicles after stimulation is due to activity-stimulated endocytosis. Since the nerve terminal plasma membrane grows in area as a result of stimulation, the mechanism for recycling must be inefficient compared with synaptic vesicle insertion. The observation that coated structures increase proportionately with the increase in surface area (Torri-Tarelli et al., 1987) suggests that coated structures are involved in recycling before and after stimulation and that the rate of coated structure formation remains proportional to nerve terminal plasma membrane area. The small number of coated vesicles detected by Torri-Tarelli et al. (1987) suggests that coated vesicles are short-lived. During a period when an average of 7×10^3 vesicles fuse with the plasma membrane per min, the total number of coated pits and vesicles is 5×10^3. The lifetime of a coated structure would have to be slightly less than 1 min to account for the numbers of vesicles in the nerve terminal. Such a lifetime is not inconsistent with measurements in nonneuronal cells.

A question that perplexes a cell biologist examining synaptic vesicle recycling is whether or not an endosome is involved as an intermediate compartment (Figure 3C). HRP-labeled endosomes can clearly be seen (e.g., Siegel and Brownell, 1986), but there is no direct evidence that they are involved in recycling synaptic vesicle membranes. It has been reported that stimulation increases cisternal organelles (Wiley et al., 1987; Brewer and Lynch, 1986) and that it does not (Torri-Tarelli et al., 1987). The conservative cell biologist might insist on the simplifying assumption that all membrane recycling goes through endosomes. On the other hand, the similarity in size between the vesicle membrane inside a coated vesicle and the synaptic vesicle makes it feasible that the nerve terminal cuts out the endosomal middleman and merely uncoats a coated vesicle to generate synaptic vesicle. Hopefully antibodies to synaptic vesicle proteins will soon resolve this issue.

Formation of the Nerve Terminal

The presence of synaptic vesicle antigens in endocrine secretory cells and of dense core granules in neurons suggests a model in which the neuronal precursor, evolutionarily or developmentally, is an endocrine cell that has regulated secretion. Many nonneuronal cells can be induced to extend processes. When a permanent
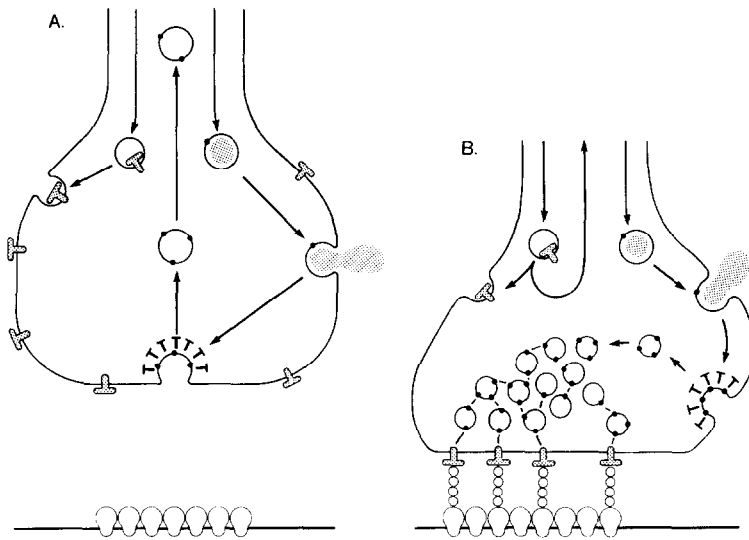
Figure 4. Suggested Mechanism of Synapse Formation

(A) Prior to contacting target, considerable constitutive membrane flow is required to add membrane to growing tips. In addition, large dense core vesicles secrete their contents. The membranes are then recovered in coated pits and form endocytotic vesicles that are returned to the cell body for reuse. (B) When contact is made, cell recognition molecules accumulate in the region of contact between neuron and target. As for epithelial cells, this is suggested to produce an organization of cytoskeletal elements at the nerve terminal plasma membrane. Synaptic vesicles on this model are initially derived by endocytosis of large dense core vesicle membranes. Accumulation of synaptic vesicles upon synaptogenesis is by selective retention, via interaction with the cytoskeleton. Since the process is no longer extending, a massive retrograde transport of constitutively transported material begins (Hollenbeck and Bray, 1987).

line of endocrine cells in induced to extend long processes, the tips of the processes are packed with regulated secretory vesicles (Matsuuchi et al., 1988; Tooze and Burke, 1987). Such cells show strong similarity to immature neurons (Buckley and Kelly, 1985) in which vesicle antigens are also found at the tips prior to synaptic contact. Large amounts of other membranous material are also transported to the tip presumably to allow process extension (Matsuuchi et al., 1988; Hollenbeck and Bray, 1987).

When the immature nerve terminal makes contact with a target, there is accumulation of vesicle antigens at the contact points (Bixby and Reichardt, 1985; Burry et al., 1986). This is likely to involve a redistribution of preexisting material since protein synthesis is not required (Burry, 1985). The amount of material transported retrogradely increases markedly, presumably because material is no longer needed for process extension (Hollenbeck and Bray, 1987). The two simplest explanations of how synaptic vesicle material might be redistributed extensively during synaptogenesis are that it is selectively sent to the nerve terminal or selectively retained. We cannot tell with confidence which is correct.

Insight into how the reorganization might be brought about may come from considering a simpler example of cell–cell interaction, the formation of an epithelium. The basolateral plasma membrane of an epithelial cell but not the apical membrane contains the $(Na^+,K^+)ATP$-ase and is associated with a cortical lining of the actin, fodrin, ankyrin cytoskeleton. As with the neuron, these components are present before contact and redistribute following cell–cell contact (Nelson and Veshnock, 1987a, 1987b). In this case, one of the elements causing the distribution is known. The transmembrane protein E cadherin (uvomorulin, L-CAM) allows selective association of epithelial cells, and antibodies to it block formation of

the junction (Boulter et al., 1985; Gumbiner and Simons, 1986). By analogy with these observations, synapse formation may involve cadherin-like molecules present in the immature neuron. The cytoplasmic domains of the clustered cadherin-like molecules could serve to hold in place the cytoskeletal elements that selectively retain synaptic vesicles at the nerve terminal.

In the speculative model (Figure 4), the possible similarity between epithelial formation and synapse formation is emphasized. The model also incorporates the notion that synaptic vesicles arise from membranes of large dense core secretory vesicles (Lowe et al., 1988) and suggests that synaptic vesicle accumulation is by selective retention.

Conclusion

Recent advances in membrane trafficking, cytoskeletal organization and function, and cell–cell interaction in nonneuronal cells have provided the cell neurobiologist with useful insights into how synapses are formed and how they might work. Although we still know depressingly little about nerve terminal components compared with the wealth of information about postsynaptic receptors, these insights coupled with the availability of nerve terminal specific antigens and the cDNAs that encode them can only make us optimistic.

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