Trans-Golgi network delivery of synaptic proteins in synaptogenesis

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Summary

Synapse formation, stabilization and maintenance comprise several remarkably precise and rapid stages. The initial steps involve delivery to the site of initial contact between axon and dendrite of transport carriers containing several sets of synaptic proteins necessary for proper synaptic function. This occurs both pre- and postsynaptically and is mediated by apparently distinct vesicular carriers that fuse with the synaptic plasma membrane to deliver receptors for neurotransmitters, ion channels, transporters and pumps. The presynaptic carriers in the developing axon give rise to synaptic vesicles. On the postsynaptic side, the so-called spine apparatus may represent a tubular reservoir that gives rise to the postsynaptic players in synaptic function. Recent evidence indicates that recognition molecules, particularly neural cell adhesion molecule (NCAM), are associated with trans-Golgi-network-derived structures and thus can provide a signal for accumulation of these transport carriers at nascent synapses.

Key words: Synaptogenesis, TGN, Transport, NCAM

Introduction

Neurons are highly polarized cells. A typical neuron in the central nervous system (CNS) consists of three distinct functional and organizational domains: a cell body (or soma); a long and slender axon of uniform diameter; and thick, tapering dendrites. The soma and dendrites receive and process information, whereas the axon transfers it through the generation of an action potential. The domains contain characteristic proteins such as microtubule-associated protein 2 (MAP2), which is largely segregated to dendrites, and tau, which is enriched in axons [Fig. 1; for reviews on neuronal polarization, see Bradke and Dotti (Bradke and Dotti, 1998; Bradke and Dotti, 2000)].

At the synapse, information is transferred when a chemical messenger is released from synaptic vesicles and activates receptors in the apposed dendritic postsynaptic domain. Neurons in the CNS can be subdivided on the basis of the type of neurotransmitter they use: glutamate or γ-aminobutyric acid (GABA). The glutamatergic and GABAergic neurons are called excitatory and inhibitory, respectively, in reference to the type of response they elicit in the postsynaptic membrane. Whereas glutamate causes depolarization of the neuron and generation of an action potential, GABA hyperpolarizes cells, reducing the probability of an action potential. Thus, glutamate receptors of the *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) types are present at excitatory synapses; whereas GABA receptors are found exclusively at inhibitory synapses (Fig. 1) (for reviews, see Kittler and Moss, 2001; Moss and Smart, 2001; Sheng and Pak, 2000). In the peripheral nervous system, another type of excitatory connection – the neuromuscular junction (NMJ) – links the axon of a motor neuron and a muscle cell. NMJs use acetylcholine as a neurotransmitter, which activates acetylcholine receptors in the postsynaptic membrane of the muscle cell (reviewed by Sanes and Lichtman, 1999).

The formation of synapses in the CNS is a highly complex process that needs to be orchestrated with high temporal and spatial precision. Synapse formation is accompanied by accumulation of synaptic organelles and proteins at the tiny sites where axons and dendrites contact each other (Ahmari et al., 2000; Friedman et al., 2000; Zhai et al., 2001; Sytnyk et al., 2002; Shapira et al., 2003). The result is the transformation of the initial contacts between filopodia of axonal or dendritic growth cones into functional synapses. Here, we summarize current knowledge of how multiple synaptic precursor structures derived from the trans-Golgi network (TGN) transport synaptic proteins to sites of synaptic contact, where they are trapped and mature into a functional synaptic machinery.

Transport vesicles mediating synaptic protein delivery

The first information about the trafficking of synapse-specific proteins in developing neurons came from studies using green fluorescent protein (GFP)-tagged synaptic proteins and in vivo time-lapse recordings (Nakata et al., 1998). The synaptic plasma-membrane-associated protein SNAP-25 (synaptosome-associated protein of 25 kDa) and the synaptic vesicle protein synaptophysin are involved in synaptic vesicle fusion at mature synapses (for reviews, see Südhof, 1995; Rizo and Südhof, 2002). In isolated axons of dorsal root ganglion neurons, these proteins are transported in intracellular tubulovesicular

membrane aggregates ~1 μm in diameter. These membranous aggregates probably represent precursors of the specialized membranes and organelles that characterize the presynaptic bouton. They are implicated both in the delivery of synaptic proteins to the synapse and in the generation of the presynaptic machinery, such as synaptic vesicles (Nakata et al., 1998). More recent studies have revealed at least two types of presynaptic precursor structure (Fig. 2). In developing axons of hippocampal neurons, pleiomorphic tubulovesicular organelles ~1 μm in diameter transport the synaptic vesicle protein VAMP2 (vesicle-associated membrane protein 2), also called synaptobrevin, to nascent synapses (Ahmari et al., 2000). These organelles colocalize with presynaptic membrane proteins such as voltage-dependent Ca^{2+} channel (VDCC) and other synaptic-vesicle-associated proteins such as SV2 (synaptic vesicle protein 2) and synapsin 1. These membranous aggregates travel along the axon and concentrate at contact sites as they transform into synapses (Ahmari et al., 2000).

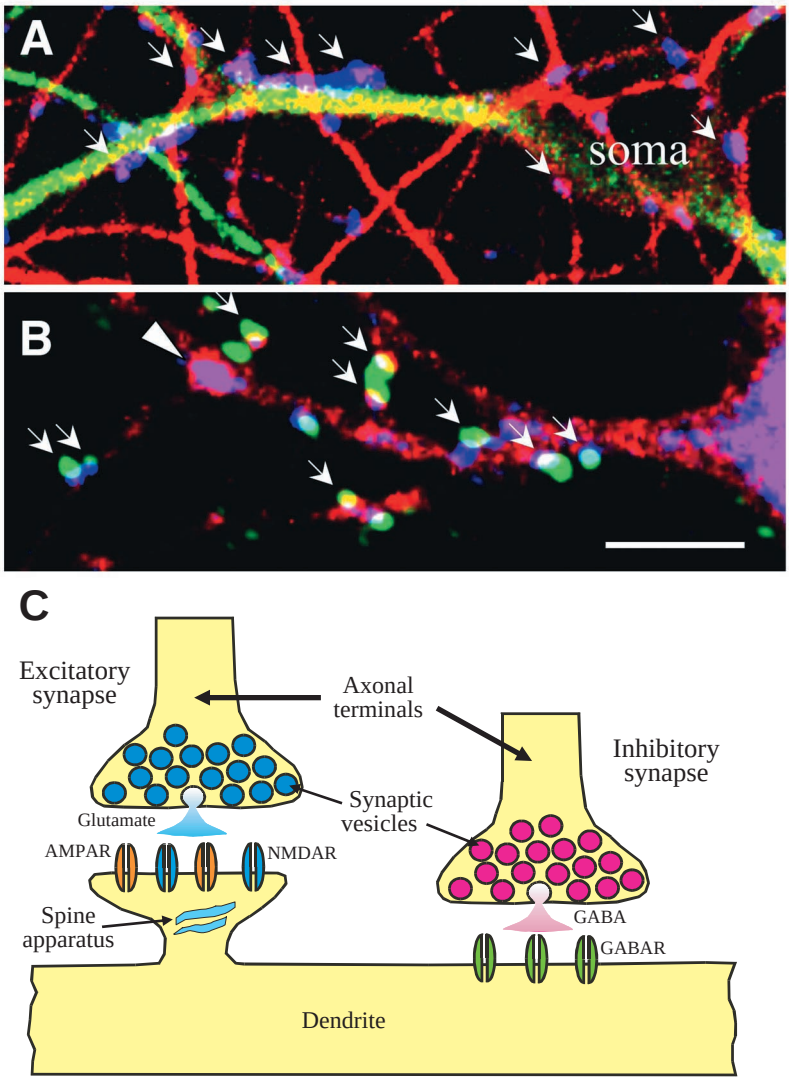

Fig. 1. Organization of mature synapses in the CNS. (A) Hippocampal neurons maintained in culture for 12 days were labeled with antibodies against cytoskeleton-associated proteins MAP2 (green), a marker of dendrites, and tau (red), a marker of axons. Antibodies against synaptic vesicle protein synaptophysin (blue) were used to label presynaptic boutons. Axons form multiple synaptic contacts with the dendrites and cell body (soma) of the neuron (arrows). (B) Hippocampal neurons maintained in culture for 12 days were stained with antibodies against synaptophysin (green) to label axonal terminals, and NMDA (blue) and AMPA (red) receptors to label excitatory postsynaptic specializations. Arrows show examples of excitatory synapses where synaptophysin-labeled presynaptic boutons are apposed to accumulations of AMPA and/or NMDA receptors. Intracellular accumulations of NMDA and AMPA receptors are also seen (arrowhead). Bar, 10 μm (A and B).
(C) Organization of excitatory and inhibitory synapses in the CNS. For more details, see the text.

Another type of axonal synaptic precursor pool consists of vesicles that are 80 nm in diameter and characterized by a dense core appearance. These dense core vesicles contain the presynaptic multi-domain proteins Piccolo and Bassoon that participate in the formation of the cytoskeletal matrix at the active zone of mature synapses (reviewed by Dresbach et al., 2001). Other molecules contained in these dense core vesicles are: the synaptic plasma membrane SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) proteins syntaxin and SNAP-25; RIM (Rab3-interacting molecule); Munc18 and Munc13, which are the mammalian homologs of the *Caenorhabditis elegans* proteins UNC-18 and UNC-13; and the cell adhesion molecule N-cadherin. Interestingly, dense core vesicles do not contain VAMP2, synaptophysin, synaptotagmin, or the perisynaptic GABA transporter GAT1 (Zhai et al., 2001; Shapira et al., 2003). Whether synaptophysin, which is also transported by pleiomorphic structures covered with a spectrin-containing cytoskeleton (Nakata et al., 1998; Sytnyk et al., 2002), is present in the VAMP-containing vesicles or vesicles that constitute another type of transport carrier remains to be determined. Another unresolved issue is the presence of spectrin at the surface of VAMP-containing or dense core vesicles. The pleiomorphic structures and dense core vesicles contain largely non-overlapping sets of synaptic proteins. However, some proteins, such as VDCC, are present in both types of vesicle, which finally accumulate at nascent synapses, where they have been detected at the ultrastructural level (Ahmari et al., 2000).

Less well characterized is the assembly of postsynaptic structures, which contain mainly neurotransmitter receptors, non-ligand-triggered ion channels, transporters, pumps and associated scaffolding proteins. However, it is evident that Golgi-related structures are present in dendrites (Sytnyk et al., 2002; Horton and Ehlers, 2003; Maletic-Savatic and Malinow, 1998) and different types of membranous carrier also exist postsynaptically. For example, in cortical neurons, the NMDA and AMPA receptors are transported at different speeds towards the nascent excitatory synapse in largely non-overlapping carriers (Washbourne et al., 2002). Vesicles transporting the NMDA receptors utilize the neuron-specific kinesin

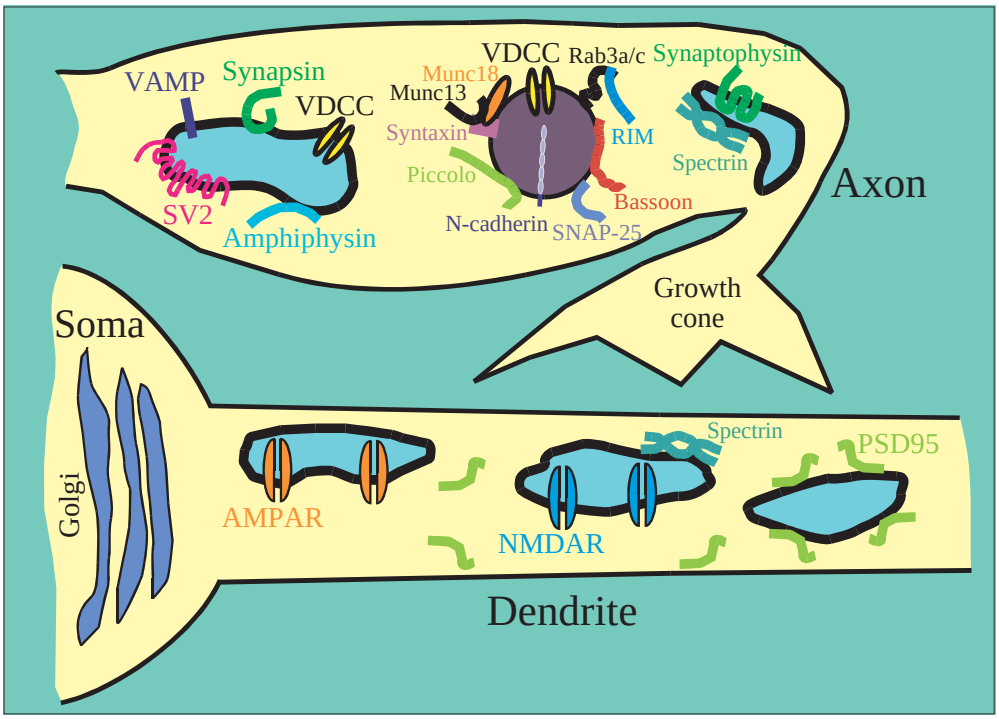

Fig. 2. Synaptic precursor organelles and cargo proteins. Presynaptically (upper part), at least two different types of synaptic precursor organelles are described. Pleiomorphic tubulovesicular organelles transport VAMP, voltage-dependent Ca^{2+} channel (VDCC), synaptic vesicle protein 2 (SV2), synapsin and amphiphysin. Another type of vesicle is characterized by its dense core and round appearance. These vesicles contain the presynaptic cytomatrix proteins Piccolo, Bassoon, the SNARE proteins syntaxin and SNAP-25, Rab3a/c and Rab3-interacting molecule (RIM), Munc18, Munc13, N-cadherin and VDCC. Synaptophysin is transported by pleiomorphic structures that are covered with spectrin-containing cytoskeleton. Whether these organelles belong to the same type as VAMP-containing vesicles or constitute another type remains to be determined. Postsynaptically (lower part), organelles containing NMDA and AMPA receptors constitute two different types of transport carriers. PSD95 is depicted in a diffuse pool, but also in association with vesicular structures that are distinct from NMDA and AMPA receptor-containing vesicles. It remains to be elucidated whether vesicular transport organelles contain NCAM pre- and postsynaptically.

motor KIF17 (Setou et al., 2000; Guillaud et al., 2003), whereas molecular motors associated with other types of carrier remain to be determined. Proteins associated with NMDA receptors in mature synapses, such as the cytoskeleton-associated protein PSD95 (postsynaptic density protein 95; also known as SAP90), may be recruited to the synaptic contact from a diffuse cytoplasmic pool after contact establishment (Bresler et al., 2001). PSD95 can also be dynamically recruited to the membranes by palmitoylation (El-Husseini et al., 2002), and has also been seen in association with carriers that are transported along growing dendrites (Prange and Murphy, 2001). However, more recent studies indicate that PSD95 moves in clusters distinct from those containing NMDA receptors (Washbourne et al., 2002).

Several lines of evidence show that the carriers that deliver synaptic proteins probably originate from the TGN, which can produce large pleiomorphic structures up to several microns in diameter (Nakata et al., 1998; Toomre et al., 1999; Toomre et al., 2000; Polishchuk et al., 2000; Stephens and Pepperkok, 2001) that are similar to the carriers that deliver presynaptic (Ahmari et al., 2000; Nakata et al., 1998; Sytnyk et al., 2002) and postsynaptic (Washbourne et al., 2002) proteins. Some of these contain proteins characteristic of the TGN, such as TGN-38 (Nakata et al., 1998). Moreover, some of those accumulating at sites of contact that eventually became synapses contain TGN-specific adaptor proteins, such as β-COP and γ-adaptin, a subunit of the AP-1 complex (Sytnyk et al., 2002). These can be loaded with the styryl dyes FM1-43 or FM4-64 following prolonged exposure, which specifically labels the TGN- or Golgi-like structures (Maletic-Savatic and Malinow, 1998; Maletic-Savatic et al., 1998; Tarabal et al., 2001; Sytnyk et al., 2002). The ability to accumulate these FM dyes also shows that the structures are connected with endosomal compartments, where FM dyes reside transiently before being transferred to TGN-like structures. A connection between the TGN and recycling endosomes has also been described in non-neuronal cells (Mallard et al., 1998; Clague, 1998) and may be important for the later stages of synaptic vesicle generation from the plasma membrane that are believed to occur after fusion of TGN-derived structures with the membrane (reviewed by Hannah et al., 1999).

TGN-derived structures probably also have a function in the formation of inhibitory synapses. Direct and indirect evidence supports the view that the $GABA_A$ receptor, in association with gephyrin, a cytoskeleton-associated linker protein, is transported in TGN-derived structures that contain the ATPase *N*-ethylmaleimide-sensitive factor (NSF), a chaperone that activates SNARE proteins (Kneussel, 2002).

Recognition molecules and synapse formation

The adult mammalian brain contains $>1\times10^{12}$ neurons, and one neuron often receives synaptic input from thousands of other neurons. These connections are formed during development of the nervous system, when axons can extend over long distances to reach their final targets. Axons are guided by a variety of extracellular cues that direct movement of a motile structure at the end of the growing axon known as the axonal growth cone. These cues are recognized by recognition molecules on the surface membrane of growth cones (for reviews, see Huber et al., 2003; Walsh and Doherty, 1997). The growth cone then interacts with the target dendrite to transform the initial contact

into a synapse. How is the initial contact site between the axon and dendrite specified, and is this contact guided by recognition molecules? Whether a particular molecule determines the precise site on a dendrite where an axon will form a synapse remains a largely unresolved issue. However, it is reasonable to assume that recognition molecules at the cell surface and in the extracellular matrix are well poised to perform this task.

Recognition molecules accumulate at synapses during development, which suggests that they might be involved in synapse stabilization through their adhesive properties. These molecules include N-cadherin, which is initially associated with all types of synapse in cell culture but then becomes restricted to excitatory synapses (Benson and Tanaka, 1998). Protocadherin-γ, a molecule related to classical cadherins, is also found in a subset of excitatory synapses (Phillips et al., 2003). Recognition molecules of the immunoglobulin superfamily, such as synaptic cell adhesion molecule (SynCAM) (Biederer et al., 2002), nectins (Mizoguchi et al., 2002) and neural cell adhesion molecule (NCAM) (Schachner, 1977; Sytnyk et al., 2002) are also present in synapses.

Work in *Drosophila* first gave direct evidence of the role of recognition molecules in the induction of synapse formation. The lack of the cell adhesion molecule fasciclin II leads to a loss of synapses that have transiently formed in early development (Schuster et al., 1996). Studies in heterogenotypic co-cultures of neurons lacking NCAM, the closest mammalian homologue of fasciclin II, and wild-type neurons indicate that the NCAM-deficient cells form fewer synapses (Dityatev et al., 2000). Overexpression of SynCAM in non-neuronal cells induces formation of synapses on the transfected cells by axons of co-cultured neurons (Biederer et al., 2002), whereas inhibition of nectin-based adhesion by an inhibitor of nectin-1, glycoprotein D, results in a decrease in synapse size and a concomitant increase in synapse number (Mizoguchi et al., 2002). Integrins, a large family of cell-surface receptors for extracellular matrix recognition molecules, are also associated with synapses (Chan et al., 2003) and have also been suggested to induce synapse formation. Application of antibodies that block integrin function reduced the number of synapses in the apical dendrites of CA1 pyramidal neurons in organotypic cultures (Nikonenko et al., 2003). Yet other recognition molecules that are present in synapses and are involved in synapse formation are the neurexins and neuroligins (Dean et al., 2003). Overexpression of neuroligin induced synapse formation on transfected non-neuronal cells (Scheiffele et al., 2000) and on cultured hippocampal neurons (Dean et al., 2003) by activation of its binding partner neurexin. The ephrin B and EphB tyrosine kinase receptor system has also been implicated in the development of excitatory synapses through phosphorylation of the cell-surface-exposed heparin sulphate proteoglycan syndecan (Ethell et al., 2001) or interaction with the NMDA receptor (Dalva et al., 2000).

Recognition molecules signal transport carriers to immobilize at sites of initial contacts

The pre- and postsynaptic transport carriers travel along neurites at remarkable speeds (up to several micrometers per minute) often in opposite directions (Nakata et al., 1998; Ahmari et al., 2000; Washbourne et al., 2002; Sytnyk et al., 2002; Shapira et al., 2003). Nevertheless, only several minutes after the first contact between axon and dendrite, these organelles start to accumulate at the contact site (Ahmari et al., 2000; Washbourne et al., 2002; Sytnyk et al., 2002). This suggests that signals from the pre- and postsynaptic membranes are required to tell carriers when and where the apposing membranes meet, and therefore recognition molecules are the best candidates to perform that task. In accordance with this idea, clustering of neurexin is sufficient to induce redistribution of synaptic vesicles (Dean et al., 2003). We recently showed that NCAM clusters in the plasma membrane directly associate with TGN-derived structures, concluding that it anchors them at the contact site (Fig. 3) (Sytnyk et al., 2002). Interestingly, axonal and dendritic TGN-derived structures are tethered to NCAM at the plasma membrane. The association between NCAM and the TGN-derived structures requires a close apposition of the intracellular domain of NCAM and these carriers. Indeed, synaptic vesicle markers, such as synaptophysin and SV2, are found in a pleiomorphic tubulo-cisternal membrane system directly beneath the plasma membranes of PC12 and CHO cells (Johnston et al., 1989; Schmidt et al., 1997). These organelles, called perisomes, are presumably the source of synaptic-vesicle-like structures in these cells.

In the squid giant axon, subaxolemmal cisternae form junctions with the axolemma. These exhibit filamentous granular bridging structures ~3 nm in diameter (Metuzals et al., 1997), which suggests that cytoskeletal elements are involved. In accordance with this idea, we found that the link between TGN-derived carriers and cell-surface NCAM180, the largest major isoform of NCAM, which has the longest cytoplasmic domain, depends on proteins associated with the membranes of the organelles, such as spectrin. Spectrin has also been implicated in accumulation of synaptic proteins and initiation of synaptic transmission in *Drosophila* (Featherstone et al., 2001). The cytoplasmic surface of Golgi- and TGN-derived structures is lined with a spectrin-actin cytoskeleton meshwork (De Matteis and Morrow, 2000; Lippincott-Schwartz, 1998; Holleran and Holzbaur, 1998). Spectrin is tightly colocalized with both TGN-derived structures and NCAM clusters, and mediates binding of the intracellular domain of NCAM180 to the organelles (Sytnyk et al., 2002).

The association between NCAM and the organelles is tight enough to form a complex that moves along neurites before contact formation (Fig. 4). Time-lapse video recordings have shown that formation of contacts between filopodia of a neurite approaching its target neurite does not coincide with the localization of NCAM-organelle complexes. However, the complexes accumulate at the initial contact sites within minutes. NCAM clusters and organelles often pass the contact site several times, but finally one or even several of the pre-formed NCAM packets become ‘trapped’ and remain at the site of contact for >1 hour. The period that contacts contain organelles is reduced ~35% in NCAM-deficient neurons compared with wild-type neurons. Moreover, the organelles move away from the contact site ~4 times more often in the NCAM-deficient neurons. NCAM thus seems to be important for stabilization of TGN-derived carriers at sites that ultimately become synapses (Sytnyk et al., 2002).

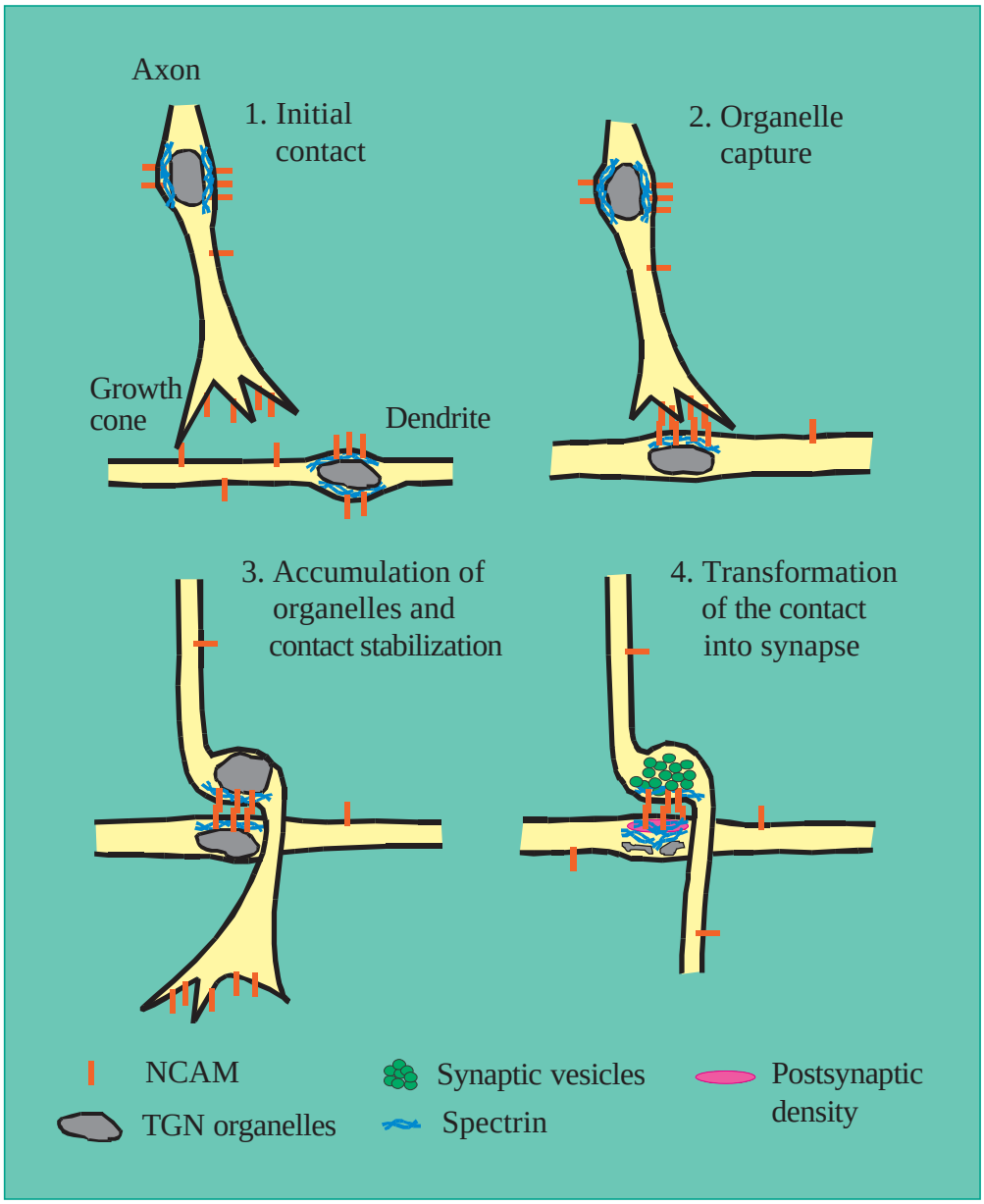

Fig. 3. Diagram of NCAM-mediated accumulation of TGN organelles at sites of contact followed by synaptic differentiation. TGN-derived carriers (gray) are bound to clusters of NCAM (red) by the plasma membrane-cytoskeleton linker protein spectrin (blue). Initially, the contact site does not contain NCAM-tethered organelles, but then captures NCAM-associated TGN organelles through NCAM-mediated interactions at the contact site. Next, TGN organelles further accumulate at stabilized contacts. Finally, the contact is transformed into a functional synapse where presynaptic transport carriers give rise to synaptic vesicles (green) and postsynaptic carriers are transformed into postsynaptic density components (black). In the mature synapse, spectrin is a prominent component of the postsynaptic membrane. Spectrin isoforms have also been detected, although less prominently, in the presynaptic compartment. Figure reproduced with permission from The Rockefeller University Press (Sytnyk et al., 2002).

Transformation of initial contact complexes to synapses

Accumulation of the transport carriers is followed by transformation of the contact sites into a functional synapse (Ahmari et al., 2000; Washbourne et al., 2002; Sytnyk et al., 2002; Zhai et al., 2001). This probably includes fusion of the carriers with the synaptic plasma membrane, which inserts membrane proteins such as Ca^{2+} channels and NMDA/AMPA receptors into the presynaptic and postsynaptic membranes, respectively. There is also the possibility that fusion of carriers with the plasma membrane occurs at least partially extrasynaptically. Limited delivery of synaptic proteins to the plasma membrane seems to occur constitutively at early stages of neuronal development before synapse formation. In isolated axons, synaptic precursor vesicles undergo multiple cycles of exo- and endocytosis that are believed to be important for synaptic vesicle biogenesis. The precursor vesicles represent immature recycling machinery (Matteoli et al., 1992; Kraszewski et al., 1995; Zakharenko et al., 1999; Dai and Peng, 1996; reviewed by Hannah et al., 1999). In dendrites, NMDA and AMPA receptors form clusters without contact between axon and dendrite (Washbourne et al., 2002). Receptors inserted into the plasma membrane extrasynaptically may then accumulate at sites of contacts by diffusion and remain there owing to interactions with the cytoskeleton (reviewed by Choquet and Triller, 2003). Besides the constitutive exocytosis that predominates early in development, regulated exocytosis occurs both in axons and in dendrites. This is activated by external stimuli, such as membrane depolarization, which elevates the intracellular Ca^{2+} concentration (Maletic-Savatic and Malinow, 1998; Lledo et al., 1998; Shi et al., 1999; Sytnyk et al., 2002). The constitutive exocytosis of synaptic precursor organelles could be important at the early stages of synapse development for insertion of some synaptic components into synaptic membranes. Regulated

exocytosis replaces this function in mature synapses and plays an important role in the regulation of synaptic strength (reviewed by Kittler and Moss, 2001).

TGN-derived carriers may also be important vehicles for delivery and release of secreted molecules such as signaling molecules of the Wnt family, which were discovered as morphogens but have recently been implicated in synaptogenesis (Hall et al., 2000; Packard et al., 2002). Another class of secreted molecules that play a role in synapse organization is components of the extracellular matrix, including tenascin-C, tenascin-R and proteoglycans. Particularly striking in this respect are certain neurons surrounded by conspicuous extracellular matrix structures, called perineuronal nets, which envelop synapses formed on the cell bodies of these cells. Another example is the secreted neuronal-activity-regulated pentraxin (Narp), which induces clustering of AMPA receptors (reviewed by Dityatev and Schachner, 2003). These secreted molecules can be delivered to the synapse by neurons (Horton and Ehlers, 2003) or by synapse-associated glial cells, which appear to regulate synapse development by providing cholesterol and soluble and contact-dependent factors that support the structural stability of the synapses (reviewed by Pfrieger, 2002). The targeting of intracellular organelles in glial cells towards nascent and functional synapses remains an intriguing possibility in synaptogenesis.

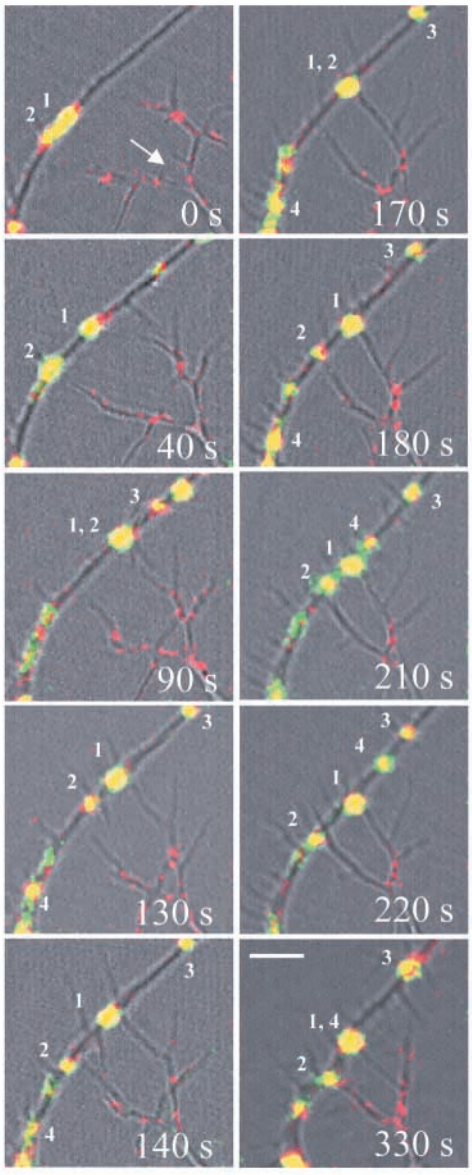

Fig. 4. Accumulation of NCAM-immunoreactive clusters and associated organelles at the contact site between two neurites. Live hippocampal neurons maintained for three days in culture were labeled with monoclonal NCAM antibodies (red). Intracellular organelles were loaded with FM1-43 applied for 24 hours before the start of recording (green). At the start of recording (0 seconds), the growth cones in the lower right hand corner (arrow) approach the diagonally oriented neurite. NCAM-immunoreactive clusters associated with intracellular organelles marked with FM1-43 and seen in yellow (marked by 1-4) move along the target neurite. During the recording time, three contacts are formed. At the end of the recording (330 seconds), each contact is associated with an NCAM-immunoreactive cluster and intracellular organelles on the target neurite. Bar, 10 μm for all frames. Figure reproduced with permission from The Rockefeller University Press (Sytnyk et al., 2002).

An important presynaptic step in synapse maturation is the segregation of synaptic plasma membrane proteins from synaptic-vesicle-associated proteins and the subsequent formation of synaptic vesicles and the presynaptic cytomatrix. Note that these proteins segregate into different transport carriers: synaptic-vesicle-associated integral membrane proteins (e.g. VAMP, SV2, synapsin and synaptophysin) are transported by the pleiomorphic tubulovesicular structures, whereas presynaptic plasma-membrane-associated proteins (e.g. syntaxin and SNAP-25) and cytomatrix proteins (Piccolo and Bassoon) are transported by dense core vesicles. Whether synaptic-vesicle-associated proteins and plasma-membrane-associated proteins are always segregated into different carrier systems remains to be determined, particularly given that other subpopulations of transport vesicles may exist. Postsynaptic transport carriers probably give rise to spine structures, including the spine apparatus, which are a reservoir of neurotransmitter receptors, recognition molecules, channels and pumps in mature synapses.

Neural recognition molecules, particularly NCAM, together with spectrin, are important not only for the initial accumulation of carriers at nascent synapses but also for synapse maturation at later stages of synapse development. During early stages of synapse stabilization, NCAM appears to be necessary both pre- and postsynaptically (Sytnyk et al., 2002), whereas at later stages synaptic strength depends predominantly on postsynaptically expressed NCAM in heterogenotypic co-cultures of hippocampal neurons (Dityatev et al., 2000). A presynaptic role of NCAM is suggested by the observation that, at neuromuscular junctions of NCAM-deficient mice, synaptic clustering of Ca^{2+} channels and synaptic vesicles is reduced (Polo-Parada et al., 2001). However, indirect postsynaptic effects from NCAM-positive muscle cells cannot be excluded. Interestingly, similar effects are seen in *Drosophila* lacking α- and β-spectrin (Featherstone et al., 2001). These findings suggest that NCAM and spectrin constitute a molecular clamp that holds the presynaptic machinery in apposition to the postsynaptic membrane by homo- or heterophilic interactions. Maturation of the synaptic machinery is severely

impaired at the neuromuscular junctions of NCAM-deficient mice, which shows that NCAM is directly involved in this process (Polo-Parada et al., 2001).

Conclusions and perspectives

Recent findings have shown that different synapse-targeted proteins are transported to nascent synapses in carriers that differ not only in their cargos but also at the ultrastructural level. Despite some progress in the characterization of these carriers, their subcellular origins are not always clear, although the majority are probably TGN derived. Detailed analysis of the different cargo proteins they deliver is required if we are to understand the differences in the time course of accumulation of synaptic proteins at nascent synapses as well as the specificity of delivery of synaptic proteins to particular types of synapse.

The observation that NCAM cooperates with spectrin to mediate accumulation of TGN-derived carriers at sites of cell-cell contact provides the first clue as to how the carriers accumulate at these sites with such remarkable precision and speed. It also begs the question of what other synaptic proteins these carriers transport (Fig. 2). Both pre- and postsynaptic transport carriers are tethered to NCAM clusters at the cell surface, which suggests that NCAM is involved in the accumulation and maturation of transport carriers both pre- and postsynaptically. The carriers associated with NCAM and their cargos remain to be characterized in detail.

The link between organelles and cell-surface NCAM180 involves proteins associated with the cytoplasmic side of the TGN-derived structures. Spectrin has been identified as one such link, being highly enriched in vesicular and tubular membranous compartments. The spectrin skeleton not only associates with NCAM (Sytnyk et al., 2002; Leshchyns'ka et al., 2003) but also contributes to the maintenance of Golgi structures and the efficiency of protein trafficking in the early secretory pathway (reviewed by De Matteis and Morrow, 2000). Whether other peripheral proteins of TGN membranes, such as adaptor proteins or additional components of the spectrin meshwork, provide links to cell-surface recognition molecules is an intriguing issue. Among the possible candidates is ankyrin, which interacts with spectrin and the neural cell adhesion molecules L1 and CHL1, both members of the immunoglobulin superfamily (reviewed by Bennett and Baines, 2001). Whether cadherins (particularly protocadherins), integrins, members of the neuroligin and neurexin families, receptor tyrosine kinases of the Eph-receptor family and their cognate ephrin ligands are also involved in targeting of TGN-derived structures to synapses also remains to be established.

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