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Targeted Trafficking of Neurotransmitter Receptors to Synaptic Sites

Sophie Marchand and Jean Cartaud*

Biologie Cellulaire des Membranes, Institut Jacques Monod, UMR 7592, CNRS/Universités Paris 6 et Paris 7, 2 Place Jussieu, 75251 Paris Cedex 05, France

Abstract

Emerging data are sheding light on the critical task for synapses to locally control the production of neurotransmitter receptors ultimately leading to receptor accumulation and modulation at postsynaptic sites. By analogy with the epithelial-cell paradigm, the postsynaptic compartment may be regarded as a polarized domain favoring the selective recruitment and retention of newly delivered receptors at synaptic sites. Targeted delivery of receptors to synaptic sites is facilitated by a local organization of the exocytic pathway, likely resulting from spatial cues triggered by the nerve. This review focuses on the various mechanisms responsible for regulation of receptor assembly and trafficking. A particular emphasis is given to the role of synaptic anchoring and scaffolding proteins in the sorting and routing of their receptor companion along the exocytic pathway. Other cellular components such as lipidic microdomains, the docking and fusion machinery, and the cytoskeleton also contribute to the dynamics of receptor trafficking at the synapse.

Index Entries: Neurotransmitter receptor; synapse; targeting; exocytic pathway; scaffolding protein; cytoskeleton; SNARE; raft; sorting sequence.

Introduction

The accumulation of neurotransmitter receptors in the postsynaptic membrane of chemical synapses is a prerequisite for fast synaptic transmission. Several mechanisms particitate in the local distribution of receptors for neurotransmitters at postsynaptic sites. Accumulating data indicate that the association of receptors to a membrane-bound scaffold of receptor-associated proteins is required for the localization and the anchoring of receptors in the postsynaptic membrane in peripheral and central synapses. At the neuromuscular junction (NMJ), rapsyn (the 43kDa-receptor associ-

* Author to whom all correspondence and reprint requests should be addressed: E-mail: cartaud@ijm.jussieu.fr

ated protein at the synapse) is linked to nicotinic acetylcholine receptors (AChRs) and is essential for receptor clustering and immobilization. It is becoming clear that neuronal neurotransmitter receptors are also linked to specific postsynaptic scaffold proteins: the 93kDa protein-gephyrin for glycine receptors (GlyRs) and γ-aminobutyric acid $GABA_A$ receptors, GABARAP ($GABA_A$ receptor-associated protein) and MAPIB (microtubule-associated protein) for $GABA_A$ and $GABA_C$ receptors, the PSD-95 (postsynaptic protein of 95kDa) family of proteins for N-methyl-D-aspartate (NMDA) receptors, GRIP (glutamate-receptor-interacting protein) and PICKI (protein kinase C [PKC] α-interacting protein) for AMPA receptors, and Homer for group I metabotropic glutamate receptors (mGluRs) *(1).*

Alternative strategies are used in the genesis of postsynaptic membrane domains. In the muscle fiber, the transcription of the genes encoding the subunits of the AChR is limited to the few specialized subneural nuclei leading to the compartmentalization of the synthesis of AChR molecules within the subneural sarcoplasm *(2–4).* In neurons, a compartmentalized distribution of several messengers including subunits of GlyRs and NMDA receptors was also observed and likely results from dendritic mRNA targeting *(5).* These data emphasize the possibility that a local biosynthesis and a direct targeting of synaptic receptors also contribute to their accumulation at synaptic sites. This hypothesis was strengthened by the observation that in skeletal muscle fibers a specialized Golgi complex and a stable network of microtubules surround subneural nuclei *(6–8),* thereby illustrating the compartimentalization of the secretory pathway. In addition, a subsynaptic machinery for protein synthesis and maturation, including endoplasmic reticulum (ER) and Golgi compartments, was identified at spine synapses in different neuron types, suggesting a local synthesis and insertion of postsynaptic components (ref. *9* and references therein). Collectively, these data support the notion that post-transcriptional mechanisms involving local synthesis, sorting, and targeting participate to the proper supply of neurotransmitter receptors at synaptic sites.

Most recent reviews on synaptic differentiation highlighted the molecular interactions involved in receptor aggregation and stabilization. Here, we summarize the recent progress in the characterization of the mechanisms of targeted delivery on newly synthesized neurotransmitter receptors to synaptic sites, with a special emphasis on membrane traffic machinery. Our current knowledge about plasma membrane protein targeting being largely based on studies of polarized epithelial cells *(10),* we will first review the basic mechanisms of membrane protein targeting to specialized domains of the cell surface. Then, we present progress in the characterization of cellular mechanisms engaged in intracellular trafficking and delivery of ionotropic and metabotropic neurotransmitter receptors at the synapse. Despite many differences between interneural synapses and NMJs, both share striking ultrastructural and functional similarities. In particular, the postsynaptic membranes are thickened and bear high concentrations of neurotransmitter receptors, sandwiched between a specialized cytoskeletal apparatus and amorphous synaptic cleft material, and in many cases, the receptors themselves are homologous. For this reason, we discuss the mechanisms of delivery of neurotransmitter receptors in central synapses as well as at the vertebrate NMJ.

Basic Mechanisms of Delivery of Membrane Proteins to Specialized Domains of the Cell Surface

Protein sorting and targeting in the exocytic pathway are constitutive mechanisms by which cells control the delivery of newly synthesized membrane proteins to various domains of the cell surface. In polarized simple epithelia, the spatial distribution of surface proteins in the apical and basolateral membrane domain is a direct consequence of their

sorting in the *trans*-Golgi network (TGN) and targeted delivery in specialized transport vesicles *(11).* Sorting signals intrinsic to apical and basolateral membrane proteins direct them to one surface or the other. Sorting signals frequently containing a critical tyrosine residue within consensus cytoplasmic sequences or consisting of a di-hydrophobic motif serve to target the protein basolaterally *(12–15).* Multiple types of signals exist for sorting to the apical surface. These include extracellular *(16),* transmembrane *(16,17),* and cytoplasmic domains *(16,18–20),* as well as the addition of oligosaccharides, both N- *(21,22)* and O-linked *(23,24),* and the lipid anchor of glycosylphosphatidylinosityl (GPI)-anchored proteins *(25).* This latter signal is important for clustering within sphingolipid-cholesterol-enriched membrane domains, or "rafts," in the exoplasmic leaflet of the Golgi *(26).* These small, highly dynamic rafts are believed to constitute platforms to which apically destinated proteins and lipids are sorted in the secretory pathway. The tetraspanning MAL/VIP17 proteolipid protein, which is found in apically directed vesicles, seems to be engaged in apical sorting *(27,28).*

Increasing evidence shows that the organization of cytoskeletal elements, and in particular that of microtubules, facilitates the targeted delivery of TGN-derived transport vesicles for long-distance carriage toward the appropriate membrane domain *(29,30).* Indeed, transport vesicles have been shown to bind to microtubules in vitro and microtubule motor proteins are required for proper delivery *(31).* To mediate the final delivery to the membrane, specific docking and fusion machineries are needed. Fusion of vesicles involves the pairing of addressing proteins named SNAREs (soluble N-ethylmaleimide-sensitive-fusion protein [NSF] attachment protein [SNAP] receptor) on the transport vesicle (termed v-SNAREs) with the cognate receptors on the target membrane (termed t-SNAREs) *(32,33).* Vesicle-target membrane fusion also requires the activity of an ATPase, the NSF factor, and of soluble SNAPs *(32,33).* In agreement with the postulated function of SNAREs in the vectorial targeting of post-TGN carriers, endogenous t-SNAREs display polarized distributions in several epithelial cell types *(34,35).*

A decade ago, Dotti and Simons proposed that neurons and polarized epithelial cells share common mechanisms of protein targeting, with apical domain equivalent to axonal domain and basolateral domain equivalent to somatodendritic domain *(36).* Although polarized epithelial cells have provided a useful model for studying neuronal polarity, and there appear to be some mechanisms in common, several data show inconsistencies with a strict analogy. For example, the dendritic targeting of the transferrin receptor is mediated by a signal distinct from that mediating basolateral targeting *(37).* In addition, recent studies presented here confirmed this point by identification in neuronal cells of novel sorting signals distinct from the already identified sorting signals in polarized epithelial cells *(38).*

Targeting of Ionotropic and Metabotropic Neurotransmitter Receptors to Synapses

Neurons are highly polarized cells with distinct domains engaged in receiving and transducing extracellular signals via the dendritic tree and the axon, respectively. Proper delivery of membrane proteins, in particular neurotransmitter receptors or ion channels to specific sites such as nodes of Ranvier, presynaptic and postsynaptic sites, is an essential functional part of neuronal polarity required for the maintenance of the complexity of synaptic connections in the nervous system. Alike, muscle fibers are highly specialized at NMJ where accumulations of nicotinic AChRs at postsynaptic sites directly across the presynaptic area of neurotransmitter release ensure rapid and efficient synaptic transmission triggered by motoneurons.

In recent years, accumulating data have revealed the implication of local synthesis, sorting in the exocytic pathway, and vectorial

targeting in the localization of neurotransmitter receptors at synaptic sites.

mRNA Localization and Local Synthesis at Synaptic Sites

Increasing evidence points to the presence in neurons of a distributed network of translational machinery. In particular, morphological examination at the EM level of dendrites from different neuron types, such as dentate granule cells, hippocampal pyramidal cells, cortical neurons, and Purkinje cells, have shown that polyribosome complexes are associated with spine synapses *(39).* These polyribosome complexes are often associated with membranous organelles, probably cisternae of the rough ER. Intermediate compartment and Golgi complexes were also localized within dendritic spines beneath synapses *(40).* These observations are coherent with biochemical in vitro and in vivo studies showing that dendrites can perform translation and glycosylation *(41,42)* and with *in situ* hybridization analyses that documented the presence of several mRNAs *(43),* in particular mRNAs encoding GlyR α1 subunit *(5)* and NMDA R1 subunit *(44,45)* within proximal portion of dendrites. Taken together, these data indicate that nonsomatic compartments—the dendritic outstations—are endowed with the capability to perform all synthesis steps of an integral membrane protein such as a neurotransmitter receptor or ion channel. This remarkable property of neurons to precisely control decentralized protein production potentially confers independent regulation of synaptic plasticity *(46–48).*

The presence of specific mRNAs at synaptic sites raises several questions: how individual mRNAs are anchored and targeted to the appropriate dendritic site, and how their eventual translation is regulated. Localizing mRNA is one of several mechanisms used to restrict proteins to particular domains of polarized cells, including neurons. *Cis*-acting sequences required for mRNA localization in dendrites are found in the 3′ untranslated region (UTR) of the α-subunit of CAMKII mRNA (the calcium- and calmodulin-dependent protein kinase involved in the synapse strengthening linked to learning and memory) *(49–51).* β-actin mRNA *(52)* and MAP-2 mRNA *(53).* In the same view, distinct regions in the 3′UTR were reported to be responsible for targeting and stabilization of utrophin transcripts via actin microfilaments in skeletal muscle cells *(54).* Primary sequence analyses of the 3′UTR revealed little or no homology among the dendritically localized mRNAs. An attractive hypothesis is that specific secondary structures in the 3′UTR region are recognized by *trans*-acting RNA binding proteins responsible for RNA transportation. Moreover, recent data support the view that mRNAs are actively targeted to the cell periphery in an activity-dependent manner *(43).* The knowledge of the dynamics of this process in neurons has benefited from studies originally conducted in Drosophila embryos or Xenopus oocytes, in which several RNA-binding proteins such as Staufen are required for assymetric distribution of specific mRNAs during oogenesis and early nervous system development *(55).* Such proteins, especially the mammalian Staufen *(56),* ZBP-1 (Zipcode Binding Protein) *(57),* and TB-RBP *(58,59)* have been localized in somatodendritic region of cultured hippocampal neurons, where their distributions overlap with that of dendritic mRNAs *(60).* These observations are consistent with a role for Staufen in dendritic mRNA targeting. Recent studies have shed light on the potential function of Staufen as a microtubule-binding factor critically involved in the movement of RNA-containing particles along microtubules *(61).* Finally, it is likely that the docking of mRNA complexes to dendritic spines involves a shift from a microtubule-based transport to a cytoskeletal-based anchor, as it has been shown in non-neuronal systems *(55).*

Contrary to neurons, muscle fibers are polynucleated cells thus likely to use different regulatory mechanisms to ensure their differentiation. As mentioned, *in situ* hybridization and promotor studies in transgenic mice have demonstrated that the restricted distribution of

messengers encoding AChR subunits at the NMJ results at least in part from differential transcription in synaptic vs extrasynaptic nuclei *(2–4).* However, in addition to this mechanism, mRNA targeting may also contribute to the precise subsynaptic localization of subset of mRNAs since the mammalian homolog of Staufen has been recently localized at the NMJ *(62).*

An important issue raised by the selective accumulation of mRNAs at synaptic sites is that their translation should be highly regulated both during transport and upon localization. Several mechanisms may likely account for local regulation of synaptic mRNAs transcription. Genetic and molecular studies in Drosophila have shown that Staufen is not only involved in mRNA transport and localization but also in the regulation of translation of transported mRNAs *(63–65).* Given the conserved nature of the molecules in mRNA transport across species, it is likely that Staufen could play a similar role in neurons. Another machinery for regulation of translation at synaptic sites is the presence in 3′UTR of mRNAs of a cytoplasmic polyadenylation element (CPE). When a protein called CPEB binds to the CPE, triggering polyadenylation, translation of this mRNA is in turn activated. CPE was found in the 3′UTR region of CAMKII *(66).* Taken together, these observations support the notion that neurons could regulate synaptic translation.

Role of Sequence Signals in Receptor Trafficking

Besides the mechanisms of mRNA localization and processing within subsynaptic regions, several post-translational regulatory processes, consisting of intrinsic molecular signals governing correct folding and assembly, sorting, transport, and anchoring of neosynthesized membrane proteins along the exocytic pathway, may participate in the precise distribution of neurotransmitter receptors at synapses.

Accumulating data resulting from chimeric and deletion experiments point to the existence of ER retention signals within the C-terminal sequences of various neurotransmitter receptor subunits. Chan et al. *(67)* identified such a sequence within mGluRlb subunit that consists in four positively charged amino acids RRKK essential for the trafficking signals. Its presence results in a much reduced cell-surface expression of the receptor and chimeric molecules in cell lines and their restricted trafficking in neurons. This motif is also present in mGluR1a, but its effect is overcome by a region of the mGluR1a-specific C-terminal sequence (amino acids 975-1098). Thus, these results suggest that these splice variants of mGluR1 utilize different targeting pathways and that this may be a general phenomenon in the metabotropic glutamate receptor gene family *(67).* In the same way, Scott et al. *(68)* identified an RXR-type ER retention/retrieval motif in the C-terminal tail of the NMDA receptor subunit NR1 that regulates receptor surface expression in heterologous cells and in neurons. An additional protein kinase C (PKC) phosphorylation site and an alternatively spliced C-terminal consensus PDZ-binding domain suppress ER retention *(68).* A similar RXR motif present in $GABA_B$ receptor GB1 subunits is masked by assembly with GB2, ensuring heterodimerization *(69).* AMPA receptors probably use such quality-control signals, as yet unidentified, because it was demonstrated that N-terminal extracellular regions are required for efficient assembly and ER exit of AMPA receptors subunits *(70).* Finally, the basic $R_{313}K_{314}$ sequence in the large cytoplasmic loop of the muscle nicotinic AChR α subunit and ubiquitination, a postranslational modification triggering proteasome-mediated degradation, govern the ER to Golgi trafficking of the subunit in heterologous cells *(71).* It has been proposed that the requirement for full assembly of the nicotinic AChR subunits for expression at the cell surface is likely due to the masking of the trafficking signals and potential ubiquitination sites exposed in the unassembled subunit *(71).* Together, these data suggest that quality-control mechanisms, consisting in part in the masking of specific ER retention/retrieval motifs during receptor

assembly, regulates the forward trafficking of neurotransmitter receptors through early stages of the secretory pathway.

As with other polarized cells, the differential localization of neurotransmitter receptors in neurons is likely to result from intrinsic targeting signals governing axonal vs somatodendritic targeting. Accumulating evidence shows that neurons are capable of segregating neurotransmitter receptors either in axonal or in somatodendritic membranes. For example, α-7 containing nicotinic receptors are exclusively located to the somatodendritic membrane of cholinergic neurons in the avian nucleus semilunaris *(72).* Metabotropic glutamate receptors are differentially targeted when expressed in cultured hippocampal neurons: mGluR1a and mGluR2 are targeted to dendrites and excluded from axons, whereas mGluR7 is targeted to axons and dendrites *(73).* Chimeras and deletions experiments point to identification of novel sorting signals distinct from the already identified ones in polarized epithelial cells. These signals consist in 60 amino acid present in the C-terminal cytoplasmic domains of receptor subunits, and mediate axon exclusion of mGluR2 vs axon targeting of mGluR7. The axon targeting signal of the tail of mGluR7 appeared dominant over the axon exclusion signal of the tail of mGluR2 *(73).* These signals define an early step in targeting, probably at the stage of sorting into vesicles from the TGN in the soma. The ultimate synaptic localization must involve additional steps in targeting such as local delivery, possibly via local vesicle fusion, to specific synaptic sites. In the same line, another recent finding indicated that the proximal C-terminus of the AMPA receptor subunit GluR1 contains a dendritic sorting signal *(74).* An important aspect of the mGluR and AMPA receptors studies is that the sequences in the C-terminal cytoplasmic tails involved in dendritic delivery does not contain previously described (Tyr or Leu-Leu) dendritic sorting motifs *(38,75,76).* Another recent study by Nadler et al. *(77)* showed that tagged M2 muscarinic AChR subtype expressed in polarized MDCK is sorted apically while M3 subtype is targeted basolaterally. Chimeras experiments led to the identification in the M3 third intracellular loop of a basolateral sorting signal consisting in a 21 residues sequence (Ser 271-Ser 291). This M3 basolateral sorting signal is dominant over apical signals in M2 and acts in a position-independent manner. Interestingly, the M3 sorting signal does not contain a critical tyrosine-based or di-hydrophobic motifs often found in basolateral sorting signals in many transmembrane proteins, and, as such, it represents a novel basolateral targeting determinant *(77).*

It was initially proposed that the apical domain of epithelial cells corresponds to neuronal axons, while the basolateral domain corresponds to cell bodies and dendrites *(36).* Although this parallel does not hold true for all proteins, recent studies have confirmed that some basolateral proteins in Madin Darby canine kidney (MDCK) cells are restricted to the somatodendritic domain of cultured hippocampal neurons and that the same signals used for basolateral targeting are also likely to mediate somatodendritic targeting *(75).* However, proteins that are apical in MDCK cells are not restricted to the axon but instead are distributed uniformly throughout the axon and dendrites of cultured hippocampal neurons *(75).* These and other studies have suggested the existence of "axon-including" signals, rather than signals that mediate targeting to the axon exclusively *(73).* The differential targeting of the M2 and M3 muscarinic AChRs in MDCK cells and neurons in vivo suggests that similar signals may operate to achieve the polarized sorting of these receptors in epithelial cells and neurons *(77).* While the basolateral sorting signals in M3 may mediate its localization to the somatodendritic domain, the apical targeting information in M2 may allow its inclusion in axons. The cytoplasmic tail domain of mGluR7 is necessary but not sufficient for polarized targeting in MDCK cells, whereas in neurons the cytoplasmic tail is sufficient for cell-surface expression but not polarization *(78,73).* Additional mechanisms are thus likely required to mediate mGluR7 neuronal polarization and synaptic clustering.

Different subtypes of nicotinic acetylcholine receptors coexist within single neurons and must be targeted to discrete synaptic sites for proper function. In chick ciliary ganglion neurons, nAChRs containing α_3 and α_5 subunits are concentrated in the postsynaptic membrane, whereas acetylcholine receptors composed of α_7 subunits are localized perisynaptically and excluded from the synapse. Williams et al. *(79)* demonstrated that the long cytoplasmic loop of α_3 targets chimeric α_7 subunits to the synapse and reduces extrasynaptic localization of nAChR, whereas the α_5 loop does neither. These data show that a particular domain of one subunit targets specific receptor subtypes to the interneuronal synapses in vivo.

These intrinsic signals may function at any stage in receptor targeting: sorting into specific vesicles via protein-protein or protein-lipid interactions, transport by association with specific motors, selection of plasma membrane target sites, or anchoring and retention within one domain. We now review the accumulative arguments suggesting the involvement of extrinsic signals in the precise targeting and localization of neurotransmitter receptors at synapses.

Role of Anchoring and Scaffolding Proteins in Receptor Delivery

Proteins characterized by the presence in their sequence of one or more PDZ (*P*SD-95, *d*iscs large, and *z*onula occludens 1) domains, motifs that mediate protein-protein interactions, are involved in the organization of intercellular junctions and in particular of excitatory central synapses *(80–83).* These molecules anchor neurotransmitter receptors and ion channels at synaptic sites but also provide a scaffold on which the different functional elements of the postsynaptic machinery can be assembled *(84–86).*

Recent contributions on vulval cell differentiation in *Caenhorhabditis elegans* demonstrated the role of this class of proteins in polarized targeting *(87).* The PDZ-containing proteins Lin-2, Lin-7, and Lin-10 act in a ternary complex to ensure the basolateral localization of the epidermal growth factor (EGF) receptor, generating the cascade leading to vulval differentiation. Interestingly, the postsynaptic localization of the glutamate receptor GluR1 in central synapses in *C. elegans* is blocked by Lin-10 mutations, suggesting that Lin-10 is a shared component of the polarized sorting pathway in neuronal and epithelial cells *(88).* The Lin-2/7/10 complex has a mammalian homolog named as CASK (calcium/calmodulin-dependent serine protein kinase)/Velis/Mint1-x11α *(89).* A fundamental point was to reveal whether the complex is directly involved in polarized sorting and targeting of selected proteins to their final subcellular destination, or whether it plays a role in tethering its interacting proteins once these have reached their appropriate location by other mechanisms. Recent studies showing the subcellular localization of Lin-10 and Mint1-x11α are consistent with both a role in tethering of the interacting proteins at the target membrane and a role in sorting membrane proteins, at the level of the Golgi complex, into distinct trafficking vesicles destined for target membranes *(90,91).*

Several extrinsic PDZ-domain containing proteins are specifically involved in the anchoring of excitatory ionotropic receptors via a direct interaction with cytoplasmic sequences of the receptors: the PSD-95 family of proteins for NMDA receptors, and GRIP and PICK1 for AMPA receptors *(85).* PSD-95 was found to transiently associate via a dual palmitoylated anchor with a perinuclear membranous compartment and traffic with vesiculotubular structures that migrate in a microtubule-dependent manner. This initial intracellular targeting may participate in postsynaptic trafficking and receptor clustering by PSD-95 *(92).* The PDZ-containing scaffolding protein, SAP97, which binds the AMPA receptors-containing the GluR1 subunit, was found to interact with its companion receptor early in the secretory pathway. These data suggest GluR1-SAP97 interactions in mechanisms underlying AMPA receptor targeting *(93).*

Stargazing, a tetraspanin protein interacting both with AMPA receptors and SAP97 regulates the delivery of AMPA receptors to the cell surface and targets the AMPA receptors to synapses *(94,95).* In the same way, GRIP is localized in post-Golgi vesicles as well as at synapses, suggesting that GRIP may be involved in transporting AMPA receptors to dendrites along the exocytic pathway *(96).* Collectively, these data indicate a common role for PDZ proteins in transport of receptor-containing vesicles destined to be conveyed to their appropriate synaptic site.

Other extrinsic molecules that do not contain PDZ motifs share the role of anchoring neurotransmitter receptors at synapses: Homer specifically for mGluRs *(97),* the 43kDa protein rapsyn for the muscle nicotinic AChRs *(98,99),* and gephyrin for GlyRs *(100,101)* and $GABA_A$ receptors *(102).* A recent view is that Homer proteins also play a role in a shared cell biological mechanism that links synaptogenesis and mGluRs receptor targeting. Homer proteins mediate the differential targeting of mGluR5 to dendritic synaptic sites and/or axons in cultured neuronal cells *(103).* Furthermore, Homer 1b in Hela cells is responsible for retention of mGluR5 into endoplasmic reticulum pools *(104),* whereas Homer 1a and Homer 1c in HEK293 cells increase cell-surface expression of mGluR1a *(105,106).* Because Homer 1b is found in dendritic spines in neurons, local retention of metabotropic receptors within dendritic ER provides a potential mechanism for regulating synapse-specific expression of group I mGluRs *(104).* In *Torpedo* electromotor synapse, a model system of the NMJ, we recently reported that neosynthesized rapsyn associates initially with the exocytic pathway, as soon as the TGN, and is cotransported with AChR in post-Golgi transport vesicles conveyed to the postsynaptic membrane *(107).* At the neuronal surface, native $GABA_A$ receptor clustering and synaptic targeting require the participation of gephyrin, a clustering protein for GlyRs *(108).* Together, these data led to the proposal that this class of proteins not only plays a role in the anchoring, clustering, and scaffolding of their companion receptor to postsynaptic membrane, but also plays a dynamic role in the sorting and trafficking of receptors along the biosynthetic pathway.

Rafts as Lipidic Platforms for Receptor Sorting and Targeting

Cholesterol-sphingolipid-enriched microdomains (lipid-rafts) are part of the machinery ensuring correct intracellular trafficking of proteins and lipids. By governing protein-protein and protein-lipid interactions, these domains selectively incorporate or exclude proteins and therefore have been proposed to function as membrane platforms for the assembly of signaling complexes and for the sorting of molecules to particular cellular structures *(109).* Studies on polarized epithelial cells suggest that sorting of apically destined proteins in the TGN is mediated by rafts, delivered to the plasma membrane with their lipid and protein cargo *(109).* Increasing evidence allows to extend the participation of this machinery in the biogenesis of neuronal cells. Membrane rafts are necessary for the formation and maintenance of the α_7 nicotinic AChR clusters in somatic spines of ciliary neurons *(110).* Similarly, AMPA-type glutamate receptor subunits were biochemically localized and associated with rafts in dendritic spines from the rat forebrain *(111).* Also, the glutamate AMPA receptor-interacting protein GRIP is recruited to rafts through association with the C-terminal PDZ target site of ephrin receptor in cultured cells *(112),* leading to hypothesize that raft machinery might be involved in the molecular organization of AMPA glutamatergic synapses. Consequently, increasing evidence suggests that neurotransmitter receptors are associated directly or via linker proteins with the cholesterol-rich membrane microdomains rafts that, in addition to ensure receptors clustering, probably control the downstream intracellular sorting of neurotransmitter receptors and associated synaptic components into specific vesicular carriers before their eventual delivery to target synap-

tic membrane. It will now be important to identify the nature of physical interaction between receptors and lipid rafts and to determine whether linker molecules participate in this interaction.

Polarized Delivery of Synaptic Receptors via the Cytoskeleton

The organization of the cytoskeleton, and in particular that of microtubules, is essential for the genesis and maintenance of neuronal polarization by facilitating the targeted delivery of carrier vesicles to the axonal and dendritic compartments. Transport of organelles is accomplished by molecular motors such as kinesin and dynein, which are capable of movement toward the opposite ends of microtubules *(113).* In neurons, numerous members of the kinesin superfamily (named KIFs) have been shown to transport organelles such as synaptic vesicle precursors and mitochondria to specific regions of the cell *(113).* In a recent work, Setou et al. *(114)* reported that the neuron-specific, microtubule-dependent molecular motor KIF17 binds directly and specifically to a PDZ domain of Lin-10 and transports the large protein complex containing the NR2B subunit, which forms the NMDA receptor with the NR1 subunit. This motor-cargo complex transports vesicles along microtubules to dendrites of neurons such as hippocampal pyramidal neurons and was proposed as the sorting machinery for NR2B. This study is of particular interest because it allows for the first time to understand the initial transport steps in the sequence of polarized trafficking of neurotransmitter receptors and associated proteins in neurons *(114).* Interestingly, along this line, the innervated muscle fibers display a local network of stable microtubules in the subneural sarcoplasm *(7).* Further studies provided evidence that the targeting of AChR to the NMJ could be contributed by a microtubule-dependent vectorial delivery in the exocytic pathway *(115,116).* The molecular motors involved in AChR transport remain to be identified.

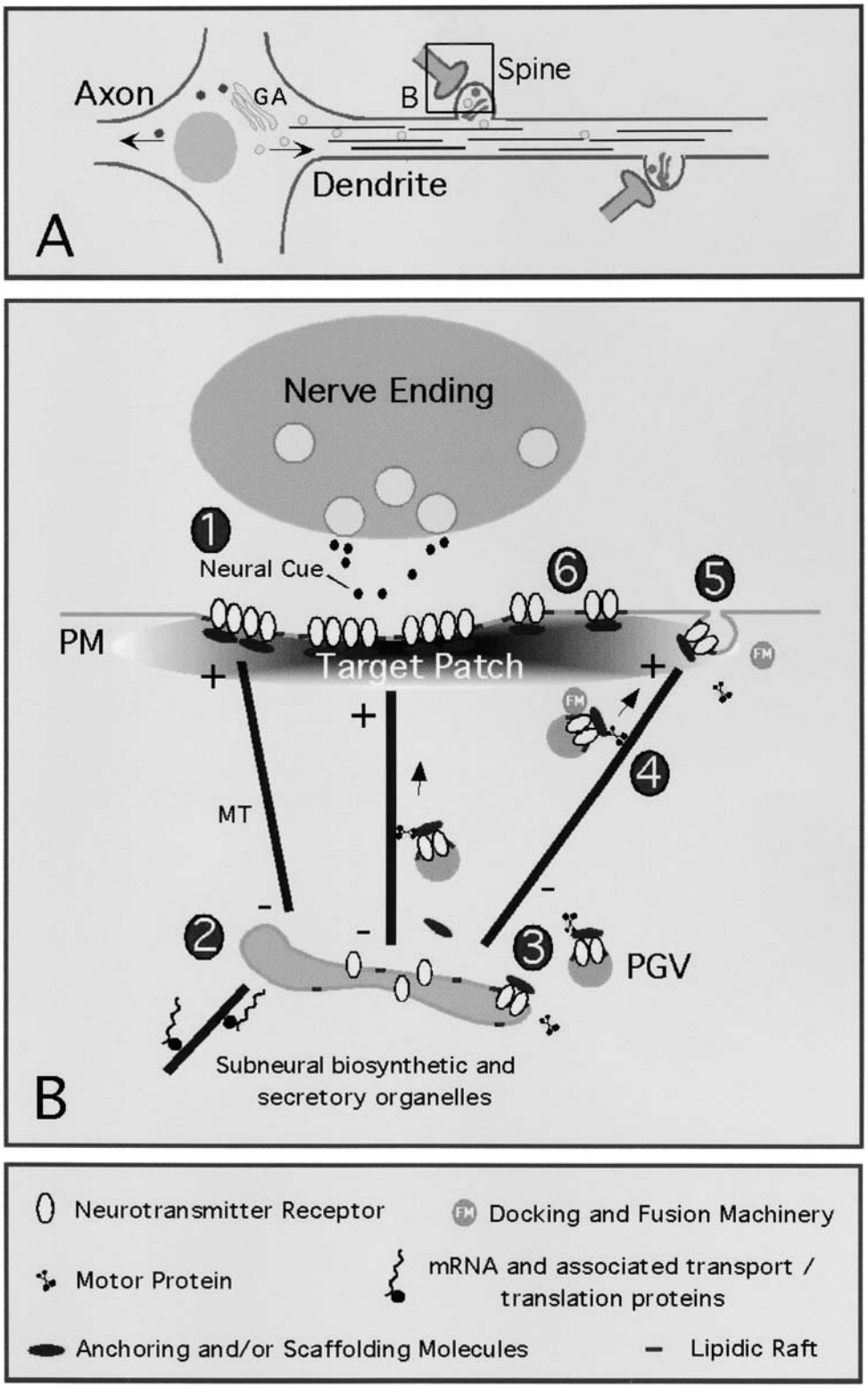
The microtubular cytoskeleton is also likely to be involved in the clustering and immobilization of receptors and ion channels at specific synaptic sites via scaffolding proteins linking these membrane proteins to the cytoskeleton *(83,117).* The NMDA receptor-associated PSD-95 family of proteins bind directly to MAP 1A *(118)* and CRIPT *(119),* both of which are microtubule-binding proteins. The inhibitory GlyRs, $GABA_A$, and $GABA_C$ receptors are thought to be attached to the microtubule cytoskeleton via gephyrin *(101,102,120),* $GABA_A$ receptor-associated protein GABARAP *(121)* and MAP1B *(122),* respectively. At the NMJ, syntrophins, components of the dystrophin-associated protein complex, associate via PDZ-PDZ domain interactions with the protein kinases MAST 205 (microtubule-associated serine/threonine kinase-205kDa) and SAST (syntrophin-associated serine/threonine kinase) that may link syntrophin and in turn the dystrophin complex to the subneural microtubule meshwork *(123).* An alternative function for the association of MAST with syntrophin would be to provide specific links with microtubules during the intracellular transport. The recently reported association of syntrophins with the AChR post-Golgi vesicles *(124)* would provide a rationale for the microtubule-dependent transportation of AChRs at the NMJ *(115).* Recently, gephyrin has been demonstrated to interact with the intracellular GABARAP protein that has been proposed to be implicated in intracellular receptor sorting and targeting processes that precede and/or initiate receptor clustering at the synapse *(121,125,126).* Indeed, GABARAP's close homology to p16, a late-acting intra-Golgi trafficking factor, and the assignment of a putative tubulin-binding site to the N-terminal region of GABARAP is consistent with this protein having a role in the transport of $GABA_A$ receptors-containing Golgi vesicles via microtubules before vesicle fusion with the plasma membrane *(125).* In the same view, the presence of PSD-95 in intracellular organelles *(92)* and its interaction with the microtubular scaffold via CRIPT led to the hypothesis that scaffolding-

associated proteins participate in the association of receptor complex-containing vesicular transporters with the microtubular network (see Fig. 1).

Postsynaptic Docking and Fusion: the SNARE/Sec Machineries

A set of additional mechanisms are needed to ensure the specific target recognition and docking of trafficking vesicles. Like epithelial cells, increasing evidence suggests that neurons are also likely to require the SNARE machinery to mediate membrane fusion of organelles. However, at variance with polarized MDCK cells in which two t-SNAREs, syntaxin 3 and TI-VAMP (tetanus neurotoxin-insensitive vesicle-associated membrane protein), specifically localize at the apical surface *(35)*, little is known on differential axonal *vs* dendritic specific localization of SNAREs, which would be expected from proteins involved in axonal or dendritic vesicle targeting. For example, syntaxin-1 and SNAP-25, SNAREs that mediate synaptic vesicle exocytosis at the axonal active zones, are also present in the soma as well as dendrites of differentiated neurons *(127,128;* but see ref. *129).* The demonstration that postsynaptic neurotransmitter receptors trafficking is dependent of SNARE exocytosis is only being reported. Indeed, NSF, a soluble factor recruited by SNAREs complex to form the mature complex ensuring membrane fusion, was shown to specifically interact with the C-terminus of the GluR2 and GluR4c subunits of AMPA receptors in vitro and in vivo *(130).* This interaction and the observation that NSF modulates the AMPA receptor function led the authors to propose a model in which NSF may be involved in the regulation of receptor insertion into the synaptic plasma membrane by regulating the docking of the AMPA receptor subunits-containing vesicles with the postsynaptic membrane fusion machinery *(130).* GABARAP, which immunoprecipitates with $GABA_A$ receptors and is enriched in Golgi fractions and postsynaptic cisternea, binds NSF, this suggesting an important role of SNARE machinery in $GABA_A$ receptor targeting *(126).* A recent study on the understanding of the molecular mechanisms underlying PKC potentiation of NMDA receptors demonstrated that PKC increases NMDA channel opening rate and favors receptor trafficking and expression

Fig. 1. Schematical representation of the targeted delivery of neurotransmitter receptors at synapses. **(A)** In neurons, a centralized somatic biosynthetic and exocytic machinery (GA) directs the sorting and targeting of postsynaptic receptors toward dendritic compartment. Delocalized dendritic outstations (B) are also involved in the local biosynthesis and targeting of receptors to synaptic sites. This local machinery is also likely to capture receptor carriers sorted in the soma and conveyed in the dendrite along microtubule tracks. **(B)** Detailed functional organization of the subsynaptic exocytic machinery. 1) Nerve-derived factors mediate a local differentiation of the surface of the target cell involving reorientation of components of the cytoskeleton and protein transport pathways toward this site. The formation of a "target patch" then accounts for selective recruitment and retention of newly delivered receptors at the postynaptic membrane (PM) and for localization of mRNAs. 2) The subneural biosynthetic machinery may be engaged in the local translation, assembly, and sorting of neurotransmitter receptors. 3) The formation of a subpopulation of receptor-enriched post-Golgi vesicles (PGV) resulted from protein segregation within cholesterol-sphingolipids-enriched rafts and recruitment of receptor-specific anchoring and/or scaffolding molecules. This molecular subcomplex may participate both in the sorting machinery and in the subsequent targeting steps. 4) The targeted delivery of synaptic receptors is facilitated by the association of PGV with the polarized subneural microtubular network via recruitment of specific molecular motors. Scaffolding molecules may also participate to the association of receptor-containing PGVs with microtubules (MT). 5) Docking and fusion of motor-cargo complex at synaptic site is monitored by the docking and fusion machinery (FM), likely including specific vesicular and target SNAREs. 6) The receptors and associated scaffolding protein microclusters embedded within rafts are recruited upon nerve signaling in subneural macroclusters and finally stabilized in the postsynaptic membrane following coupling with the cytoskeleton. Such a machinery may function both at neuron-neuron synapses (dendritic spine) as well as at the vertebrate NMJ.


Axon
GA
B
Spine
Dendrite
A
Nerve Ending
1
Neural Cue
6
5
PM
Target Patch
FM
FM
4
MT
2
3
PGV
Subneural biosynthetic and
secretory organelles
B
Neurotransmitter Receptor
Docking and Fusion Machinery
Motor Protein
mRNA and associated transport /
translation proteins
Anchoring and/or Scaffolding Molecules
Lipidic Raft

to dendrites and dentritic spines in recombinant systems and in neurons *(131).* Inhibition of PKC potentiation by botulinum neurotoxin A and a dominant-negative mutant of SNAP-25 suggested that NMDA receptor trafficking to dendrites upon potentiation by PKC occurs via SNARE-dependent exocytosis *(131).*

In parallel to the SNARE machinery, it has been proposed that the Sec6/8 complex may play a role in the targeting of trafficking vesicles in neurons. The Sec6/8 complex is enriched in growth cones and clusters on the axonal plasma membrane in developing hippocampal neurons. These clusters colocalize with synaptic vesicles markers early in development, suggesting that the complex might play a role in the targeting of synaptic proteins to the potential active zones during synaptogenesis *(132).* The potential role of the Sec6/8 in the polarized neurotransmitter receptors targeting remains to be determined.

Concluding Remarks: the Synaptic "Target Patch" Hypothesis

In both central and peripheral synapses, the postsynaptic apparatus is characterized by accumulations of neurotransmitter receptors and associated molecules. In recent years, significant progress has been made in the elucidation of cellular and molecular mechanisms that participate in the differentiation of the postsynaptic membrane during synaptogenesis. In particular, nerve-induced receptor aggregations have been described in central neurons and at the NMJ *(4)* and several proteins have been characterized that cluster neurotransmitter receptors and stabilize them via interaction with the subneural skeleton. In addition, it is reasonable to assume that similar mechanisms account for receptor delivery in both systems, these involving specialized aspects of the exocytic pathway.

From the convergent data reported in this review, a working model for receptor biosynthesis, trafficking, and delivery at synaptic sites is proposed. A first body of experimental evidence points to the existence in neurons of a sorting machinery in the soma accounting for dendritic delivery of synaptic receptors. This is achieved by sorting/retention signals residing in the sequence of the protein. A second, decentralized, biosynthetic machinery exists at synaptic sites. These "outstations" likely favor precise delivery and modulation of receptor density at a given synapse. The relative contribution of these two mechanisms to receptor supply at a single synaptic site remains a mystery.

Figure 1 presents a model illustrating the possible mechanisms involved in neurotransmitter receptor biosynthesis and delivery accounting for their accumulation at postsynaptic sites. In differentiating neurons or muscle fibers, neurotransmitter receptors are initially randomly distributed at the cell surface, then clustered by nerve terminals. Neural cues such as agrin at the NMJ are responsible for the orchestration of the differentiation of the postsynaptic apparatus. In muscle fibers, the signaling pathway triggered by agrin is mediated by the muscle-specific receptor tyrosine kinase MuSK. MuSK triggers a cascade of signalization, ultimately leading to the clustering of AChRs, and organizes the subsynaptic scaffold via a direct interaction with the PDZ-domain containing protein MAGI-1c, a membrane-associated guanylate kinase (MAGUK) with an inverted domain organization *(133).* As proposed by Nelson *(11,134),* one could hypothesize that this local differentiation of the cell surface of the target cell constitutes a "target patch" accounting for the local reorganization of the exocytic pathway, which ultimately will favor the selective recuitment and retention of newly delivered receptors at synaptic sites. In muscle fibers, the target patch would account for the remarkable differentiation of the sarcoplasm beneath the NMJ. In neurons, multiple target patches would recruit the delocalized biosynthetic "outstations" in dendritic spines, allowing local translation of synaptic mRNAs, and assembly and targeting of receptors. These target patches may also participate in the precise synaptic targeting of receptors previously sorted in the somatic Golgi compartment and conveyed in the den-

drite, providing that an adequate docking and fusion machinery is associated with the receptor-cargo carrier.

From these recent progress, it appears that several concerted mechanisms acting at the cell surface (signaling by nerve-released factors), at the plasma membrane level (clustering and stabilization of neurotransmitter receptors, coupling to the cytosleleton), and in the subneural cytoplasm (reorganization of the exocytic pathway and microtubular network) contribute to the accumulation of neurotransmitter receptors at synaptic sites. It seems likely that this profusion of regulatory mechanisms also participate in the regulation of receptor expression underlying synaptic plasticity in the CNS.

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