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Selective Targeting of Glutamate Receptors in Neurons

Maria E. Rubio

Department of Molecular Biology of Neuronal Signals, Max-Plank-Institute for Experimental Medicine, 37075 Göttingen, Germany

Abstract

Glutamate receptors mediate the majority of excitatory responses in the central nervous system (CNS). Neurons express multiple subtypes and subunits of glutamate receptors, which are differentially distributed at pre- and postsynaptic sites. This allows the cell to respond differentially depending on the subunit composition of receptors at the postsynaptic membrane. The process by which receptors are targeted selectively to the appropriate synapse is poorly understood. Evidence exists that targeting of glutamate receptors to the different neuronal compartments is regulated at multiple levels involving a general targeting step; a local step where receptor-containing organelles are moved to the synapse; and a step where the receptors are stabilized at the synapse, which may involve interaction with an anchoring protein.

Index Entries: AMPA; NMDA; metabotropic glutamate receptors; targeting; dendrite; cytoskeleton; endoplasmic reticulum; brain.

Introduction

Neurons, like other eukaryotic cells, possess intracellular organelles involved in the synthesis and maturation of proteins, such as the endoplasmic reticulum (ER) and the Golgi apparatus. They also possess a rich cytoskeletal network that acts as a support and mediates the transport of organelles. However, neurons are highly polarized cells with an apparatus of emmision, the axon, and an apparatus of reception, the dendrite (Ramon y Cajal, 1923). The morphology of the neuron is essential to its function. Each morphological specialization of the neuron, the dendrite, the dendritic spines, the axon, the axon hillock, the nodes of Ranvier, the active zone, and the synaptic vesicle appears to have a unique protein composition. Therefore, to understand the function of neurons in molecular terms, we need to know how unique proteins involved in it are distributed. However, the molecular mechanisms underneath such a process are still poorly understood. Targeting of proteins to the differ-

*Address to which all correspondence and reprint requests should be sent. E-mail: mrubio@gwdg.de

ent neuronal compartments may involve a general targeting step, where signal sequences sort receptors early after synthesis; a local step where receptor containing organelles are moved to the synapse; and a step where the receptors are stabilized at the synapse, which may involve interaction with an anchor. This review will try to update the current ideas about receptor targeting, focusing on glutamate receptors.

Glutamate Receptor Family: General Introduction

Glutamate is the major neurotransmitter in the central nervous system (CNS). By molecular cloning, it is known that the family of glutamate receptors is complex and formed by multiple subtypes and subunits. There are two major types of glutamate receptors: the ionotropic and the metabotropic. Ionotropic glutamate receptors are made up of four or five subunits, which assemble to form functional heteromeric or homomeric complexes (Wenthold et al., 1992; Ferrez-Montiel and Montal, 1995; Rosenmund et al., 1998). They include the α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) subtype of glutamate receptor that mediates most fast excitatory transmission in the CNS. AMPA receptors are formed by four genes, GluR1-4 or GluRA-D. The kainate receptors with five subunits, GluR5-7 and KA1-2; the delta receptors with two subunits, delta 1-2 (δ1–2); and the *N*-methyl-D-aspartate (NMDA) receptors with six subunits, NR1 (ζ1), NR2A-D (ε1–4), and NR3 (χ-1 or NMDA-L; *see* for review Hollmann and Heinemann, 1994; Hollmann, 1999; Lerma, 1999). Many subunits also have variant forms generated through alternative splicing. Metabotropic glutamate receptors (mGluR) can be divided into three groups based on G-protein coupling, pharmacology, and sequence homology (Nakanishi 1994; Pin and Duvoisin, 1995). Group I is formed by mGluR1 and mGluR5; group II by mGluR2 and mGluR3; and group III by mGluR4, mGluR6, mGluR7, and mGluR8. New lines of evidence indicate that mGluRs can be also linked to Src-family tyrosine kinases (Heuss et al., 1999).

By *in situ* hybridization and immunocytochemistry, it is known that glutamate receptors are highly expressed in CNS and that most neurons express multiple subtypes and subunits (*see* Petralia et al., 1999b), with the pattern of expression changing during development (*see* reviews by Bahn and Wisden, 1997; Watanabe, 1997). Glutamate receptors are predominantely postsynaptic, although numerous studies report a population of presynaptic receptors as well as a population in glia (*see* Petralia and Wenthold, 1992; Martin et al., 1993; Gallo et al., 1994; Petralia et al., 1994, 1996a,b,c; Clark et al., 1997; Rodriguez-Moreno et al., 1997; Rubio and Wenthold, 1999a). Physiological and immunocytochemical studies have shown that two or more glutamate receptors can co-exist at the same postsynaptic structure (Nusser et al., 1994; Matsubara et al., 1996; Rubio and Wenthold, 1997; Wang et al., 1998). Therefore the first fundamental question concerns the mechanism by which various glutamate receptors are distributed within a single neuron. Do all glutamatergic synapses on a neuron share the same glutamate receptor composition? Or presumably, has the neuron developed a mechanism to target selectively its multiple receptor types to different populations of synapses that allow multiple physiological responses? Evidence exists with both excitatory and inhibitory receptors that supports the last scenario (Nusser et al. 1996a,b; Landsend et al., 1997; Rubio and Wenthold, 1999a, 1997; Zhao et al., 1997, 1998; Toth and McBain, 1998).

Differential Distribution of Glutamate Receptors in Neurons. Are Individual Receptors Sorted Differentially?

Differential Distribution of Glutamate Receptors in Caenorhabditis elegans *(C. elegans)*

Evidence on glutamate receptor targeting has been analyzed in the nematode *C. elegans.*

Using degenerate polymerase chain reaction (PCR), a cDNA was identified that encoded a potential glutamate receptor *(glr-1),* which is most similar to the vertebrate AMPA-type ionotropic glutamate receptors (Maricq et al., 1995). *glr-1* is expressed in motor neurons and interneurons, including interneurons implicated in the control of locomotion. Deletions of *glr-1* rendered worms unable to withdraw backwards when mechanically stimulated, whereas they withdrew normally in response to chemical repellents (Maricq et al., 1995). The ASH sensory neurons are known to mediate withdrawal responses both to mechanical stimuli and chemical repellents. Also, ASH neurons make chemical synapses with *glr-1*-expressing interneurons. Thus, these results suggested that postsynaptic interneurons may use different neurotransmitter receptors to process different sensory stimuli detected by the same sensory neurons.

Differential Distribution of Glutamate Receptors in the CNS

Owing to the complex cytoarchitecture of the CNS, there are not many studies on neuronal receptor targeting. We may think that a differential distribution of glutamate receptors probably occurs in different dendritic processes such as apical basal dendrites, or within dendrites receiving at least two excitatory synaptic inputs. In the first case, differential distribution of glutamate receptors has been studied in detail in a particular bipolar neuron of the cochlear complex: the fusiform cell of the dorsal cochlear nucleus (Rubio and Wenthold, 1997). The fusiform cell possesses a dendritic tree divided in apical and basal dendrites, receiving two different excitatory synaptic inputs: parallel fibers from the granule cells on apical dendrites, and the primary input from the auditory nerve on basal dendrites. By retrograde tracing and post-embedding immunogold labeling, fusiform cells were shown to express different glutamate receptors at these two synapse populations (Table 1). Subunits like GluR2/3 and NR2A/B were equally abundant at both synaptic populations, whereas GluR4 and mGluR1α were present only at the basal dendrite synapses. Delta 1/2 was about 4 times more abundant at apical dendrite synapses. Functional studies have identified NMDA receptors, but not metabotropic glutamate receptors, on apical dendrites of fusiform cells (Manis and Monitor, 1996; Molitor and Manis, 1997). The presence of the GluR4 subunit at auditory nerve synapses and its absence from parallel fiber synapses is reflected in a recent study that showed that AMPA receptors postsynaptic to the auditory nerve differ from those postsynaptic to parallel fibers in channel-gating, kinetics and in their permeability to calcium (Gardner et al., 1999).

Differential distribution of glutamate receptors has also been described in neurons that receive two excitatory synaptic input onto the same dendritic process, i.e., Purkinje cells of the cerebellum and two kinds of neurons of the CA3 region of the hippocampus. Dendrites of Purkinje cells have two excitatory inputs—climbing fiber synapses originating from inferior olivary neurons, and parallel fiber synapses, originating from granule cells of the cerebellum. Delta 2 receptors are abundant at parallel fiber synapses, but are rare or absent from climbing fiber synapses (Landsend et al., 1997; Zhao et al., 1997). In contrast, GluR2/3 subunits of AMPA receptors are more common in climbing fiber synapses than in parallel fiber synapses (Zhao et al., 1998). Delta 2 is believed to play a specific role in synaptic plasticity of adult parallel fiber synapses, because long-term depression of parallel fiber synapses is impaired in mice lacking delta 2 gene (Kashiwabuchi et al., 1995).

In the apical dendrites of pyramidal cells of the CA3 region of the hippocampus, postsynaptic immunolabeling for NR1 subunits is more common at small-spine synapses than at mossy-terminal synapses (Petralia et al., 1994; Siegel et al., 1994; Takumi et al., 1999). In contrast, immunolabeling for NR2A/B subunit is different, as has been shown by studies using separate antibodies for NR2A and NR2B in CA3 pyramidal cell apical dendrites. In this dendritic process, NR2A is present in both small-spine

Table 1
Summary of the Post-Embedding Immunoreactivity for Glutamate Receptor Subunits at the Auditory Nerve and Parallel Fiber Synapses

	Auditory nerve synapses (Basal dendrites)		Parallel fiber synapses (Apical dendrites)	
Receptors	Number of PSDs[c]	Number of gold particles per µm of PSD[c] ± SE	Number of PSDs[c]	Number of gold particles per µm of PSD[c] ± SE
GluR2/3[a,b]	18	17.7 ± 4.0	17	16.5 ± 3.2
GluR2[b]	25	9.1 ± 1.1	17	7.2 ± 1.2
GluR4[b]	17	19.1 ± 2.2	17	0
GluR4 (10 nm gold)	9	9.2 ± 1.9	8	0
NR2A/B[b]	19	6.4 ± 1.4	14	9.8 ± 1.3
mGluR1α[b]	35	8.0 ± 1.3	25	0
Delta1/2[b]	31	8.3 ± 1.2	25	33.9 ± 3.1

[a] Monoclonal antibodies.
[b] 5 nm gold was used for immunogold labeling quantification with all the antibodies selective for the glutamate receptor subunits, except for GluR4, which was analyzed using 5 and 10 nm.
[c]Postsynaptic membrane.
Adapted with permission from Table 2 in Rubio and Wenthold (1997).

and mossy-terminal synapses, whereas NR2B is only present in small-spine synapses (Fritschy et al., 1998; Watanabe et al., 1998). Thus, NMDA receptor composition may differ between small-spine and mossy-terminal synapses in CA3 pyramidal cell apical dendrites. In support of this, most apical-dendrite synapses exhibit NMDA-receptor-dependent long-term-potentiation (LTP), whereas mossy terminals have NMDA-receptor-independent LTP (Zalutsky and Nicoll, 1990; Derrick et al., 1991), even though they do have some functional NMDA receptors (Spruston et al., 1995). Differential distribution of NMDA receptors with distinct subunit composition is also supported by developmental studies (Bahn and Wisden, 1997; Watanabe, 1997). The function of AMPA receptors varies according to subunit composition. For example, the presence of GluR2 in an AMPA receptor complex can render the receptor essentially impermeable to Ca^{2+} (Verdoon et al., 1991). In the CA3 region, differential distribution occurs in interneurons, which have Ca^{2+}-permeable (GluR2 lacking?) AMPA receptors at mossy-fiber terminals and Ca^{2+}-impermeable (GluR2 containing?) AMPA receptors at commissural/associational axon terminals (Toth and McBain, 1998). Furthermore, different populations of AMPA receptors, containing GluR1 plus GluR2, GluR2 plus GluR3, or GluR1 only, are found in the CA1/CA2 region, although their synaptic distribution is not known (Wenthold et al., 1996). In addition, some synapses in the hippocampus and other regions may have NMDA receptors but lack AMPA receptors, whereas most synapses on the same neurons have both NMDA and AMPA receptors. The former synapses are called "silent synapses" and have been proposed to recuit AMPA receptors following adequate activation (e.g., review by Malenka and Nicoll, 1997; Nusser et al., 1998; Petralia et al., 1999a; Shi et al., 1999).

Intracellular Pool of Glutamate Receptors

These studies of differential distribution of glutamate receptors at the postsynaptic membrane raise the question of how receptor target-

ing can be achieved. Like other transmembrane proteins, glutamate receptors follow the secretory pathway (Pelhman and Munro, 1993) to be transported to the plasma membrane. Therefore, receptor expression at the postsynaptic plasma membrane requires an effective mechanism to move selectively the intracellular pool of receptors to their appropriate location throughout the somatodendritic compartment.

The existence of an intracellular pool of glutamate receptors is well-established (Petralia and Wenthold, 1992; Kharazia et al., 1996; Doherty et al., 1997; Hall and Soderling, 1997; Mammen et al., 1997; Huh and Wenthold, 1999). Analyses of the turnover of glutamate receptors showed that the assembled AMPA and NMDA types of receptors have long half-lives (approx 30 h; Mammen et al., 1997; Huh and Wenthold, 1999), whereas unassembled subunits are rapidly degraded, as in the case of NRI in cultured granule cells (Huh and Wenthold, 1999). More recently and supporting transport of glutamate receptors from the cell body throughout dendrites, experiments with GFP-tagged receptors showed that newly synthesized intracellular pool of receptors could be transported to the synapse following adequate activation (Shi et al., 1999).

In addition, analysis of the distribution of AMPA and metabotropic receptors in the dendrites of fusiform cells in the cochlear complex showed the intracellular receptor pool to be related to the synaptic pool, such that dendritic segments lacking a particular synaptic receptor had a much lower level of intracellular receptors (Rubio and Wenthold, 1999a). As noted earlier, basal synapses contain GluR2/3, GluR4, and mGluR1α, whereas apical synapses contain GluR2/3. Quantitatively, it was shown that a significantly higher concentration of receptors occurred in the dendritic branches that contained synaptic receptors (Fig. 1), but these receptors were not concentrated near the synapse (Rubio and Wenthold, 1999a) as it was previously suggested. GluR4 and mGluR1α were both higher in intracellular pools of basal dendrites, whereas they were not present in apical dendrites. On the other hand, GluR2/3, which was present at synapses in both apical and basal dendrites, had similar intracellular pools in both dendrites. This may indicate that a mechanism exits to selectively sort and target receptors early after synthesis. This differential targeting between two different dendritic segments can be compared to the compartmentalization that is found in other systems, such as polarized epithelial cells (Drubin and Nelson, 1996; Wozniak and Limbird, 1996) and axonal vs dendritic sorting that takes place in neurons and occurs at the level of the Golgi apparatus (Kelly and Grote, 1993).

As mentioned previously, neurons posses a variety of specialized organelles. Thus, it is important to know where this intracellular pool of glutamate receptors is located and how this is organized. By early studies at the electron microscope level, the immunohistochemical reaction for glutamate receptors was observed as electrondense patches throughout dendrites. These patches of receptors were associated to intracellular membranes presumably from the ER (Petralia and Wenthold, 1992; Kharazia et al., 1996). More recently, using immunogold labeling to localize the intracellular pool of glutamate receptors in fusiform cells of the dorsal cochlear nucleus, Purkinje cells of the cerebellum and pyramidal cells of the hippocampus (Rubio and Wenthold, 1999a), it was shown that groups of gold particles labeling AMPA and metabotropic glutamate receptors were associated with tubulovesicular membranes of the ER and cytoskeleton (Fig. 1). Interestingly, this association was not limited to the trans-Golgi network in the cell body, but continued further throughout dendrites and dendritic spines. The extension of ER membranes from the cell body to the most distal dendrites, including dendritic spines, has been well-documented (Walton et al., 1991; Martone et al., 1993; Terasaki et al., 1994; Krijnse-Locker et al., 1995; Spacek and Harris, 1997). Thus, this membrane system in dendrites may represent a major conduit for the transport of membrane proteins from the cell body to their functional location (Petralia and Wenthold, 1992; Kharazia et al., 1996;

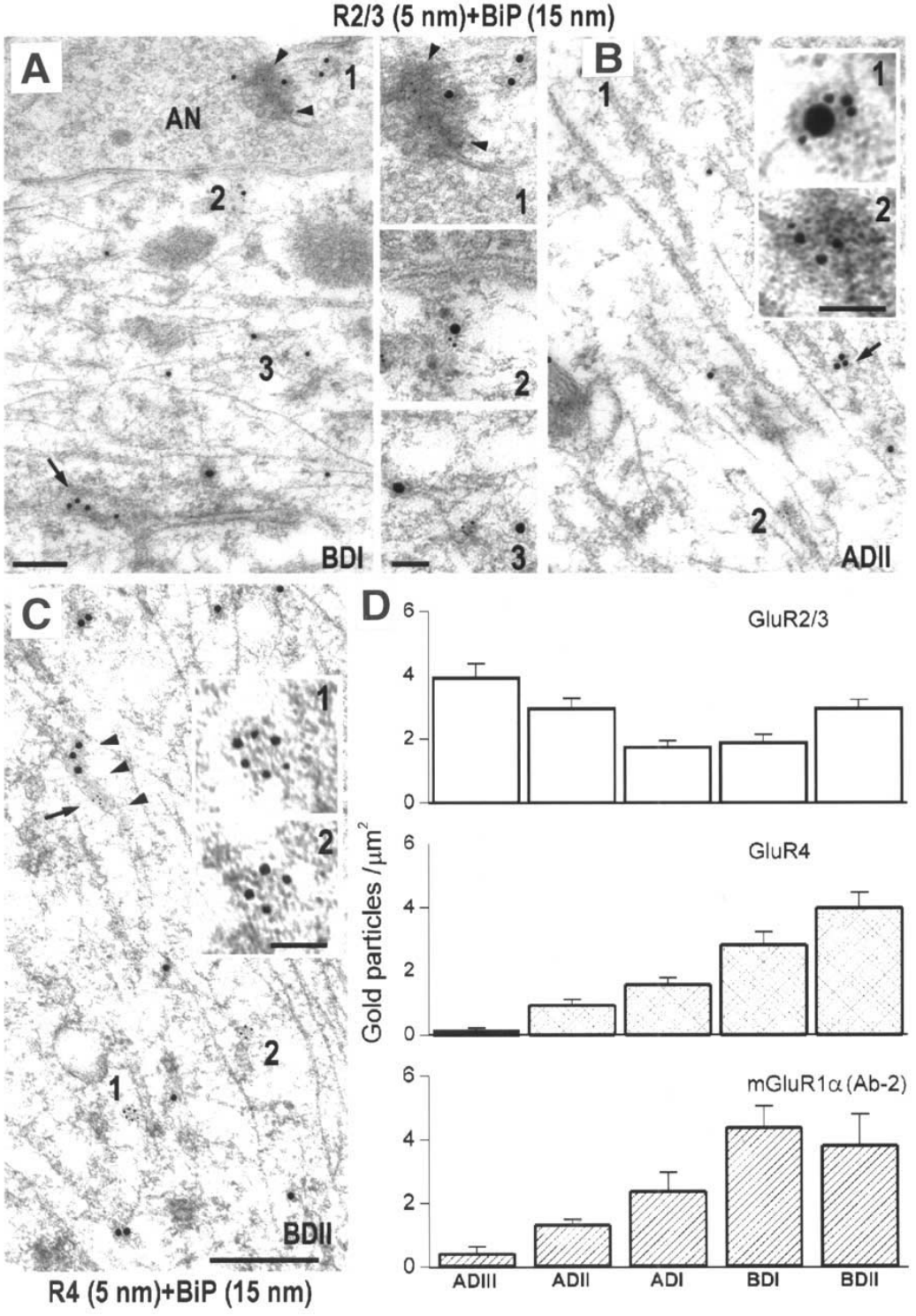
R2/3 (5 nm)+BiP (15 nm)
A
AN
1
2
3
BDI
B
ADII
C
BDII
R4 (5 nm)+BiP (15 nm)
D
GluR2/3
GluR4
mGluR1α (Ab-2)
Gold particles /μm²
ADIII
ADII
ADI
BDI
BDII

Fig. 1. Double immunogold labeling with polyclonal antibodies (PAbs) for AMPA receptor subunits and BiP or calnexin in fusiform cell dendrites. **(A, B)** Double immunogold labeling with a PAb for GluR2/3 (5 nm gold particles) and a monoclonal antibody (MAb) for BiP (15 nm) in a basal proximal dendrite (A, BDI), and in a secondary apical dendrite (B, ADII). Insets in both dendrites, membranes of the endoplasmic reticulum immunogold-labeled with BiP (15 nm) as well as GluR2/3 (5 nm). Some membranes that labeled only for BiP (*arrows* in A and B) and only for GluR2/3 (B, 2) are also observed. In A, an auditory nerve terminal (AN) is seen making synaptic contact on a dendritic spine *(1)* on basal dendrites. The postsynaptic membrane *(arrowheads)* contains 5 nm gold particles specific for GluR2/3, and the cytoplasm of the spine (1) contains 15 nm gold particles specific for BiP. **(C),** Distal basal dendrite (BDII), after double immunogold labeling with a PAb specific for GluR4 (5 nm) and a MAb for BiP (15 nm). Membranes of the endoplasmic reticulum showed immunogold labeling for BiP (15 nm) dispersed in the cytoplasm of the dendrite. Gold particles labeling GluR4 (5 nm; C, *arrow*) are associated with the same membranes labeled for BiP *(arrowheads),* but mostly are seen forming groups of particles associated with membranes that do not contain labeling for BiP. Insets show higher magnification of A, 1–3. Scale bar, 0.25 μm; insets, 50 nm; E, 0.12 μm. **(D)** Histograms showing the relative density of gold particles and SEM for GluR2/3, GluR4, and mGluR1α (Ab-2) in different dendritic segments of fusiform cells. GluR2/3 immunolabeling is present in all dendritic segments, but with the highest level in both distal apical and basal dendritic segments (the difference between these two distal dendritic segments is not statistically significant, $p > 0.01$; the difference among the rest of the dendritic segments is statistically significant, $p < 0.01$). On the other hand, only basal dendrites show relatively high levels for GluR4 and mGluR1α. The labeling decreases toward the apical distal dendrites and is statistically significant ($p < 0.01$). The density of gold particles was compared for all the dendritic segments, and the difference of gold labeling was statistically significant for all cases ($p < 0.01$). ADIII, apical tertiary dendrite; ADII, apical secondary dendrite; ADI, apical primary dendrite; BDI, proximal primary basal dendrite; BDII, distal secondary basal dendrite. Adapted with permission from Rubio and Wenthold (1999a; copyright 1999 by the Society of Neuroscience).

Rubio and Wenthold, 1999a,b). By immunocytochemistry, we also know that these membranes are rich in ER and Golgi proteins including immunoglobulin binding protein (BiP), and calnexin (Jareb and Banker, 1998; Gardiol et al., 1998; Rubio and Wenthold, 1999a,b). In other systems, like the spinal cord, the presence of ER proteins in dendrites has been related to the local synthesis of some proteins (e.g. the α-subunit of the glycine receptor; Racca et al., 1997). Yet the distribution of mRNA suggests that glutamate receptors are synthesized predominantly in the neuronal cell body (Eshhar et al., 1993; Hunter et al., 1993; Lauri and Seeburg, 1994; Bahn and Wisden, 1997). This may indicate that proteins synthesized in the cell body can undergo post-translational processing, such as glycosylation and assembly, in the dendrite (Rubio and Wenthold, 1999b). This is supported by the close relationship between ER chaperones like BiP and calnexin with AMPA receptor subunits as shown by immunogold labeling and biochemical analysis. The existence of local synthesis of glutamate receptors in dendrites, however, remains a controversial subject.

Molecular Basis of Receptor Targeting

Role of Interacting Proteins

The mechanisms by which neurons regulate the assembly of subunits to form functional receptor complexes and the targeting of these complexes to, and their maintenance at, the appropriate synapses involve interactions with a series of proteins. The functions of these proteins range from that of a molecular chaperone that aids in the folding and assembly of a receptor complex to that of protein that anchors the receptor at the synapse.

Mature forms of AMPA receptor subunits interact with BiP and calnexin (Rubio and Wenthold, 1999b), and this association occurs

after translation of individual subunits has taken place in the ER. The molecular chaperones, calnexin and BiP, have been implicated in the quality-control and architectural editing of proteins (Hurtley and Helenius, 1989; Klausner, 1989; Zhang et al., 1997), and have been shown to cooperate sequentially in facilitating folding of nascent proteins within the ER (Hammond and Helenius, 1994; Kim and Arvan, 1995). It is known that both chaperones interact with individual receptor subunits. Furthermore, BiP and calnexin also facilitate the assembly and surface expression of heterogeneous receptor complexes (Chang et al., 1997). This raises the interesting possibility that these chaperones, known to participate in protein folding, also have a role in the maturation, targeting, and assembly of AMPA receptor subunits.

Glutamate receptors are also associated with many different molecules involved in the formation of synaptic cytoskeletal networks and signal transduction cascades. A growing family of PDZ-domain-containing proteins have an apparently critical role in synaptic targeting and localization of neurotransmitter receptors and ion channels (Kornau et al., 1997; Kim and Huganir, 1999). PDZ-domains mediate protein–protein interactions by binding to the carboxyterminal amino-acid sequence T/SXV of membrane-bound and membrane-associated proteins. The first interacting protein to be identified was PSD-95 (Kennedy, 1997), which has three N-terminal PDZ domains. By a yeast two-hybrid assay, it was shown that the first two PDZ domains (PDZ1 and PDZ2) of PSD-95 interact with the C-terminus of the NMDA NR2 subunits. Subsequentally, three additional homologous proteins were identified that interact with the C-terminus of NR2 subunits, and interacting proteins have been described for AMPA, kainate, delta, and metabotropic glutamate receptors (Table 2). Some of these may not play a role in synaptic anchoring, but rather in the dendritic organization of the receptor, and the movement of the receptor between intracellular organelles.

As mentioned earlier, interaction with PDZ domain proteins has been suggested as a means of targeting and anchoring ionotropic glutamate receptors. However, mutant mice expressing NMDA receptors lacking the intracellular C-terminal domain (Sprengel et al., 1998), had functional synaptic NMDA receptors, even though they lacked binding sites of the anchoring proteins. Thus, the synaptic expression of the mutant NMDA receptors suggested that other factors were involved in the anchoring of glutamate receptors at synapses. Moreover, it has been recently shown that PSD-95 family proteins are not essential for the maintenance of the synaptic localization of NMDA receptors (Migaud et al., 1998; Passafaro et al., 1999). This indicates that the family of PDZ interacting proteins might have additional roles and are not absolutely necessary for the synaptic expression of the receptor.

Domains of the Receptor Molecule That Are Important for Targeting

Dotti and Simons (1990) proposed that neurons and polarized epithelial cells share mechanisms of protein targeting, with apical side of epithelial cells equivalent to axons and basolateral side equivalent to the somatodendritic region of neurons. Studies of epithelial cells have identified basolateral sorting signals in the cytoplasmic tails of a large number of proteins (Mellman, 1996; *see also* Winckler and Mellman, 1999). These signals are often, but not always, based on the presence of a critical tyrosine or dileucine motif. In the case of apical targeting, sequences in transmembrane regions, or by *N*- or *O*-glycans in MDKC cells, direct the transport. There is another element to apical targeting. Detergent-insoluble glycolipid domains, or "rafts" (Simons and Ikonen, 1997; Ledesma et al., 1998) appear to serve as sorting platforms for apically directed proteins.

Studies analyzing molecular mechanisms of protein targeting in neurons have been rare owing to technical difficulties. However, recent studies using cultured neurons and viral infec-

Table 2
Glutamate Receptor-Interacting Protein

Receptor/subunit	Interacting protein	Reference
NMDA		
NR1	α-actinin	Wyszynski et al. (1997)
	Neurofilament-L	Lin et al. (1998)
	Yotiao	Ehlers et al. (1996)
	Spectrin	Wechsler and Teichberg (1998)
NR2	PSD95/SAP90	Kornau et al. (1995)
	PSD93/Chapsin 110	Kim et al. (1996)
	SAP102	Muller et al. (1996)
	CIPP	Kurschner et al. (1998)
	Spectrin	Wechsler and Teichberg (1998)
AMPA		
GluR1	SAP97	Leonard et al. (1998)
	Calnexin	Rubio and Wenthold (1999b)
	BiP	Rubio and Wenthold (1999b)
GluR2, 3	Grip1, 2	Dong et al. (1997, 1999)
	NSF	Nishimune et al. (1998), Osten et al. (1998)
	Calnexin	Rubio and Wenthold (1999b)
	BiP	Rubio and Wenthold (1999b)
GluR2, 4C	ABP	Srivastava et al. (1998)
GluR2, 3, 4C	PICK	Xia et al. (1999)
Kainate	PSD95/SAP90	Garcia et al. (1998)
Metabotropic		
MGluR1α, 5	Homer	Brakeman et al. (1997)
Delta	PSD93/Chapsin110	Roche et al. (1999)

tion have elucidated specific signals implicated in neuronal targeting of proteins including glutamate receptors. Jareb and Banker (1998) confirmed the hypothesis proposed by Dotti and Simons (1990) showing that there are some mechanisms of protein targeting in common between epithelial cells and neurons. They reported that some basolateral targeting signals such as those in the polyimmunoglobulin receptor and the low-density lipoprotein (LDL) receptor also mediate targeting to the somatodendritic surface. However, in other proteins such as the transferrin receptor, dendritic targeting is mediated by a signal distinct from the basolateral targeting, indicating that neurons may have additional mechanisms for receptor targeting.

The C-terminus of some types of glutamate receptors has been reported to contain signals implicated in targeting (Stowell and Craig, 1998; Okabe et al., 1999). Using hippocampal cell cultures and virus expression, the axon/dendrite targeting signals of mGluRs (Stowell and Craig, 1998) were analyzed. First, it was shown that group I mGluR1α and group II mGluR2 metabotropic receptors were targeted to dendrites and excluded from axons, whereas group III mGluR7 was targeted to axons and dendrites of transfected cells. Analysis of chimeric constructs showed that the differential axon targeting vs axon exclusion of mGluR7 vs mGluR2 was dependent on approx 60 C-terminal amino acids primarily, but not exclusively on the distal 30 amino acids. Both C-terminal regions contained essential targeting signals; mGluR2 C-terminus was required for axon exclusion, and mGluR7 C-terminus was required for axon targeting of the native protein. At the same time,

mGluR7 C-terminus was shown to be dominant and could induce axonal targeting of full-length mGluR2, whether inserted at the extreme C-terminus or proximal to the membrane. Thus, the mGluR7 C-terminus can be considered as the first identified cytoplasmic axon targeting signal and may be generally useful for redirecting any dendritic membrane protein to axons (Stowell and Craig, 1998). The EVH domain proteins of the Homer/Vesl family bind to the motif PPXXFR, which is present within the C-terminus of mGluR1α and mGluR5 but not in mGluR2 or mGluR7 (Brakeman et al., 1997; Kato et al., 1998; Tu et al., 1998). Whether Homer/Vesl has any function in subcellular targeting, and whether there are related proteins that bind to other mGluR groups, is still not known. Interestingly, these targeting regions of mGluRs differ from the region involved in G-protein coupling (the second intracellular loop). The cytoplasmic axon targeting signal of mGluR7 is also distinct from the axon targeting signal of the other neuronal membrane proteins. These data suggest the existence of different specific signal sequences in the other members of the glutamate receptor family. However, to date these signal sequences are still unknown.

Role of the Cytoskeleton and Associated Proteins in Receptor Targeting

We have discussed the different steps that may contribute to the localization of glutamate receptors at the synapse. We described the association of intracellular glutamate receptors with tubulovesicular membranes of the ER and the putative role of these membranes in receptor transport. Neurons also posses microtubules, which are dynamic polymers made up of tubulin subunits. The microtubules provide architectural support to eukaryotic cells, and act as railways along which cytoplasmic components are actively transported. Microtubules have intrinsic polarity. Axons contain plus-end oriented microtubules, and dendrites have mixed microtubule polarity, and in most distal dendrites, plus-end oriented microtubules (Baas et al., 1988). Certain cytoplasmic components are transported preferentially toward the plus-end of the microtubule, whereas others are transported preferentially toward the minus-end (Fig. 2). In neurons, the majority of long-distance organelle transport events in axons and dendrites are thought to be achieved by the active movements of microtubule-associated motor proteins, such as kinesins and dyneins, along microtubule tracks (Hirokawa 1998; Foletti et al., 1999). Motor proteins that are plus-end directed transport organelles throughout the axon in an anterograde manner, whereas motor proteins that are minus-end directed transport organelles in a retrograde manner. It has been assumed that only minus-end oriented kinesins can enter into dendrites because they are excluded from axons. However, KIF21B a plus-end oriented motor protein, has been also detected in dendrites (Marszalek et al., 1999). This can be explained if inactivated KIF21B is transported by a minus-end motor, where it would remain sequesterd until needed or alternatively stability of KIF21B is differentially regulated in axonal vs dendritic processes. Thus, the factors that can be involved in the movement of organelle and associated proteins in dendrites may include (Fig. 2): (1) the mixed orientation of microtubules in dendrites that support organelle transport (Overly et al., 1996; Baas et al., 1988); (2) motor proteins of the kinesin family (*see* Note Added in Proof), such as KIFC2 and KIF21B, that have been identified in dendrites (Marszalek et al. 1999), and (3) the observation in vitro that organelles can reverse direction by changing motor protein activation or association, or by switching to another microtubule (Brady et al., 1982; Smith and Forman, 1988). Myosins, which are involved in organelle transport in many systems, are also found in dendrites, and may play a role in dendritic protein movement (Mermall et al., 1998). There is recent evidence showing that Myosin V activity is not necessary for long-range transport in axons. Yet, Myosin

Va activity appears to be necessary for local movement and processing of organelles in regions, such as presynaptic terminals that lack microtubules (Bridgman, 1999). Therefore, Myosin Va may play a role in local movement of proteins also in dendrites, such as receptor insertion at the postsynaptic plasma membrane.

Are Dendrites Involved in Glutamate Receptor Synthesis?

Dendrites possess functional elements equivalent to the endoplasmic reticulum, Golgi, and trans-Golgi network. In recent years, a variety of mRNA species have been detected in neuronal processes indicating that a decentralized translation machinery might be also operative, at least in dendrites that posses protein-synthetizing capacity (Steward, 1994). Hence, some types of glutamate receptors may be synthesized and assembled within the dendrite, near the synapse. The first mRNA identified in dendrites was the transcript encoding the microtubule-associated protein (MAP) 2, a marker of the dendritic cytoskeleton. Until now, a variety of mRNA transcripts (Table 3) have been reported in this location (*see* reviews by Mohr, 1999; Schumann, 1999). However, as noted earlier, most evidence supports a cell-body localization of glutamate receptor mRNA. Nevertheless, by reverse-transcription PCR (RT-PCR) on material isolated from individual dendritic segments of culture hippocampal neurons, mRNA encoding various glutamate receptors has been amplified (Miyashiro et al., 1994). It is unclear, however, whether the presence of mRNA transcript is functionally significant (*see* discussion by Steward, 1994, 1997). Gazzaley and colleagues (1997) also reported an upregulation of NRI mRNA in dendrites of cultured hippocampal neurons, and possibly in dendrites in sections from the dentate gyrus molecular layer following perforant transection. They suggested that NRI mRNA normally exists at very low levels in dendrites and can be upregulated during denervation-induced synaptic plasticity. Until now, these are the only data supporting the presence of glutamate receptor mRNA in dendrites, and they are in disagreement with numerous studies done in vivo (Eshhar et al., 1993; Hunter et al., 1993; Lauri and Seeburg, 1994; Bahn and Wisden, 1997). Future studies are needed to resolve this discrepancy.

Summary

In summary, it can be hypothesized that the newly synthesized glutamate receptors possess specific signal sequences that might sort them to, or exclude them from, a particular neuronal compartment (e.g. axon, dendrite, apical vs basal dendrites). Then receptors might be transported by intracellular organelles or tubulovesicular membranes of the ER. These intracellular membranes transporting receptors are the cargo of specific motor proteins (kinesin and kinesin-like family proteins) associated with the cytoskeleton. Thus, motor proteins move receptors assembled in vesicular membranes along microtubules throughout dendrites. Once the receptors arrived at the target synapse, they are incorporated into the plasma membrane (Fig. 2).

Note Added in Proof: Very recently it has been shown that the NR2B subunit of the NMDA receptor is transported along microtubules by KIF17, a neuron-specific molecular motor in neuronal dendrites (Seton et al., 2000).

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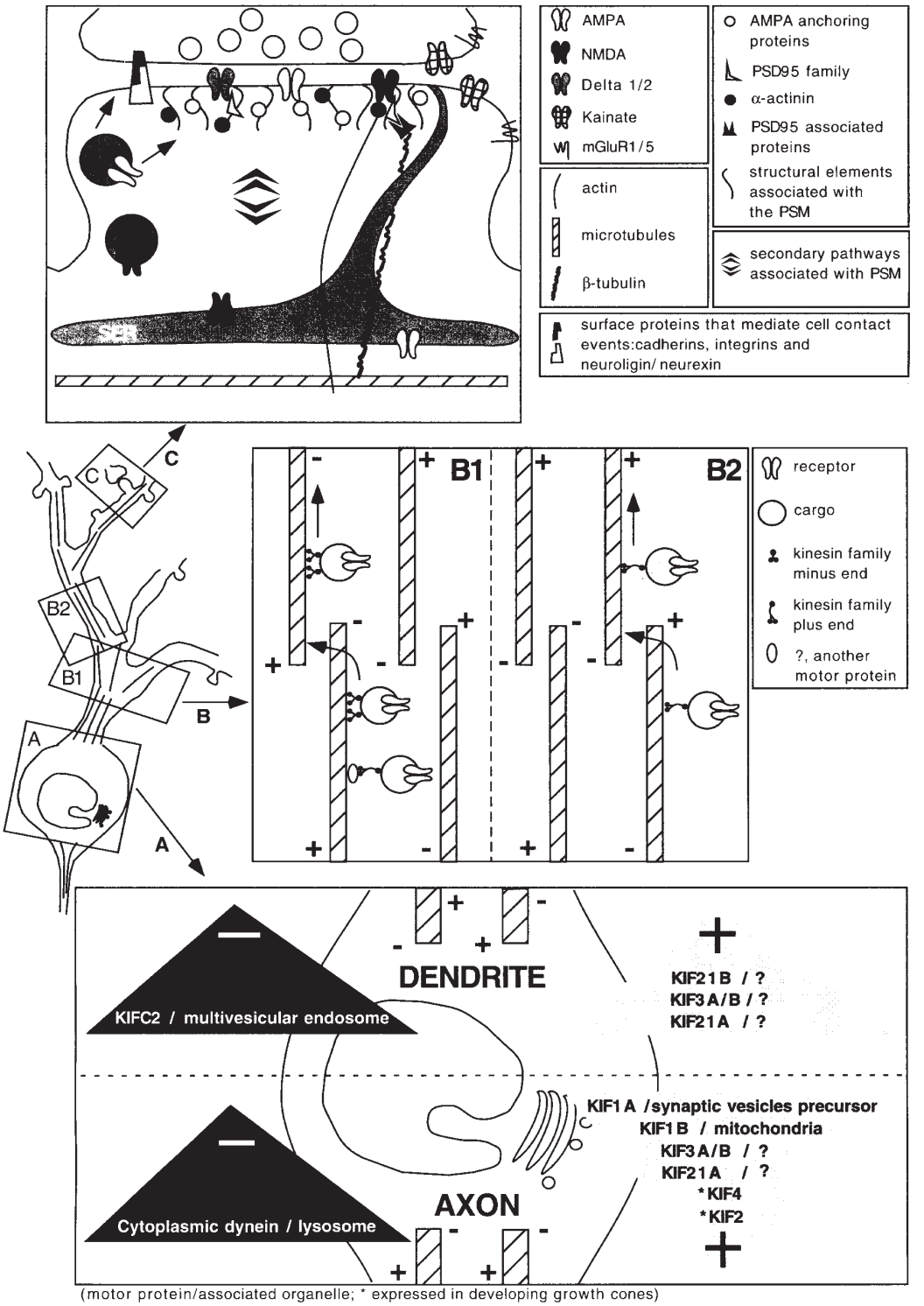

(motor protein/associated organelle; * expressed in developing growth cones)

Fig. 2. Multiple elements that may be involved in glutamate receptor targeting. **(A)** The cell body showing the difference of microtubule polarity between dendrites and axons. Also the family of kinesin motor proteins with their cargo and their orientation to axon dendrites are shown. **(B)** (1 Proximal dendritic segment with mixed microtubule polarity. In these segments minus-end oriented motor proteins can transport the cargo organelles and the corresponding proteins towards more distal dendritic segments. (2 Distal dendritic segment where microtubules are plus-end oriented. Also, the putative movement of plus-end directed kinesins and their cargo throughout the dendrite is represented. **(C)** Excitatory dendritic spine with all the elements that have been implicated in synapse formation, including glutamate receptors, postsynaptic membrane elements, associated proteins, cytoskeleton, and smooth endoplasmic reticulum (SER).

Table 3
RNAs Located in Dendrites

Dendritic RNAs	Tissue	Reference
Arc/Arg3.1	Hippocampus	Lyford et al. (1995); Link et al. (1995)
BC1	Hippocampus	Tiedge et al. (1991)
BDNF	Hippocampus	Dugich-Djordjevic et al. (1992)
CaMKIIα	Hippocampus	Burgin et al. (1990)
Dendrin	Forebrain	Herb et al. (1990)
GAP 43	Brain	Landry et al. (1994)
Glycine receptor α-subunit	Spinal cord	Racca et al. (1997)
Glutamate receptors	Hippocampus	Myashiro et al. (1994); Gazzaley et al. (1997)
InsP3 receptor	Cerebellum	Furuichi et al. (1993)
MAP2	Hippocampus	Garner et al. (1988)
Neurogranin RC3	Brain	Landry et al. (1994)
Oxytocin	Hypothalamus	Mohr et al. (1995)
Pcp-2/L7	Cerebellum	Bian et al. (1996)
Vasopressin	Hypothalamus	Mohr et al. (1995)

Arc, activity-regulated cytoskeleton-associated protein; Arg, activity-regulated gene; BC, brain cytosolic; BDNF, brain-derived neurotrophic factor; CaMKIIα, α-subunit of the Ca^{2+} calmodulin-dependent protein kinase II; GAP, growth-associated protein; InsP3, inositol trisphosphate; MAP, microtubule-associated protein; pcp, Purkinje cell protein.

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