Annu. Rev. Pharmacol. Toxicol. 2003. 43:335–58
doi: 10.1146/annurev.pharmtox.43.100901.135803

TRAFFICKING OF NMDA RECEPTORS[1]

Robert J. Wenthold, Kate Prybylowski, Steve Standley, Nathalie Sans, and Ronald S. Petralia
Laboratory of Neurochemistry, NIDCD, NIH, Bethesda, Maryland 20892; email: wenthold@nidcd.nih.gov

Key Words PDZ proteins, synapse, postsynaptic density, glutamate receptor, subunit assembly

■ **Abstract** The NMDA receptor (NMDAR) plays a central role in the function of excitatory synapses. Recent studies have provided interesting insights into several aspects of the trafficking of this receptor in neurons. The NMDAR is not a static resident of the synapse. Rather, the number and composition of synaptic NMDARs can be modulated by several factors. The interaction of PDZ proteins, generally thought to occur at the synapse, appears to occur early in the secretory pathway; this interaction may play a role in the assembly of the receptor complex and its exit from the endoplasmic reticulum. This review addresses recent advances in our understanding of NMDAR trafficking and its synaptic delivery and maintenance.

INTRODUCTION

Since its identification in the late 1970s, the NMDA receptor (NMDAR) has been the object of an intense and diverse research effort, which has implicated this receptor in multiple neuronal functions ranging from synapse formation to ischemic damage to learning and memory. The demonstration in 1983 that antagonists of the NMDAR could block long-term potentiation (LTP) in the hippocampus elevated the NMDAR to the role of a key player in synaptic plasticity (1). This role was supported by studies showing the voltage dependency of the magnesium block and calcium permeability of the channel (2, 3). With the cloning of the NMDAR subunits, first NR1 in 1991 (4), the NR2 subunits in 1992 (5, 6), and the NR3 subunit in 1995 (7), the molecular bases for these functional properties could be defined. However, our understanding of the cell biology of the NMDAR has lagged, and questions concerning the assembly of the receptor complex, its trafficking in the neuron, and the mechanisms controlling its addition to and removal from the synapse have only recently been addressed. Its companion glutamate receptor, the AMPA receptor, has been shown to be added to and removed from the synapse

[1]The U.S. Government has the right to retain a nonexclusive, royalty-free license in and to any copyright covering this paper.

based on activity, providing a possible molecular mechanism for LTP and long-term depression (LTD), which are cellular models of learning and memory (8). Although the NMDAR has been viewed as a more stable component of the synapse, recent data indicate that the number of receptors can be changed rapidly by internalization (9–12). NMDARs are present at synapses early in development, and the addition of AMPA receptors is dependent on the activity of NMDARs, forming the basis of the "silent synapse hypothesis" (13). These studies point to the central role of trafficking in the regulation of functional NMDARs and provide a clear incentive to understand the mechanisms underlying these events.

STRUCTURE, SUBUNIT COMPOSITION, AND FUNCTIONAL PROPERTIES OF NMDA RECEPTORS

Subunit Assembly

To form a functional receptor complex, the NR1 subunit must assemble with one of the four NR2 subunits (NR2A, NR2B, NR2C, NR2D) into what is generally thought to be a tetrameric complex. The NMDA receptor is unique among ligand-gated ion channels in its requirement for two agonists, glutamate and glycine. The NR1 and NR2 subunits contain the glycine and glutamate binding sites, respectively. There are three sites of alternative splicing in the NR1 subunit, one in the N terminus and two in the C terminus, resulting in a total of eight possible splice variants. The C-terminal splice variant compositions affect the early trafficking of this subunit and are discussed in detail later. In addition, the NR3 subunit assembles with NR1 and NR2, resulting in a receptor with diminished activity (14), and NR3 subunits can assemble with NR1 alone to create a functional glycine receptor (15). However, it appears that most NMDARs are made up of an NR1 subunit and one or more NR2 subunits. The NR2A and 2B subunits are the major and most widespread NR2 subunits, with NR2C largely restricted to the cerebellum and NR2D most heavily expressed early in development. The NR2B subunit predominates early in development and then gradually decreases, whereas expression of NR2A is low shortly after birth but continues to increase. Therefore, NR2B is the major subunit during the early period of a neuron's life, whereas NR2A is predominant in the later stages, suggesting that the NR2B to 2A switch is responsible for the transition of a synapse from a more plastic to a less plastic state. The importance of having the correct number and composition of NMDAR subunits is demonstrated by genetic studies. Targeted disruption of the NR2A subunit produces mice with a reduction in LTP and deficiencies in some learning tests (16). Animals with enhanced production of NR2B show superior ability in learning and memory in behavioral tasks (17), whereas a dramatic decrease in the amount of NR1 produces animals with symptoms characteristic of schizophrenia (18).

Neurons have a large excess of the NR1 subunit relative to the NR2 subunits; this pool of NR1 is unassembled with NR2, exists primarily as a monomer, does not reach the cell surface, and is rapidly degraded if it does not assemble (19, 20).

This suggests that the availability of the NR2 subunits is the limiting factor in production of functional receptors. This seemingly inefficient mechanism, in which most of the NR1 is never used to produce a functional receptor, stresses the critical importance of mechanisms that regulate rapid changes in availability of NR2 subunits. In cortical neurons of four-week-old rats, three types of NMDARs were identified: those made up of NR1/NR2A, NR1/NR2B, and NR1/NR2A/NR2B (21). Because these receptor complexes have different functional characteristics, their localization and ontogeny could dramatically affect the neuron's response to synaptic stimulation. It is not known if the assembly process is simply based on the availability of subunits or regulated in some fashion, but based on similar data on AMPA receptors, it is suggested that the assembly process is regulated and may vary with cell type and stage of development (22).

Proteins that Interact with NMDA Receptors

The targeting of the receptor to the synapse and its localization at the synapse depend on a series of interactions with other proteins. Many of these interactions may involve the receptor's carboxy terminal domain, which is located in the cytoplasm (Figure 1), and are likely candidates to influence the trafficking of the receptor. The carboxy terminal domains are large compared to those of other ionotropic glutamate receptors. For example, the NR2A subunit has 627 amino acids and the NR1-1 subunit has 105 amino acids. The first yeast two-hybrid screen with the NR2 C-terminal tail identified PSD-95, a highly abundant protein in the postsynaptic density (PSD), as an interacting protein that associates with the last four amino acids of the NR2 subunit (23). Additional members of the PSD-95 family were identified and include SAP102, SAP97, and PSD-93 [see (24) for review]. Like PSD-95, they all have three PDZ domains, one SH3 domain, and one guanylate kinase (GK) domain. Proteins in this family are referred to as membrane-associated guanylate kinases (MAGUKs) and are proposed to anchor NMDARs at the synapse. The NR2 subunits have been shown to bind to the first two PDZ domains of PSD-95, PSD-93, and SAP102 (24). The NR1 splice variant, NR1-4, contains a consensus PDZ interacting domain that interacts with all these MAGUKs (25).

Although the PDZ interacting domain of NR2 subunits has received most of the attention, a number of other proteins have been shown to interact with both the NR1 and NR2 subunits (Figure 1), and this number will certainly grow. For example, recently two sites on the NR2B tail, one near TM4 and the other near the C terminus, have been shown to be involved in clathrin-mediated internalization of the receptor (9, 10). These are consensus sequences for binding to AP-2 adaptor proteins that initiate internalization of membrane proteins. In addition, a number of phosphorylation sites that affect the function or trafficking of the receptor have been identified on the C terminus. The roles of these sites are discussed in more detail later. In addition, although the C terminus is critical for mediating many protein-protein interactions, co-assembly of subunits into a complex has been

shown to involve interactions of the N terminus of NR1 and NR2 subunits (26), and interactions of NMDARs with the EphB receptor appear to be mediated by the N terminus of NR1 (27).

PROCESSING OF NMDA RECEPTORS IN INTRACELLULAR COMPARTMENTS

Because both NR1 and NR2 subunits assemble together to form a functional receptor complex, a mechanism must exist to ensure that only complexes that contain the proper combination of both subunits are allowed to reach the cell surface. For other complex proteins, this is often achieved by retaining the unassembled subunits in the endoplasmic reticulum (ER); a similar mechanism appears to play a role in NMDAR assembly.

Subunit Assembly and Quality Control in the Endoplasmic Reticulum

Newly synthesized proteins undergo essential folding, maturation, and oligomerization in the ER. The ER uses a rigorous quality control process to ensure that both lumenal and transmembrane proteins conform to their proper states before exiting. Thus, unfolded, misfolded, and unassembled proteins can be retained in the ER by molecular chaperones [see (28) for review]. Although some multimeric proteins require only chaperone-based quality control for proper oligomerization, many transmembrane proteins contain a short amino sequence on their cytoplasmic or ER lumenal domains that acts as an ER retention signal. ER retention signals were first defined in single transmembrane domain Type-I transmembrane proteins (N terminus in the ER lumen and C terminus in the cytoplasm) that form heteromultimers and Type-II transmembrane proteins (C terminus in the ER lumen and N terminus in the cytoplasm) [see (29) for review]. Different motifs are distinguished as functional in either Type I (KKXX-COOH; di-lysine motif), or Type II (NH_2-XXRR; di-arginine, and KDEL/HDEL-COOH) proteins. Detailed analyses of several proteins that contain retention signals and form complexes that are destined for the plasma membrane have shown that ER exit is achieved by steric masking of retention signals. Assembly of the subunits, therefore, results in masking of the retention signals and exit from the ER.

Recently, another ER retention signal, RXR, has been defined in multitransmembrane domain proteins. In this case, X is less well defined, but can be any basic and some neutral amino acids depending on the protein. RXR retention motifs have been characterized in a number of different proteins, the first being the ATP-sensitive potassium channel (K_{ATP}) (30). Fully assembled channels contain eight subunits; four core α-subunits (Kir 6.1/6.2) that form the pore and four β-auxiliary subunits (SUR1). All of the subunits contain the RXR ER retention motif, and through analysis of mutants and surface expression in *Xenopus* oocytes, Zerangue et al. (30) were able to demonstrate an even more complex interplay between

subunits and steric blockade of ER retention. ER retention signals on core α-subunits require the β-auxiliary subunits for steric masking. α-Subunits do not reciprocate by blocking the β-subunit's signal. Rather, individual β-subunits occlude the α-subunit and contribute to masking of the neighboring β-subunit. This sets up exquisite criteria for ER exit; only fully assembled octamers are able to pass quality control by ER retention. If α- and β-subunits were capable of mutual retention blockade, one might imagine that individual α- and β-subunits could pass quality control by this mechanism. Zerangue et al. (30) discovered that by mutating all of the retention signals in every subunit, functional channels were still formed. The mutant channels showed higher basal responses that were insensitive to modulation by ATP. However, the channels were expressed in greater abundance on the surface. Considering the investment that cells put into regulating trafficking via ER retention, these signals might have the additional role of reducing cell-surface expression and creating a larger intracellular pool that could act as a buffer to meet a wide range of demands for cell-surface expression. Thus, the presence of ER retention motifs may not only reduce surface expression, but also may allow fine regulation of the number of proteins delivered to the cell surface.

$GABA_B$ receptors possess distinctly different subunits, GB1 and GB2, that are required for functional expression of the $GABA_B$ complex. The GB1 subunit has a retention signal similar to the RXR (RXR[R]) (31). The GB2 has no ER retention signal and is expressed on the surface in heterologous cells, but it does not seem to form a functional receptor in the absence of GB1. Classical steric masking through a proximal coiled-coil domain in each cytoplasmic tail has been identified as the mechanism for blocking the retention signal on GB1 (31). GB2 does not appear to have a function independent of GB1, as no GB2 homodimers can be found on the neurons (32). Supposing that GB2's synthesis is rate-limiting to expression of GB1, GB1 retention acts as a "checkpoint" along the secretory pathway for normal assembly (31). Essentially, GB1 proteins could remain in ER-residence and become associated with any newly synthesized GB2.

ER Retention of the NR1 Subunit

NR2 subunits do not form functional receptors when expressed alone in heterologous cells (5), and those that have been studied (NR2A and NR2B) do not reach the cell surface (33). NR1 subunits also do not form functional receptors alone, but their cell surface expression depends on the splice variant. As mentioned above, there are eight splice variants of NR1, and four of these are generated by alternative splicing of the C terminus. The NR1 C terminus (Figure 1) is made up of four cassettes. C0 is present in all splice variants. C1 is optional and is present in four of the eight splice variants. Alternative splicing also determines if a C2 or C2′ cassette is present, and each is present in four of the eight variants. The most abundant variant contains C0, C1, and C2. This variant, NR1-1, does not reach the cell surface when expressed without NR2 in heterologous cells and is retained in the ER. NR1 with the other three combinations of C termini will all reach the

cell surface. The ER retention of NR1 is dependent on an RXR motif in one of the alternatively expressed cassettes, C1 (25, 34, 35).

Although the ER retention of NR1-1 (C1C2) and the cell surface expression of NR1-2 (C2) and NR1-4 (C2′) are clearly explained by the presence of the ER retention motif in C1, it does not explain the surface expression of NR1-3 (C1C2′). It suggests that another signal, present in C2′, can negate the RXR retention signal in C1. This signal was shown to be the PDZ-binding domain at the distal end of C2′ (STVV). This could result from masking of the RXR motif by the PDZ proteins that interact with the C2′ cassette, or the PDZ protein could be acting as an export protein that simply overrides the ER retention. The last six amino acids of C2′ are sufficient to suppress ER retention, as shown by deleting the rest of C2′, but experiments to lengthen the distance between the RXR motif and the PDZ binding domain remain to be done. Such experiments may help determine the mechanism by which the retention is negated. Soluble fusion proteins containing the PDZ interacting domain of C2′ can effectively block the surface expression of NR1-4, showing that saturation of the trafficking pathway leads to an intracellular buildup of NR1-4 (36). Subunits that lack the C2′ cassette are not affected by this treatment. In addition to providing an interesting mechanism to overcome ER retention, these results show that the PDZ protein can interact early in the secretory pathway in addition to serving as an anchor at the synapse.

The functional significance of the multiple C-terminal configurations of NR1 and their various behaviors in the ER remain unclear. Homomeric NR1 has not been detected on the surface of neurons. It is interesting that NR1 can assemble with NR3 to produce functional glycine receptors; this assembly is also sufficient to relieve the ER retention of NR1 (37).

Assembly Releases ER Retention of NR1 and NR2 Subunits

The NR2 subunit is retained in the ER, and like NR1, its retention appears to depend on a signal in the C terminus. The mechanism by which assembly overcomes ER retention of both subunits is not clear. Cytoplasmic tails of each subunit type have been truncated in a number of experiments in different preparations; the sum of the observations do not support simple masking of the ER retention signal via complementary cytoplasmic tails (10, 38–40). With the entire NR1 C-terminal domain truncated, functional heteromeric receptors are expressed on the cell surface (10, 39, 40). Similar results are obtained when NR2 C-terminal domains are truncated. For example, mice lacking nearly all of the C-terminal cytoplasmic domain of NR2A still have functional NMDARs containing this subunit, although they fail to be appropriately clustered at the synapse (41). If complementary masking is not involved, a number of other possibilities must be considered. First, like subunits may mask each other. The argument against this is that dimerization of NR1 subunits is believed to be an early step in assembly; in heterologous cells, NR1-1 can form dimers in the absence of NR2, but they are still retained in the ER (26). Thus, masking of their retention signals does not occur. However co-expression of

NR1-1 and NR1-4 resulted in a limited surface expression of NR1-1, showing that the subunits interacted and in some way negated the ER retention of NR1-1 (42). Second, NMDAR C termini may undergo sustained conformational changes upon proper quaternary folding, acting as a "folding sensor" that occludes the motifs. Finally, an unknown protein may interact with one or both cytoplasmic tails and thereby suppress ER retention, as PDZ proteins do for the NR1-3 variant. This may or may not involve masking of the retention signals.

Trafficking of NMDA Receptors from the ER to the Plasma Membrane

After they are released from the ER, membrane proteins such as the NMDAR are further processed in the Golgi apparatus and then distributed to the *trans* Golgi network (TGN) and endosomes. Because functional ER and Golgi complexes are present in many dendrites and even dendritic spines, it is possible that some of the processing related to these compartments actually occurs near the synapse. This would provide a mechanism for a rapid and local response. Most receptors, however, are likely processed in the cell body and then transported to the synapse. In determining the function of a novel kinesin (KIF17), Setou et al. (43) found KIF17 to be indirectly associated with NMDARs. Their data suggest that a complex of mLin-7, mLin-2, and mLin-10 associates with a cargo vesicle containing NMDARs and with KIF17, which transports it along microtubules in dendrites to the synapse. Little is known about the nature or formation of these vesicles that contain the newly processed receptors. Because it would require creating a large number of discrete vesicles, it is unlikely that each type of receptor is parceled into its own vesicle population, but whether or not the packaging follows any particular organization remains to be determined.

Several findings suggest that the NMDAR interacts with PDZ proteins before it reaches the synapses and that these interactions may be involved in the synaptic delivery (25, 43). A yeast two-hybrid analysis showed that one of the PDZ partners of the NR2 subunit, SAP102, interacts through its PDZ domains with sec8, a protein of the exocyst or sec6/8 complex (44). The exocyst is a complex of eight proteins first identified in yeast and later in mammals that was shown to be involved in targeting of secretory vesicles to the plasma membrane (45). The initial site of interaction is unclear, with some studies indicating the ER and others the Golgi or TGN (45–47); the site of interaction may vary with the cell type or particular protein. The fact that SAP102 interacts with sec8 suggests that the NMDAR may indirectly associate with the exocyst complex through SAP102. Several studies, including functional analyses, confirm that the NMDAR, SAP102, and sec8 form a complex in the brain and suggest that formation of this complex is involved in the synaptic delivery of NMDARs. Receptors without the PDZ interacting domain bypass the exocyst interaction and reach the cell surface. This raises the possibility that there are two mechanisms for delivery of NMDARs to the cell surface, one which involves the PDZ interacting domain and one that does not.

REGULATION OF NMDA RECEPTORS AT THE SYNAPSE

Many factors could conceivably influence the number and composition of NMDA receptors at the synapse. These include the availability of receptors, either in an intracellular pool or a nonsynaptic surface pool, the stability of receptors at the synapse, and the removal of receptors from the synapse either through endocytosis or diffusion in the plasma membrane.

NMDA Receptors and Organization of the Synapse

NMDARs are found at both synaptic and extrasynaptic sites, but are present at a much higher density at the synapse. This clustering at the synapse is believed to involve an interaction of the receptor with proteins that are part of the PSD. Identification of the interacting proteins and how these interactions are regulated is central to understanding the trafficking of NMDARs at the synapse.

The PSD is an electron-dense organelle that is localized along the postsynaptic membrane of excitatory synapses. A major component of the PSD is the PSD-95 family of MAGUKs (PSD-95, PSD-93, SAP97, and SAP102) (24) (Figure 2). Through their PDZ domains, these proteins can bind directly to NMDARs (at least two other NMDAR-associated proteins, S-SCAM and CIPP, have been reported) (48). From this basic scaffold, other proteins can bind in chains that link the NMDAR to the other glutamate receptors and to ion channels in the postsynaptic reticulum. For example, GKAP can bind to the GK-like domain that is found in MAGUKs and S-SCAM (49); GKAP then can bind to Shank, which can bind to dimers of Homer that bind to type 1 metabotropic glutamate receptors in the postsynaptic or adjacent perisynaptic membrane. Shank also can bind to Homer dimers that bind to inositol 1,4,5-triphosphate (IP3) receptors in extensions of the reticulum that lie subjacent to the PSD (24, 50). Another chain of proteins can be formed between NMDARs and AMPA receptors via a combination of calcium/calmodulin-dependent protein kinase II (CaMKII) and an AMPA receptor channel anchoring an assembly of proteins, which consists of actinin, actin, 4.1N protein, and SAP97/GluR1 [see (51) for review]. An additional method of linking NMDA and AMPA receptors is through PSD-95 and other MAGUKs via Stargazin, which binds to the PDZ domains of the MAGUKs and mediates synaptic targeting of AMPA receptors (52). Finally, the postsynaptic complex is linked to actin filaments, which control the overall structure of the postsynaptic spine and may form pathways for transport of proteins to and from the postsynaptic membrane. At least three such kinds of connections exist in addition to the one associated with GluR1: actinin-actin-NMDARs, GKAP-Shank-cortactin-actin, and PSD-95-SPAR-actin [see (53) for review]. SPAR is a Rap-specific GTPase-activating protein; the latter proteins are implicated in regulation of MAP kinase cascades, cell adhesion, and activation of integrins. Like GKAP, SPAR can bind to the GK domain of PSD-95 (54). It regulates spine morphology, both through its direct interaction with F-actin and also probably via Rap

signaling. Nevertheless, whereas actin-protein associations play significant roles in synaptic structure and function, anchoring of NMDAR/PSD-95 complexes at synapses appears to be independent of actin associations (55).

Many other proteins associate with the NMDAR complex. For example, at least two proteins that may regulate the MAP kinase pathway can bind to the PDZ domains of the major scaffolding proteins: SynGAP (synaptic Ras-GTPase activating protein) binds to PSD-95 (56, 57) and nRap GEP (neural GDP/GTP exchange protein for Rap1 small G-protein) binds to S-SCAM (58). MAGUIN, which binds to both PSD-95 and S-SCAM, also may regulate the MAP kinase pathway (59). SynGAP is believed to maintain a low steady-state level of active Ras near the synapse by catalyzing rapid hydrolysis of Ras-GTP to Ras-GDP. Calcium entry through NMDARs activates CaMKII, which can inactivate SynGAP via phosphorylation. Without the influence of SynGAP, Ras-GTP accumulates and increases the activation of the MAP kinase cascade, which is associated with long-term potentiation of synaptic function. Another PSD-95-associated protein, Citron, is limited to certain populations of neurons in the brain, and is a target of Rho, which is a small GTPase that regulates actin cytoskeleton organization (60, 61). Citron may mediate forms of NMDAR-dependent synaptic plasticity that are limited to certain specialized groups of neurons.

Finally, pre- and postsynaptic components of glutamatergic synapses can be linked by proteins, including neuroligin/neurexin complexes, L1, and cadherins (48). Neuroligin/neurexin complexes cross the synaptic cleft and connect to other PDZ domain-containing proteins in the presynaptic terminal. Cadherins dimerize in the synaptic cleft and link via catenins to the actin cytoskeleton. Both cadherins and catenins are part of the NMDA receptor complex (62). This dimerization of cadherin is linked directly to NMDAR activation, so that the stability of the synaptic contact may be regulated by NMDARs (63). Interestingly, NMDARs plus PSD-95 also are found in cadherin-based attachment plaques in cerebellar glomeruli, suggesting that the overall stability of the glomerulus is controlled similarly, in this case, via glutamate spillover from adjacent synapses in the glomerulus (64).

Kinase/Phosphatase Interactions with NMDA Receptors

Phosphorylation of NMDARs produces a wide variety of effects under various experimental conditions [see (65) for review], suggesting that this may be a major mechanism for regulating receptor trafficking at the synapse. In some cases, the effect may be directly on the ion channel. In this section, we limit the discussion to effects that alter the distribution of the NMDAR or its interaction with other proteins.

CaMKII can associate with NR1, NR2A, and NR2B. Binding of CaMKII to NR2A and NR2B does not involve the direct binding of CaMKII to its major phosphorylation site on the NMDAR subunit—serine 1289 for NR2A (66) or serine 1303 for NR2B (67). Association of CaMKII with NMDARs is believed to occur following autophosphorylation of CaMKII due to calcium entry from activated

NMDARs and induction of LTP. It is believed that this association between CaMKII and NMDARs brings CaMKII into close proximity with AMPA receptors. Subsequent phosphorylation of AMPA receptors results in potentiation of the synapse by inducing synaptic insertion and increasing single-channel conductance of AMPA receptors (67, 68). However, autophosphorylation of CAMKII (via T286) is not necessary for binding to NR2B. In fact, following stimulation by calcium and calmodulin, CaMKII can bind to NR2B (68). NR2B then regulates the function of the bound CaMKII, putting it in an autonomous, calmodulin-trapping state that cannot be reversed by phosphatases, and suppresses inhibitory autophosphorylation of T305/306, thus preventing dissociation of the CaMKII from the synapse. This association may also induce autophosphorylation of neighboring CaMKII molecules. CaMKII forms stable complexes with NR1 and NR2B but not with NR2A; stimulation of NMDARs increases this association (67). Another study shows that CaMKII and PSD-95 compete for binding to NR2A in a hippocampal PSD preparation (66). They also show that in LTP-potentiated hippocampal slices, both CaMKII-dependent activity and CaMKII association with NR2A/B (ratio of CaMKII/PSD-95 after NR2A/B precipitation) increases, with a concomitant decrease in association between PSD-95 and NR2A/B. Clearly, there are complex relationships of CaMKII with NMDARs and these vary even with different NR2 subunits.

Studies on the effects of protein kinase C (PKC) on NMDARs have yielded conflicting results, probably because PKC has multiple effects depending on cell type, sites of action, and variable associations of NMDARs with other proteins. Liao et al. (69) found that PSD-95 has a profound influence on insulin/PKC and Src potentiation of currents of NMDARs expressed in *Xenopus* oocytes. Potentiation of NR1/NR2A currents by Src requires co-expression of PSD-95. In contrast, PSD-95 co-expression eliminates insulin—(probably via PKC) or phorbol ester (PE; activates PKC)—mediated potentiation of NR1/NR2A currents. Furthermore, both of these responses differ from those involving NR1/NR2B currents, which are potentiated by insulin or PE with or without co-expression of PSD-95 (Src failed to potentiate these currents in either case). The effect of PKC on NR1/NR2A receptors in *Xenopus* oocytes involves an increase in NMDAR channel opening rate and delivery of new NMDAR channels to the surface through regulated exocytosis (70). PKC activation of NMDARs also influences the association of CaMKII with NMDARs. PKC-dependent phosphorylation [due to stimulation with PE or the metabotropic glutamate receptor (mGluR)-specific agonist, t-ACPD] of NR2A at serine 1416 inhibits CaMKII binding, thus promoting the dissociation of the CaMKII-NR2A complex (71). In addition, Fong et al. (72) found that PE activation of PKC induces translocation of CaMKII to synapses in cultured hippocampal neurons; perhaps related to this, immunogold studies indicate that CaMKII in the PSD increases fivefold following depolarization (73). Fong et al. (72) also found that the PKC activation induces rapid dispersal of NMDARs from the synapse to the extrasynaptic membrane; this may be a way that phosphorylation can downregulate synaptic NMDARs. Overall, the last four studies (in addition to studies

described in the previous paragraph) suggest that synapse potentiation involves an initial recruitment of CaMKII to the synapse. CaMKII would first associate with NMDARs; this association would have different consequences for binding to NR2A versus NR2B. Ultimately, this would translate into an optimum effect on the potentiation of the synapse by inducing synaptic insertion and increasing single-channel conductance of AMPA receptors, as noted above.

Other important kinases include the Src protein tyrosine kinase (PTK) family, cyclin-dependent kinase-5 (Cdk5), and cyclic AMP-dependent protein kinases (PKAs). PTKs include five members in the CNS—Src, Fyn, Lyn, Lck, and Yes [see (74) for review]. Tyrosine phosphorylation of NR2A by Fyn is promoted by PSD-95, possibly because PSD-95 acts as a physical intermediate to bring Fyn close to NR2A (75). Other PTKs including Src, Yes, and Lyn associate with PSD-95. Indeed, Liao et al. (69) showed that PSD-95 is required for the Src-mediated potentiation of NR1/NR2A receptor current in *Xenopus* oocytes. Also, Fyn and other Src PTKs may be important in mediating the action of ephrins and their receptors, the Eph tyrosine kinases, which are involved in the establishment of axon-dendrite connections during development (76). Cdk5 phosphorylates NR2A at serine 1232 and appears to be necessary for LTP (77). PKAs, which are known to increase the activity of NMDARs, can phosphorylate NR1, NR2A, and NR2B (78) and can induce synaptic targeting of NMDARs (79). PKA can overcome constitutive type 1 protein phosphatase (PP1) activity, resulting in rapid enhancement of NMDAR currents (80). This is due to a selective anchoring of PKA to NMDARs via yotiao, a protein that binds to the C1 exon cassette, found in the C-terminal of some NR1 splice variants.

Compared to kinases and phosphorylation of NMDARs, less is known about the role of phosphatases and dephosphorylation of NMDARs, but overall evidence suggests that phosphatases downregulate the function of NMDARs (80).

Subunit Composition of Synaptic and Extrasynaptic Receptors

Functional and immunocytochemical studies have shown that NMDARs are present at both synaptic and extrasynaptic sites. It is possible that the extrasynaptic population simply represents receptors that have been delivered to the plasma membrane and are awaiting incorporation into the synapse. This idea is supported by recent work that shows that NMDARs can rapidly move between synaptic and extrasynaptic sites, possibly by lateral diffusion of the receptors (81). However, the possibility remains that there is a distinct population of extrasynaptic receptors with a specific function. Extrasynaptic receptors respond differently to excitotoxic drugs (82) and may be activated under physiological conditions (83). There is also evidence of a selective coupling of NMDA channels at extrasynaptic sites to inhibitory currents, which may limit excitation during periods of intense activity (84).

Synaptic and extrasynaptic receptors may also differ in their subunit compositions. The kinetics of NMDA excitatory postsynaptic currents (EPSCs) become faster during development in the CNS and correlate with an increase in expression

of the NR2A subunit (85) and a decrease in the sensitivity to NR2B-selective antagonists (86, 87). At ages when EPSCs are relatively insensitive to NR2B-selective antagonists, however, extrasynaptic receptors still show considerable block by these agents (87–89). In addition, the decay kinetics of the EPSC are significantly faster than the decay kinetics of direct glutamate application to extrasynaptic receptors (87), providing evidence for NR2B expression in the extrasynaptic pool. These results are consistent with synaptic and extrasynaptic receptor pools having different subunit compositions and forming distinct populations of receptors. Although NR2A and NR2B are thought to be the major subunits comprising synaptic receptors, the reduced Mg^{++} sensitivity and slower deactivation kinetics of NMDA-EPSCs at the mature mossy fiber synapse implies that NR2C-containing receptors are present at this synapse (90). A recent study (90b) showed that NR2A- and NR2B-containing receptors are added to the synapse using different mechanisms. Ligand binding to synaptic NMDARs leads to the delivery of NR2A-containing receptors to the synapse, but not of NR2B-containing receptors. Furthermore, NR2A-containing receptors can replace synaptic NR2B-containing receptors, but not vice versa. These results are consistent with the changes in subunit composition seen in synaptic NMDARs during development and begin to reveal the mechanism by which this occurs.

Recent work has indicated that subunit composition at the synapse can regulate synapse maturation through communication of the pre- and postsynaptic neuron by integrins (91). This study found that blocking activity in hippocampal neurons in culture kept the neurons in an immature state with high expression of the NR2B subunit. Expression of the NR2B subunit was correlated with a high release probability that decreased as the neurons matured; NR2B expression and high release probability could be maintained in the culture by blocking integrin signaling. Although this concept of cross-talk between the pre- and postsynaptic neurons via integrins is only beginning to be studied, it appears to be a critical means by which subunit composition at the postsynaptic density can control NMDA-EPSC properties.

Sensory experience can strongly regulate NMDAR subunit expression and incorporation at the synapse. Ocular dominance plasticity is a well-described phenomenon (92), and dark-reared rats have significantly lower expression of the NR2A subunit (93). Visual experience causes a decrease in NR2B-antagonist sensitivity of EPSCs along with a shortening of EPSC duration; deprivation of visual input has the opposite effect (94). Although the deprivation-induced change to NR2B-like characteristics occurred over a time course of days, visual experience could cause increased expression of the NR2A protein and a change to NR2A-like characteristics in synaptic transmission within hours, implying distinct mechanisms for these changes (93, 94). Interestingly, it has been shown that in NR2A knockout mice, the critical period of plasticity at thalamocortical synapses closes even when the NMDA channels retain NR2B-like characteristics (95). Therefore, changes in synaptic NMDAR subunit composition may be influenced by sensory experience, but the role of these changes in controlling plasticity is not fully clear.

Activity-Dependent Changes in NMDA Receptor Subunit Localization

Blockade of NMDAR activity can cause a dramatic increase in expression of NR2 subunits and an increase in surface expression of the NR1 subunit (79, 96, 97). Blockade of synaptic activity or treatment with NMDAR antagonists has been shown to increase NMDAR colocalization with PSD-95 (a marker of excitatory synapses) without an apparent increase in the number of synapses (97). However, those authors have also shown that significant synaptic rearrangement can occur in the absence of protein synthesis, implicating a role for changes in subunit trafficking (79). The data indicate that PKA may control this activity-dependent change in receptor localization downstream of NMDAR activation.

Functional studies have also tested changes in NMDAR properties in response to receptor blockade. Blockade of NMDARs during development in culture leads to a large increase in the frequency of NMDAR-mediated miniature excitatory postsynaptic currents (NMDA-mEPSCs) with no change in amplitude (98). In contrast, blockade of synaptic transmission with tetrodotoxin (TTX) leads to an increase in the amplitude of NMDA-mEPSCs with no change in frequency (98, 99). With changes in synaptic activity, there were parallel changes in AMPA and NMDAR currents, implying that there is activity-dependent scaling of the number of AMPA and NMDARs at the synapse to maintain a constant ratio between these two receptor types (99). Excitatory synaptic innervation is critical for clustering of AMPA receptors, but PSD-95 and NMDARs can cluster in the absence of this input (100). Therefore, these data indicate that both synaptic activity and NMDAR activation are critical regulators of excitatory synapses, but that the initial formation of the postsynaptic density may be independent of this activity.

Synaptic Targeting of NMDA Receptors

Studies with transgenic mice have been used extensively to study NMDAR trafficking [see (101) for review]. Knockout mice lacking NR1 and NR2B die shortly after birth, but in both cases, synaptic morphology appears normal. If embryonic cultures are made from NR2B knockout mice, NMDAR responses are seen that are significantly faster than normal, indicating that the NR2A subunit can be delivered normally to the synapse in the absence of the NR2B subunit (102). Knockout mice lacking NR2A have NMDARs with slow kinetics and show reduced LTP (16, 103), and mice lacking the NR2C subunit show increased amplitude of NMDAR-EPSCs with faster kinetics (104). However, motor discoordination in mice is seen only with loss of both NR2A and NR2C, with knockouts of either individual subunit showing normal motor coordination (103). NR2D knockout mice develop normally, but have deficits in certain locomotor activities and monoamine metabolism (105, 106). The ability of mice lacking different NR2 subunits to form functional synapses implies that subunit trafficking to the synapse can occur effectively even in the absence of the normal complement of NR2 subunits.

Transgenic mice expressing NR2A with a deletion of its C terminus showed an absence of NMDARs from the synapse, with receptor expression limited to extrasynaptic sites (41). Mice expressing truncated NR2B had a perinatally lethal phenotype, but in cultures made from these animals, there was a decrease in localization of NR2B at synapses (38, 107). In both of these cases, because the transgenic mice had a loss of a large region of the NR2 C terminus, the actual region(s) responsible for synaptic delivery cannot be determined. The PDZ-binding domain on the NR2 subunits would be a likely candidate for controlling synaptic delivery because it mediates interaction with several MAGUK proteins that are localized to the synapse (108). Several studies in which these proteins were deleted or overexpressed, however, were inconclusive in establishing their role in the delivery or stabilization of NMDARs at the synapses (109–111). This may be due to compensatory mechanisms for regulating other MAGUKs when one is increased or lost (112).

Recent work has studied the effect of NMDAR subunit overexpression by transfection on NMDAR properties in cultured cerebellar granule cells. Overexpression of the NR1 subunit does not alter the total number of functional channels in neurons, but overexpression of either NR2A or NR2B causes an increase in receptor number, implying that synthesis of NR2 subunits controls the number of functional channels expressed by neurons (113). In contrast, the amplitude of NMDAR-EPSCs was not increased by overexpression of NR2 subunits, indicating that subunit availability is not the major factor in determining the number of synaptic NMDARs. Transfected NR2 subunits are targeted to the synapse because the deactivation kinetics of NMDA-EPSCs were controlled by the NR2 subunit overexpressed. NR2 subunits lacking the *PDZ-binding* domain did not alter synaptic kinetics, however, indicating that the PDZ domain is necessary for entry of receptors into the functional pool at the synapse. These findings would be consistent with the interpretation that the receptor without its PDZ interacting domain cannot be clustered at the synapse. However, these results also raise the possibility that there are two distinct mechanisms for delivery of synaptic and extrasynaptic NMDARs, requiring and not requiring the PDZ-binding domain, respectively. The latter interpretation would support recent work that defines a role for MAGUKs in the early trafficking of NMDAR subunits from the ER/Golgi via association with the exocyst complex through the PDZ-binding domain of the sec8 protein (44).

Subunit overexpression has also been studied in transgenic mice. Overexpression of the NR2B subunit in the forebrain produced the "smart mouse," which showed improved performance on memory tasks and larger NMDA-mediated currents in the hippocampus (17). Interestingly, studies have also shown that this mouse had an increased sensitivity to pain (114), emphasizing the complex systems controlled by NMDA-mediated transmission. Recent studies have shown in this same strain of mice that there is no change in NMDA-EPSC properties in the visual cortex (115). Thus, the ability of NR2 subunit overexpression to modulate synaptic NMDA responses may be dependent on the brain region studied. In contrast, overexpression of the NR2D subunit in mice causes a marked impairment

of LTP generation with NMDA-evoked currents that were slower and had smaller amplitude (116). Therefore, maintenance of the subunit composition may be a critical means of determining NMDA receptor responses at synapses.

Internalization of NMDA Receptors

NMDARs are relatively stable components of the postsynaptic membrane. For example, the basal rate of endocytosis of surface AMPA receptors in cultured cortical neurons is nearly threefold that of NMDARs (20, 117). Thus, it is not surprising that synaptic plasticity is linked more to changes in numbers and distribution of surface AMPA receptors than it is to NMDARs; consequently, studies on glutamate receptor internalization have focused more on AMPA receptors than on NMDARs.

What regulates NMDAR internalization and where does it occur? Mechanisms of NMDAR internalization involve at least three different regions of the receptor molecule, including a PDZ-binding motif and two different tyrosine motifs. First, NMDAR internalization is regulated by its association with PSD-95 and other PDZ proteins. These proteins form the backbone of the NMDAR complex at the postsynaptic membrane. NMDARs that are bound to these proteins are stabilized and are less likely to be internalized, whereas NMDARs that are not bound to these proteins may be internalized readily. This would be a useful mechanism for removing surface NMDARs that are not bound at the synapse. This phenomenon has been demonstrated recently: When a chimera of surface integral membrane protein Tac and the distal C terminus of NR2B is transfected into cultured hippocampal neurons, only 15% of the cells show internalized constructs after a 15-minute incubation at 37°C. In contrast, when the neurons are transfected with modified constructs that lack the last seven amino acid residues [TacNR2BΔ7], two thirds of the cells show distinct internalization. The latter constructs lack the PDZ-binding domain and thus cannot be stabilized by association with the MAGUKs (9). Furthermore, when the same constructs are transfected into HeLa cells with/without cotransfection of PSD-95, the latter protein significantly inhibits internalization of TacNR2B and stabilizes it at the cell surface. The control of internalization of NMDARs via binding to PSD-95 is likely a dynamic event. First, Roche et al. (9) suggest that internalization of the receptor/PSD-95 complex may occur under conditions leading to the dissociation of PSD-95 from other components of the postsynaptic complex. Second, binding of NMDARs to PSD-95 appears to be affected by phosphorylation of the receptor, as discussed in the previous section. Interestingly, the main site of tyrosine phosphorylation of NR2B is Y1472, which is very close to the PDZ-binding domain on the C-terminal (118). Increased phosphorylation of this site, as occurs following tetanic stimulation of Schaffer collaterals in the CA1 region of the hippocampus, might interfere with PSD-95 binding, and perhaps makes the receptor more available for internalization.

Indeed, the latter tyrosine is part of a motif, YEKL, that signals clathrin-mediated endocytosis by binding to AP-2 adaptor complexes. Roche et al. (9)

transfected TacNR2B, TacNR2BΔ7 (which lacks the last 7 amino acids) and TacNR2BΔ11 (lacking the last 11 amino acids) into HeLa cells; the last construct, which lacks YEKL, conferred a 50% inhibition of internalization. The authors also tried a TacNR2BΔ11 with a mutated dileucine motif, which can signal clathrin-mediated endocytosis in some systems, but this mutation did not produce any additional inhibition of internalization. Thus, the remaining 50% internalization must be regulated by some other motif. Internalization of NMDARs also involves a ring of tyrosines on the C termini of NMDAR subunits, just distal to the last transmembrane domain. These include tyrosine 837 of NR1 and 842 of NR2A (10). Dephosphorylation of these tyrosines may allow AP-2 binding, leading to clathrin-mediated endocytosis of the NMDAR. Interestingly, agonist binding to the NMDAR mediates this dephosphorylation independent of ion flux. This suggests that, in this way, an NMDAR could trigger intracellular signal cascades, independent of its function as an ion channel, and thus would behave, in some ways, like metabotropic glutamate receptors (mGluRs). In fact, group 1 mGluRs also can induce NMDAR internalization, perhaps by indirectly affecting the binding of NMDARs to PSD-95 (11).

Identification of the site of glutamate receptor exo- and endocytosis has always been problematic. There is at least circumstantial evidence that glutamate receptors move to and from synapses from either the sides of the spine or from the edge of the active zone (50, 119–120). Passaforo et al. (121) used a thrombin cleavage assay in cultured hippocampal neurons to provide evidence that GluR2/3 AMPA receptors are inserted at the synapse. They also suggest that endocytosis of these receptors would occur at an extrasynaptic site; this method would avoid co-internalization of the latter receptors with synaptic NMDARs and other membrane proteins. Nevertheless, endocytosis of at least some kinds of glutamate receptors directly from the synaptic active zone could be possible. Dynamin 2, which is involved in endocytosis, can specifically interact with Shank proteins and is prevalent in the postsynaptic density (122). In addition, we see some evidence of clathrin, adaptin, and dynamin immunogold labeling at and just below the postsynaptic density, and show some associations there with AMPA and NMDA receptors (123).

CONCLUSION

As is apparent from this review, there is considerable information available on the trafficking of NMDARs in neurons. But most of it is fragmentary and fails to address in detail any one step in the assembly, processing, and synaptic delivery of the receptor. For one of the best-characterized NMDAR interacting proteins, PSD-95, it is still unknown where and when this interaction occurs and what regulates its association with the NMDAR. More than 70 proteins have been shown to interact either directly or indirectly with the NMDAR (62). Our challenge is to determine how each of these proteins, in addition to many others yet to be identified, participates in the trafficking of the NMDAR.

The *Annual Review of Pharmacology and Toxicology* is online at http://pharmtox.annualreviews.org

LITERATURE CITED

1. Collingridge GL, Kehl SJ, McLennan H. 1983. Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. *J. Physiol.* 334:33–46
2. Nowak L, Bregestovski P, Ascher P, Herbet A, Prochiantz A. 1984. Magnesium gates glutamate-activated channels in mouse central neurones. *Nature* 307: 462–65
3. Mayer ML, Westbrook GL, Guthrie PB. 1984. Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones. *Nature* 309:261–63
4. Moriyoshi K, Masu M, Ishii T, Shigemoto R, Mizuno N, Nakanishi S. 1991. Molecular cloning and characterization of the rat NMDA receptor. *Nature* 354:31–37
5. Monyer H, Sprengel R, Schoepfer R, Herb A, Higuchi M, et al. 1992. Heteromeric NMDA receptors: molecular and functional distinction of subtypes. *Science* 256:1217–21
6. Kutsuwada T, Kashiwabuchi N, Mori H, Sakimura K, Kushiya E, et al. 1992. Molecular diversity of the NMDA receptor channel. *Nature* 358:36–41
7. Ciabarra AM, Sullivan JM, Gahn LG, Pecht G, Heinemann S, Sevarino KA. 1995. Cloning and characterization of chi-1: a developmentally regulated member of a novel class of the ionotropic glutamate receptor family. *J. Neurosci.* 15:6498–508
8. Luscher C, Nicoll RA, Malenka RC, Muller D. 2000. Synaptic plasticity and dynamic modulation. *Nat. Neurosci.* 3:545–50
9. Roche KW, Standley S, McCallum J, Ly CD, Ehlers MD, et al. 2001. Molecular determinants of NMDA receptor internalization. *Nat. Neurosci.* 4:794–802
10. Vissel B, Krupp JJ, Heinemann SF, Westbrook GL. 2001. A use-dependent tyrosine dephosphorylation of NMDA receptors is independent of ion flux. *Nat. Neurosci.* 4:587–96
11. Snyder EM, Philpot BD, Huber KM, Dong X, Fallon JR, et al. 2001. Internalization of ionotropic glutamate receptors in response to mGluR activation. *Nat. Neurosci.* 4:1079–85
12. Grosshans DR, Clayton DA, Coultrap SJ, Browning MD. 2001. LTP leads to rapid surface expression of NMDA but not AMPA receptors in adult rat CA1. *Nat. Neurosci.* 5:27–33
13. Malinow R, Mainen ZF, Hayashi Y. 2000. LTP mechanisms: from silence to four-lane traffic. *Curr. Opin. Neurobiol.* 10: 352–57
14. Das S, Sasaki YF, Rothe T, Premkumar LS, Takasu M, et al. 1998. Increased NMDA current and spine density in mice lacking the NMDA receptor subunit NR3A. *Nature* 393:377–81
15. Chatterton JE, Awobuluyi M, Premkumar LS, Takahashi H, Talantova M, et al. 2002. Excitatory glycine receptors containing the NR3 family of NMDA receptor subunits. *Nature* 415:793–98
16. Sakimura K, Kutsuwada T, Ito I, Manabe T, Takayama C, et al. 1995. Reduced hippocampal LTP and spatial learning in mice lacking NMDA receptor epsilon 1 subunit. *Nature* 373(6510):151–55
17. Tang YP, Shimizu E, Dube GR, Rampon C, Kerchner GA, et al. 1999. Genetic enhancement of learning and memory in mice. *Nature* 401(6748):63–69
18. Mohn AR, Gainetdinov RR, Caron MG, Koller BH. 1999. Mice with reduced NMDA receptor expression display behaviors related to schizophrenia. *Cell* 98:427–36

19. Chazot PL, Stephenson FA. 1997. Molecular dissection of native mammalian forebrain NMDA receptors containing the NR1 C2 exon: direct demonstration of NMDA receptors comprising NR1, NR2A, and NR2B subunits within the same complex. *J. Neurochem.* 69(5): 2138–44
20. Huh K-H, Wenthold RJ. 1999. Turnover analysis of glutamate receptors identifies a rapidly degraded pool of the N-methyl-D-aspartate receptor subunit, NR1, in cultured cerebellar granule cells. *J. Biol. Chem.* 274:151–57
21. Kew JN, Richards JG, Mutel V, Kemp JA. 1998. Developmental changes in NMDA receptor glycine affinity and ifenprodil sensitivity reveal three distinct populations of NMDA receptors in individual rat cortical neurons. *J. Neurosci.* 18:1935–43
22. Wenthold RJ, Petralia RS, Blahos J II, Niedzielski AS. 1996. Evidence for multiple AMPA receptor complexes in hippocampal CA1/CA2 neurons. *J. Neurosci.* 16:1982–89
23. Kornau HC, Schenker LT, Kennedy MB, Seeburg PH. 1995. Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. *Science* 269:1737–40
24. Sheng M, Sala C. 2001. PDZ domains and the organization of supramolecular complexes. *Annu. Rev. Neurosci.* 24:1–29
25. Standley S, Roche KW, McCallum J, Sans N, Wenthold RJ. 2000. PDZ domain suppression of an ER retention signal in NMDA receptor NR1 splice variants. *Neuron* 28:887–98
26. Meddows E, Le Bourdelles B, Grimwood S, Wafford K, Sandhu S, et al. 2001. Identification of molecular determinants that are important in the assembly of N-methyl-D-aspartate receptors. *J. Biol. Chem.* 276:18795–803
27. Dalva MB, Takasu MA, Lin MZ, Shamah SM, Hu L, et al. 2000. EphB receptors interact with NMDA receptors and regulate excitatory synapse formation. *Cell* 103:945–56
28. Ellgaard L, Helenius A. 2001. ER quality control: towards an understanding at the molecular level. *Curr. Opin. Cell. Biol.* 13:431–37
29. Teasdale RD, Jackson MR. 1996. Signal-mediated sorting of membrane proteins between the endoplasmic reticulum and the Golgi apparatus. *Annu. Rev. Cell Dev. Biol.* 12:27–54
30. Zerangue N, Schwappach B, Jan YN, Jan LY. 1999. A new ER trafficking signal regulates the subunit stoichiometry of plasma membrane K(ATP) channels. *Neuron* 22:537–48
31. Margeta-Mitrovic M, Jan YN, Jan LY. 2000. A trafficking checkpoint controls GABA(B) receptor heterodimerization. *Neuron* 27:97–106
32. Benke D, Honer M, Michel C, Bettler B, Mohler H. 1999. γ-Aminobutyric acid type B receptor splice variant proteins GBR1a and GBR1b are both associated with GBR2 in situ and display differential regional and subcellular distribution. *J. Biol. Chem.* 274:27323–30
33. McIlhinney RA, Le Bourdelles B, Molnar E, Tricaud N, Streit P, Whiting PJ. 1998. Assembly intracellular targeting and cell surface expression of the human N-methyl-D-aspartate receptor subunits NR1a and NR2A in transfected cells. *Neuropharmacology* 37:1355–67
34. Scott DB, Blanpied TA, Swanson GT, Zhang C, Ehlers MD. 2001. An NMDA receptor ER retention signal regulated by phosphorylation and alternative splicing. *J. Neurosci.* 21:3063–72
35. Xia H, Hornby ZD, Malenka RC. An ER retention signal explains differences in surface expression of NMDA and AMPA receptor subunits. *Neuropharmacology* 41:714–23
36. Holmes KD, Mattar PA, Marsh DR, Weaver LC, Dekaban GA. 2002. The N-methyl-D-aspartate receptor splice variant NR1-4 C-terminal domain. Deletion

analysis and role in subcellular distribution. *J. Biol. Chem.* 277:1457–68
37. Perez-Otano I, Schulteis CT, Contractor A, Lipton SA, Trimmer JS, et al. 2001. Assembly with the NR1 subunit is required for surface expression of NR3A-containing NMDA receptors. *J. Neurosci.* 21:1228–37
38. Sprengel R, Suchanek B, Amico C, Brusa R, Burnashev N, et al. 1998. Importance of the intracellular domain of NR2 subunits for NMDA receptor function in vivo. *Cell* 92:279–89
39. Krupp JJ, Vissel B, Thomas CG, Heinemann SF, Westbrook GL. 1999. Interactions of calmodulin and alpha-actinin with the NR1 subunit modulate Ca^{2+} dependent inactivation of NMDA receptors. *J. Neurosci.* 19:1165–78
40. Zheng X, Zhang L, Wang AP, Bennett MV, Zukin RS. 1999. Protein kinase C potentiation of N-methyl-D-aspartate receptor activity is not mediated by phosphorylation of N-methyl-D-aspartate receptor subunits. *Proc. Natl. Acad. Sci. USA* 96:15262–67
41. Steigerwald F, Schulz TW, Schenker LT, Kennedy MB, Seeburg PH, Kohr G. 2000. C-terminal truncation of NR2A subunits impairs synaptic but not extrasynaptic localization of NMDA receptors. *J. Neurosci.* 20(12):4573–81
42. Okabe S, Miwa A, Okado H. 1999. Alternative splicing of the C-terminal domain regulates cell surface expression of the NMDA receptor NR1 subunit. *J. Neurosci.* 19:7781–92
43. Setou M, Nakagawa T, Seog DH, Hirokawa N. 2000. Kinesin superfamily motor protein KIF17 and mLin-10 in NMDA receptor-containing vesicle transport. *Science* 288:1796–802
44. Sans N, Prybylowski K, Petralia RS, Chang K, Wang Y-X, et al. 2002. NMDA receptor trafficking depends on an interaction between the MAGUKs and the exocyst complex. *Soc. Neurosci. Abstr.* In press
45. Hsu SC, Hazuka CD, Foletti DL, Scheller RH. 1999. Targeting vesicles to specific sites on the plasma membrane: the role of the sec6/8 complex. *Trends Cell Biol.* 9:150–53
46. Yeaman C, Grindstaff KK, Wright JR, Nelson WJ. 2001. Sec6/8 complexes on trans-Golgi network and plasma membrane regulate late stages of exocytosis in mammalian cells. *J. Cell. Biol.* 155:593–604
47. Shin DM, Zhao XS, Zeng W, Mozhayeva M, Muallem S. 2000. The mammalian Sec6/8 complex interacts with $Ca^{(2+)}$ signaling complexes and regulates their activity. *J. Cell Biol.* 150:1101–112
48. Cantalops I, Cline HT. 2000. Synapse formation: if it looks like a duck and quacks like a duck. *Cur. Biol.* 10:R620–23
49. Hirao K, Hata Y, Ide N, Takeuchi M, Irie M, et al. 1998. A novel multiple PDZ domain-containing molecule interacting with N-methyl-D-aspartate receptors and neuronal cell adhesion proteins. *J. Biol. Chem.* 273:21105–10
50. Petralia RS, Wang Y-X, Sans N, Worley PF, Hammer III JA, et al. 2001. Glutamate receptor targeting in the postsynaptic spine involves mechanisms that are independent of myosin Va. *Eur. J. Neurosci.* 13:1722–32
51. Lisman J, Schulman H, Cline H. 2002. The molecular basis of CaMKII function in synaptic and behavioural memory. *Nat. Rev.* 3:175–90
52. Chen L, Chetkovich DM, Petralia RS, Sweeney NT, Kawasaki Y, et al. 2000. Stargazin regulates synaptic targeting of AMPA receptors by two distinct mechanisms. *Nature* 408:936–43
53. Ehlers MD. 2002. Molecular morphogens for dendritic spines. *Trends Neurosci.* 25: 64–67
54. Pak DTS, Yang S, Rudolph-Correia S, Kim E, Sheng M. 2001. Regulation of dendritic spine morphology by SPAR, a PSD-95-associated RapGAP. *Neuron* 31:289–303

55. Allison DW, Chervin AS, Gelfand VI, Craig AM. 2000. Postsynaptic scaffolds of excitatory and inhibitory synapses in hippocampal neurons: maintenance of core components independent of actin filament s and microtubules. *J. Neurosci.* 20:4545–54
56. Chen H-J, Rojas-Soto M, Oguni A, Kennedy MB. 1998. A synaptic Ras-GTPase activating protein (p135 SynGAP) inhibited by CaM kinase II. *Neuron* 20:895–904
57. Kim JH, Liao D, Lau L-F, Huganir RL. 1998. SynGAP: a synaptic RasGAP that associates with the PSD-95/SAP90 protein family. *Neuron* 20:683–91
58. Ohtsuka T, Hata Y, Ide N, Yasuda T, Inoue E, et al. 1999. nRap GEP: a novel neural GDP/GTP exchange protein for Rap1 small G protein that interacts with synaptic scaffolding molecule (S-SCAM). *Biochem. Biophys. Res. Commun.* 265:38–44
59. Yao I, Hata Y, Ide N, Hirao K, Deguchi M, et al. 1999. MAGUIN, a novel neuronal membrane-associated guanylate kinase-interacting protein. *J. Biol. Chem.* 274:11889–96
60. Furuyashiki T, Fujisawa K, Fujita A, Madaule P, Uchino S, et al. 1999. Citron, a rho-target, interacts with PSD-95/SAP-90 at glutamatergic synapses in the thalamus. *J. Neurosci.* 19:109–18
61. Zhang W, Vasquez L, Apperson M, Kennedy MB. 1999. Citron binds to PSD-95 at glutamatergic synapses on inhibitory neurons in the hippocampus. *J. Neurosci.* 19:96–108
62. Husi H, Ward MA, Choudhary JS, Blackstock WP, Grant SGN. 2000. Proteomic analysis of NMDA receptor-adhesion protein signaling complexes. *Nat. Neurosci.* 3:661–69
63. Tanaka H, Shan W, Phillips GR, Arndt K, Bozdagi K, et al. 2000. Molecular modification of N-cadherin in response to synaptic activity. *Neuron* 25:93–107
64. Petralia RS, Wang Y-X, Wenthold RJ. 2002. NMDA receptors and PSD-95 are found in attachment plaques in cerebellar granular layer glomeruli. *Eur. J. Neurosci.* 15:583–87
65. Kennedy MB, Manzerra P. 2001. Telling tails. *Proc. Natl. Acad. Sci. USA* 98: 12323–24
66. Gardoni F, Schrama LH, Kamal A, Gispen WH, Cattabeni F, et al. 2001. Hippocampal synaptic plasticity involves competition between Ca^{2+}/calmodulin-dependent protein kinase II and postsynaptic density 95 for binding to the NR2A subunit of the NMDA receptor. *J. Neurosci.* 21:1501–9
67. Leonard AS, Lim IA, Hemsworth DE, Horne MC, Hell JW. 1999. Calcium/calmodulin-dependent protein kinase II is associated with the N-methyl-D-aspartate receptor. *Proc. Natl. Acad. Sci. USA* 96: 3239–44
68. Bayer K-U, Koninck PD, Leonard AS, Hell JW, Schulman H. 2001. Interaction with the NMDA receptor locks CaMKII in an active conformation. *Nature* 411:801–5
69. Liao G-Y, Kreitzer MA, Sweetman BJ, Leonard JP. 2000. The postsynaptic density protein PSD-95 differentially regulates insulin- and Src-mediated current modulation of mouse NMDA receptors expressed in *Xenopus* oocytes. *J. Neurochem.* 75:282–87
70. Lan J, Skeberdis VA, Jover T, Grooms SY, Lin Y, et al. 2001. Protein kinase C modulates NMDA receptor trafficking and gating. *Nat. Neurosci.* 4:382–90
71. Gardoni F, Bellone C, Cattabeni F, Di Luca M. 2001. Protein kinase C activation modulates a-calmodulin kinase II binding to NR2A subunit of N-methyl-D-aspartate receptor. *J. Biol. Chem.* 276: 7609–13
72. Fong DK, Rao A, Crump FT, Craig AM. 2002. Rapid synaptic remodeling by protein kinase C: reciprocal translocation of NMDA receptors and calcium/calmodulin-dependent kinase II. *J. Neurosci.* 22:2153–64

73. Dosemeci A, Tao-Cheng J-H, Vinade L, Winters CA, Pozzo-Miller L, et al. 2001. Glutamate-induced transient modification of the postsynaptic density. *Proc. Natl. Acad. Sci. USA* 98:10428–32
74. Ali DW, Salter MW. 2001. NMDA receptor regulation by Src kinase signaling in excitatory synaptic transmission and plasticity. *Curr. Opin. Neurobiol.* 11:336–42
75. Tezuka T, Umemori H, Akiyama T, Nakanishi S, Yamamoto T. 1999. PSD-95 promotes Fyn-mediated tyrosine phosphorylation of the N-methyl-D-aspartate receptor subunit NR2A . *Proc. Natl. Acad. Sci. USA* 96:435–40
76. Takasu MA, Dalva MB, Zigmond RE, Greenberg ME. 2002. Modulation of NMDA receptor-dependent calcium influx and gene expression through EphB receptors. *Science* 295:491–95
77. Li B-S, Sun M-K, Zhang L, Takahashi S, Ma W, et al. 2001. Regulation of NMDA receptors by cyclin-dependent kinase-5. *Proc. Natl. Acad. Sci. USA* 98:12742–47
78. Leonard AS, Hell JW. 1997. Cyclic AMP-dependent protein kinase and protein kinase C phosphorylate N-methyl-D-aspartate receptors at different sites. *J. Biol. Chem.* 272:12107–15
79. Crump FT, Dillman KS, Craig AM. 2001. cAMP-dependent protein kinase mediates activity-regulated synaptic targeting of NMDA receptors. *J. Neurosci.* 21:5079–88
80. Westphal RS, Tavalin SJ, Lin JW, Alto NM, Fraser IDC, et al. 1999. Regulation of NMDA receptors by an associated phosphatase-kinase signaling complex. *Science* 285:93–96
81. Tovar KR, Westbrook GL. 2002. Mobile NMDA receptors at hippocampal synapses. *Neuron* 34:255–64
82. Sinor JD, Du S, Venneti S, Blitzblau RC, Leszkiewicz DN, et al. 2000. NMDA and glutamate evoke excitotoxicity at distinct cellular locations in rat cortical neurons in vitro. *J. Neurosci.* 20(23):8831–37
83. Chen S, Diamond JS. 2002. Synaptically released glutamate activates extrasynaptic NMDA receptors on cells in the ganglion cell layer of rat retina. *J. Neurosci.* 22(6):2165–73
84. Isaacson JS, Murphy GJ. 2001. Glutamate-mediated extrasynaptic inhibition: direct coupling of NMDA receptors to $Ca^{(2+)}$-activated K^+ channels. *Neuron* 31(6):1027–34
85. Flint AC, Maisch US, Weishaupt JH, Kriegstein AR, Monyer H. 1997. NR2A subunit expression shortens NMDA receptor synaptic currents in developing neocortex. *J. Neurosci.* 17(7):2469–76
86. Stocca G, Vicini S. 1998. Increased contribution of NR2A subunit to synaptic NMDA receptors in developing rat cortical neurons. *J. Physiol.* 507(Pt.1):13–24
87. Rumbaugh G, Vicini S. 1999. Distinct synaptic and extrasynaptic NMDA receptors in developing cerebellar granule neurons. *J. Neurosci.* 19(24):10603–10
88. Tovar KR, Westbrook GL. 1999. The incorporation of NMDA receptors with a distinct subunit composition at nascent hippocampal synapses in vitro. *J. Neurosci.* 19(10):4180–88
89. Momiyama A. 2000. Distinct synaptic and extrasynaptic NMDA receptors identified in dorsal horn neurones of the adult rat spinal cord. *J. Physiol.* 523(Pt. 3):621–28
90. Cull-Candy S, Brickley S, Farrant M. 2001. NMDA receptor subunits: diversity, development and disease. *Curr. Opin. Neurobiol.* 11(3):327–35
90b. Barria A, Malinow R. 2002. Subunit-specific NMDA receptor trafficking to synapses. *Neuron* 35:345–53
91. Chavis P, Westbrook G. 2001. Integrins mediate functional pre- and postsynaptic maturation at a hippocampal synapse. *Nature* 411(6835):317–21
92. Bear MF, Rittenhouse CD. 1999. Molecular basis for induction of ocular dominance plasticity. *J. Neurobiol.* 41(1):83–91
93. Quinlan EM, Olstein DH, Bear MF.

1999. Bidirectional, experience-dependent regulation of N-methyl-D-aspartate receptor subunit composition in the rat visual cortex during postnatal development. *Proc. Natl. Acad. Sci. USA* 96(22):12876–80

94. Philpot BD, Sekhar AK, Shouval HZ, Bear MF. 2001. Visual experience and deprivation bidirectionally modify the composition and function of NMDA receptors in visual cortex. *Neuron* 29(1): 157–69
95. Lu HC, Gonzalez E, Crair MC. 2001. Barrel cortex critical period plasticity is independent of changes in NMDA receptor subunit composition. *Neuron* 32(4):619–34
96. Follesa P, Ticku MK. 1996. NMDA receptor upregulation: molecular studies in cultured mouse cortical neurons after chronic antagonist exposure. *J. Neurosci.* 16(7):2172–78
97. Rao A, Craig AM. 1997. Activity regulates the synaptic localization of the NMDA receptor in hippocampal neurons. *Neuron* 19(4):801–12
98. Luthi A, Schwyzer L, Mateos JM, Gahwiler BH, McKinney RA. 2001. NMDA receptor activation limits the number of synaptic connections during hippocampal development. *Nat. Neurosci.* 4(11):1102–7
99. Watt AJ, van Rossum MC, MacLeod KM, Nelson SB, Turrigiano GG. 2000. Activity coregulates quantal AMPA and NMDA currents at neocortical synapses. *Neuron* 26:659–70
100. Rao A, Cha EM, Craig AM. 2000. Mismatched appositions of presynaptic and postsynaptic components in isolated hippocampal neurons. *J. Neurosci.* 20:8344–53
101. Sprengel R, Single FN. 1999. Mice with genetically modified NMDA and AMPA receptors. *Ann. NY Acad. Sci.* 868:494–501
102. Tovar KR, Sprouffske K, Westbrook GL. 2000. Fast NMDA receptor-mediated synaptic currents in neurons from mice lacking the epsilon2 (NR2B) subunit. *J. Neurophysiol.* 83:616–20
103. Kadotani H, Hirano T, Masugi M, Nakamura K, Nakao K, et al. 1996. Motor discoordination results from combined gene disruption of the NMDA receptor NR2A and NR2C subunits, but not from single disruption of the NR2A or NR2C subunit. *J. Neurosci.* 16:7859–67
104. Ebralidze AK, Rossi DJ, Tonegawa S, Slater NT. 1996. Modification of NMDA receptor channels and synaptic transmission by targeted disruption of the NR2C gene. *J. Neurosci.* 16:5014–25
105. Ikeda K, Araki K, Takayama C, Inoue Y, Yagi T, et al. 1995. Reduced spontaneous activity of mice defective in the epsilon 4 subunit of the NMDA receptor channel. *Brain Res. Mol. Brain Res.* 33:61–71
106. Miyamoto Y, Yamada K, Noda Y, Mori H, Mishina M, Nabeshima T. 2002. Lower sensitivity to stress and altered monoaminergic neuronal function in mice lacking the NMDA receptor epsilon 4 subunit. *J. Neurosci.* 22:2335–42
107. Mori H, Manabe T, Watanabe M, Satoh Y, Suzuki N, et al. 1998. Role of the carboxy-terminal region of the GluR epsilon2 subunit in synaptic localization of the NMDA receptor channel. *Neuron* 21:571–80
108. Sheng M. 2001. The postsynaptic NMDA-receptor–PSD-95 signaling complex in excitatory synapses of the brain. *J. Cell Sci.* 114:1251
109. Migaud M, Charlesworth P, Dempster M, Webster LC, Watabe AM, et al. 1998. Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. *Nature* 396:433–39
110. McGee AW, Topinka JR, Hashimoto K, Petralia RS, Kakizawa S, et al. 2001. PSD-93 knock-out mice reveal that neuronal MAGUKs are not required for development or function of parallel fiber synapses in cerebellum. *J. Neurosci.* 21:3085–91
111. El-Husseini AE, Schnell E, Chetkovich

DM, Nicoll RA, Bredt DS. 2000. PSD-95 involvement in maturation of excitatory synapses. *Science* 290:1364–68
112. Misawa H, Kawasaki Y, Mellor J, Sweeney N, Jo K, et al. 2001. Contrasting localizations of MALS/LIN-7 PDZ proteins in brain and molecular compensation in knockout mice. *J. Biol. Chem.* 276:9264–72
113. Prybylowski K, Fu Z, Losi G, Hawkins LM, Luo JH, et al. 2002. Relationship between availability of NMDA receptor subunits and their expression at the synapse. *J. Neurosci.* In press
114. Wei F, Wang GD, Kerchner GA, Kim SJ, Xu HM, et al. 2001. Genetic enhancement of inflammatory pain by forebrain NR2B overexpression. *Nat. Neurosci.* 4:164–69
115. Philpot BD, Weisberg MP, Ramos MS, Sawtell NB, Tang YP, et al. 2001. Effect of transgenic overexpression of NR2B on NMDA receptor function and synaptic plasticity in visual cortex. *Neuropharmacology* 41:762–70
116. Okabe S, Collin C, Auerbach JM, Meiri N, Bengzon J, et al. 1998. Hippocampal synaptic plasticity in mice overexpressing an embryonic subunit of the NMDA receptor. *J. Neurosci.* 18:4177–88
117. Ehlers MD. 2000. Reinsertion or degradation of AMPA receptors determined by activity-dependent endocytic sorting. *Neuron* 28:511–25
118. Nakazawa T, Komai S, Tezuka T, Hisatsune C, Umemori H, et al. 2001. Characterization of Fyn-mediated tyrosine phosphorylation sites on GluRepsilon 2 (NR2B) subunit of the N-methyl-D-aspartate receptor. *J. Biol. Chem.* 276: 693–99
119. Spacek J, Harris KM. 1997. Three-dimensional organization of smooth endoplasmic reticulum in hippocampal CA1 dendrites and dendritic spines of the immature and mature rat. *J. Neurosci.* 17:190–203
120. Cooney JR, Hurlburt JL, Selig DK, Harris KM, Fiala JC. 2002. Endosomal compartments serve multiple hippocampal dendritic spines from a widespread rather than a local store of recycling membrane. *J. Neurosci.* 22:2215–24
121. Passafaro M, Piëch V, Sheng M. 2001. Subunit-specific temporal and spatial patterns of AMPA receptor exocytosis in hippocampal neurons. *Nat. Neurosci.* 4:917–26
122. Okamoto PM, Gamby C, Wells D, Fallon J, Vallee RB. 2001. Dynamin isoform-specific interaction with the shank/proSAP scaffolding proteins of the postsynaptic density and actin cytoskeleton. *J. Biol. Chem.* 276:48458–65
123. Petralia RS, Wang Y-X, McCallum J, Roche KW, Wenthold RJ. 2000. Morphological characterization of glutamate receptor internalization. *Soc. Neurosci. Abstr.* 26:425.12
124. Tingley WG, Ehlers MD, Kameyama K, Doherty C, Ptak JB, et al. 1997. Characterization of protein kinase A and protein kinase C phosphorylation of the N-methyl-D-aspartate receptor NR1 subunit using phosphorylation site-specific antibodies. *J. Biol. Chem.* 272:5157–66
125. Zheng F, Gingrich MB, Traynelis SF, Conn PJ. 1998. Tyrosine kinase potentiates NMDA receptor currents by reducing tonic zinc inhibition. *Nat. Neurosci.* 1:185–91
126. Strack S, McNeill RB, Colbran RJ. Mechanism and regulation of calcium/calmodulin-dependent protein kinase II targeting to the NR2B subunit of the N-methyl-D-aspartate receptor. *J. Biol. Chem.* 275:23798–806
127. Wyszynski M, Kharazia V, Shanghvi R, Rao A, Beggs AH, et al. 1998. Differential regional expression and ultrastructural localization of alpha-actinin-2, a putative NMDA receptor-anchoring protein, in rat brain. *J. Neurosci.* 18:1383–92
128. Ehlers MD, Zhang S, Bernhadt JP, Huganir RL. 1996. Inactivation of NMDA receptors by direct interaction of calmodulin with the NR1 subunit. *Cell* 84:745–55

129. Lin JW, Wyszynski M, Madhavan R, Sealock R, Kim JU, Sheng M. 1998. Yotiao, a novel protein of neuromuscular junction and brain that interacts with specific splice variants of NMDA receptor subunit NR1. *J. Neurosci.* 18:2017–27
130. Ehlers MD, Fung ET, O'Brien RJ, Huganir RL. 1998. Splice variant-specific interaction of the NMDA receptor subunit NR1 with neuronal intermediate filaments. *J. Neurosci.* 18:720–30
131. Wechsler A, Teichberg VI. 1998. Brain spectrin binding to the NMDA receptor is regulated by phosphorylation, calcium and calmodulin. *EMBO J.* 17:3931–39
132. van Rossum D, Kuhse J, Betz H. 1999. Dynamic interaction between soluble tubulin and C-terminal domains of N-methyl-D-aspartate receptor subunits. *J. Neurochem.* 72:962–73
133. Strack S, Colbran RJ. 1998. Autophosphorylation-dependent targeting of calcium/calmodulin-dependent protein kinase II by the NR2B subunit of the N-methyl-D-aspartate receptor. *J. Biol. Chem.* 273:20689–92
134. Kurschner C, Mermelstein PG, Holden WT, Surmeier DJ. 1998. CIPP, a novel multivalent PDZ domain protein, selectively interacts with Kir4.0 family members, NMDA receptor subunits, neurexins, and neuroligins. *Mol. Cell. Neurosci.* 11:161–72
135. Yu XM, Askalan R, Keil GJ 2nd, Salter MW. 1997. NMDA channel regulation by channel-associated protein tyrosine kinase Src. *Science* 275:674–78
136. Gurd JW, Bissoon N. 1997. The N-methyl-D-aspartate receptor subunits NR2A and NR2B bind to the SH2 domains of phospholipase C-gamma. *J. Neurochem.* 69:623–30
137. Wu XS, Rao K, Zhang H, Wang F, Sellers JR, et al. 2002. Identification of an organelle receptor for myosin-Va. *Nat. Cell Biol.* 4:271–78

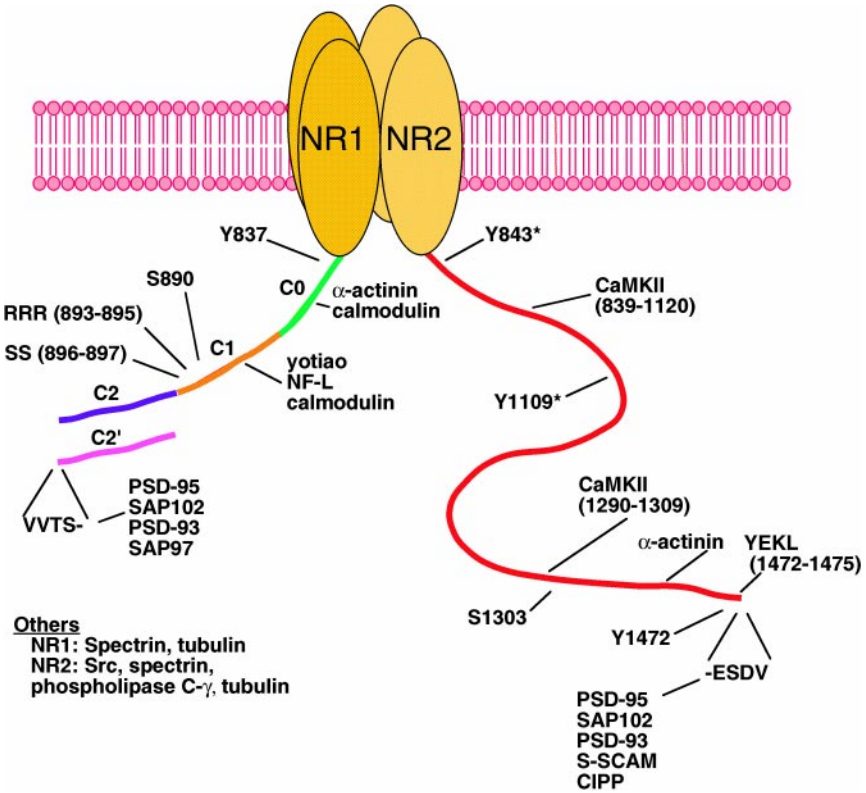
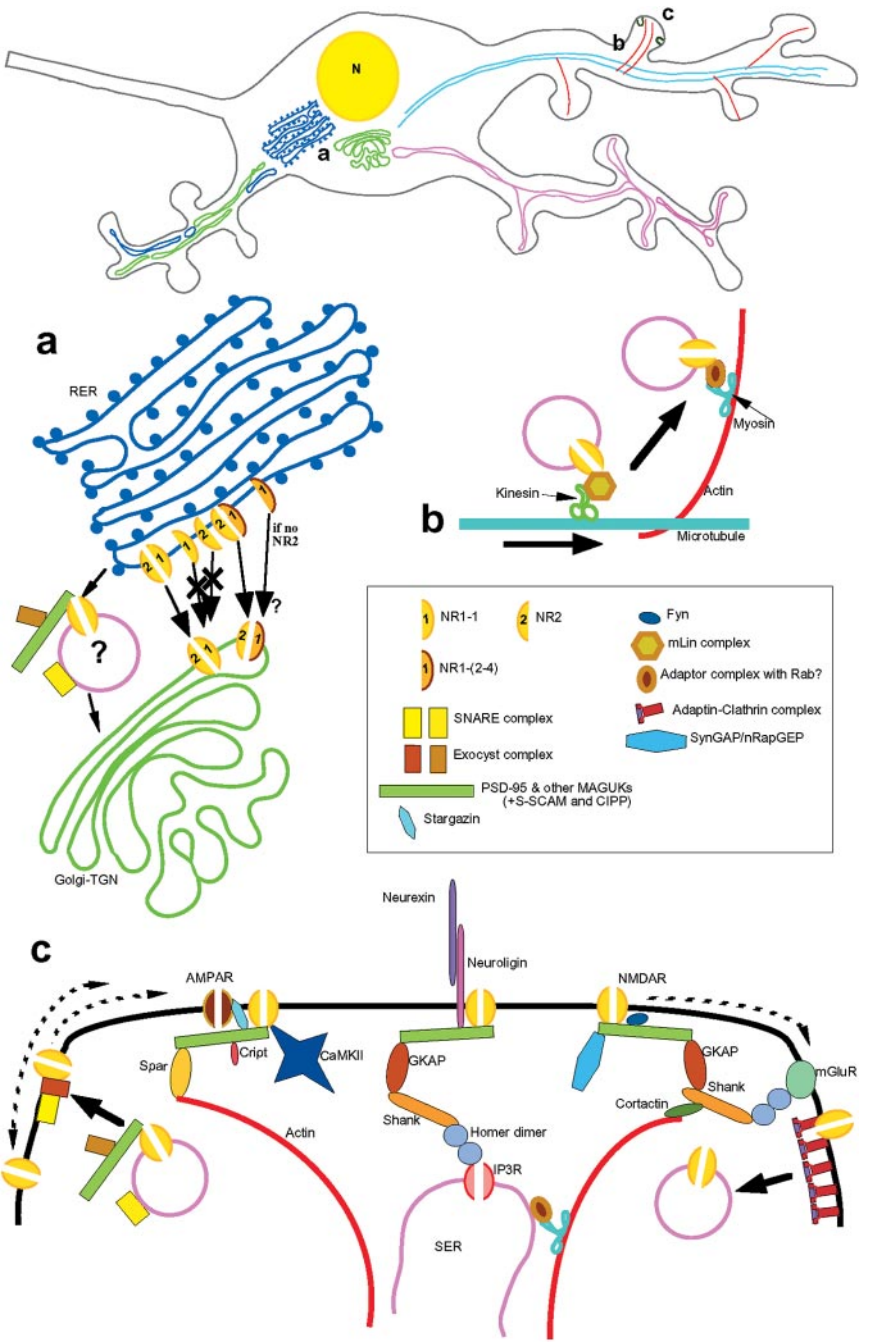


Figure 1 Important motifs and proteins that interact with the C-terminus domains of NR1 and NR2B (residues 839–1482). The splice variant NR1-1 (residues 834–938) is illustrated along with an alternatively spliced cassette, C2′. In NR1, Y837 has been implicated in endocytosis of the receptor (10). S890 and S896 are PKC phosphorylation sites and S897 is a PKA phosphorylation site (124). RRR is an ER retention signal in the C1 cassette of NR1. In NR2, Y843 has been implicated in endocytosis and may be phosphorylated (10). Y1472 is the major phosphorylation site of Fyn (118). Y1109 and Y1281 are phosphorylated by Src (125) and S1303 by CaMKII (126). Some phosphorylation sites were demonstrated on the homologous subunit, NR2A, and corresponding sites (*asterisks*) are present on NR2B. A cdk5 site (S1232) found on the NR2A subunit is not present on NR2B. YEKL is a consensus AP-2 adaptor binding site and plays a role in the endocytosis of the NMDAR (9). Proteins that interact with NR1 include α-actinin (alpha-actinin) (127), calmodulin (128), yotiao (129), neurofilament-L (130), spectrin (131), tubulin (132), and the MAGUKs (PSD-95, SAP102, PSD-93, and SAP97) (25). Proteins that interact with NR2 include CaMKII (133), α-actinin (127), PSD-95 (24), SAP102 (24), PSD-93 (24), S-SCAM (49), CIPP (134), Src (135), spectrin (131), phospholipase C-γ (136) and tubulin (132).



See legend on next page

Figure 2 (See figure on previous page) Diagram representing the major steps in the trafficking of NMDARs. The lower magnification drawing represents a typical neuron with an axon and three dendrites. As is typical of cells, there are major concentrations of RER and Golgi/TGN adjacent to the nucleus (*N*). Dendrites contain cytoskeletal elements (*upper dendrite*) and several systems of tubulovesicular organelles (*lower dendrites*) including ER, Golgi, TGN, and endosomes. For clarity, these elements are divided amongst the three dendrites in the drawing; major areas are indicated and enlarged in drawings (*a*)–(*c*). (*a*) Exit of NMDARs from the ER and subsequent processing and delivery to the cell membrane depends on subunit composition; NR2 subunits must combine (heteromeric complexes) with one or more NR1 variants (NR1-1 to NR1-4) for exit. In addition, NR1-2 to NR1-4 (presumably in homomeric complexes) are expressed on the surface in heterologous cells lacking NR2, but it is not clear whether this occurs in vivo. NMDARs leaving the ER probably traffic with the assistance of SNARE and exocyst complexes. In addition, PDZ-containing proteins, including MAGUKs, may link the NMDAR to components of the exocyst complex during this trafficking. (*b*) Passage of NMDARs along dendrites requires kinesin motors, which are linked to the receptors via an mLin complex. The receptors would travel on the surface of a vesicle or tubulovesicular organelle. Presumably, the receptor switches to myosin motors traveling along actin for passage into the spine and to the spine synapse. The proteins that connect NMDARs to myosin motors are not known but perhaps involve Rab proteins, as described for melanosome trafficking (137). The SNARE and exocyst complex components and MAGUKs, which probably also are associated with the receptors in these stages, have been excluded from the drawing for clarity. (*c*) NMDARs can reach the synapse via exocytosis onto the side of the synaptic spine and perhaps close to or within the active zone itself. At the synapse, NMDARs are linked to many other proteins and are endocytosed, probably from the sides of the spines, typically via adaptin/clathrin complexes. NMDARs move between the synapse, extrasynaptic sites, and sites of exocytosis and endocytosis (*dotted arrows*).