

AMPA Receptor Trafficking and the Control of Synaptic Transmission

Minireview

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Glutamate is the major excitatory neurotransmitter in mammalian brain. After release from the presynaptic terminal, glutamate acts on specific receptors that are clustered in the postsynaptic membrane. The AMPA-type glutamate receptor, a ligand-gated cation channel that opens upon glutamate binding, mediates most of the excitatory (depolarizing) postsynaptic response in glutamatergic synapses. Thus, changing the activity of AMPA receptors is a powerful way to control the strength of synaptic transmission, which is important for information storage in the brain.

AMPA receptors are formed from heteromeric (probably tetrameric) combinations of subunits GluR1–4. GluR subunits can be divided into two groups, GluR1 and GluR4, and GluR2 and GluR3, based on sequence similarity of their C-terminal cytoplasmic domains. In the hippocampus, GluR2 and GluR3 have short cytoplasmic tails of around 50 amino acids with a conserved C-terminal sequence (–SVKI) that binds to cytoplasmic PDZ proteins GRIP/ABP and PICK-1 (Sheng and Pak, 2000; Scannevin and Haganir, 2000). The longer GluR1 cytoplasmic tail (terminating in –ATGL) binds to a distinct set of proteins (Figure 1). In the hippocampus (part of the brain important for learning and memory and where many experiments on synaptic transmission are conducted), endogenous AMPA receptors are composed mainly of GluR1/GluR2 and GluR2/GluR3 heteromers (Wenthold et al., 1996). Recent work from Roberto Malinow and colleagues, culminating in a paper in *Cell* (Shi et al., 2001), has defined an important set of subunit-specific rules governing the delivery of AMPA receptors to synapses. These rules provide new insights into the postsynaptic trafficking of AMPA receptors and open inroads into the molecular mechanisms that tune synaptic strength.

Silent Synapses

A simple way to modify synaptic responses is to change the number of postsynaptic AMPA receptors available for activation by released glutamate. Electrophysiological and morphological evidence for such a mechanism has accumulated over the past few years, driven by the discovery of “silent synapses.” A subset of glutamatergic synapses in many parts of the CNS lack AMPA receptor currents. These so-called “silent synapses” nevertheless contain functional NMDA receptors, another type of ionotropic glutamate receptor that is per-

meable to calcium and that controls synaptic plasticity. Activation of NMDA receptors leads to the appearance of functional AMPA receptors (“unsilencing”) in previously silent synapses, thereby potentiating synaptic transmission (Malenka and Nicoll, 1999; Malinow et al., 2000). This postsynaptic potentiation could be due to the activation of nonfunctional AMPA receptors already existing in synapses (e.g., by phosphorylation), or by the delivery of new AMPA receptors to the postsynaptic membrane. Supporting the latter idea, the level of AMPA receptors in synapses is influenced by synaptic activity and varies greatly between different synapses, with some synapses being devoid of AMPA receptors (Nusser, 2000).

AMPA Receptor Delivery

Recent studies have revealed that AMPA receptors can translocate from nonsynaptic to synaptic sites, providing a cell biological basis for controlling the synaptic level of AMPA receptors and hence postsynaptic responsiveness (Lüscher et al., 2000; Malinow et al., 2000). The first direct evidence for movement of AMPA receptors came when Shi et al. (1999) showed that following strong synaptic stimulation and NMDA receptor activation, GFP-tagged GluR1 translocated from the main shaft of dendrites into spines, specialized dendritic protrusions on which excitatory synapses are formed.

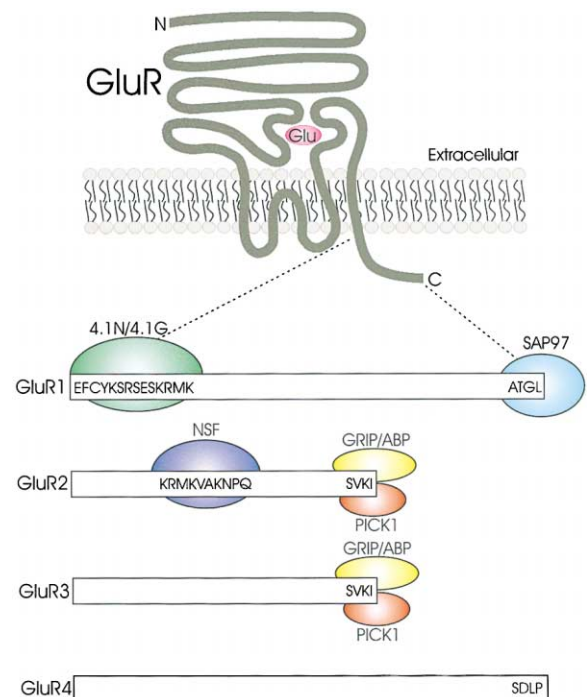


Figure 1. Membrane Topology and Cytoplasmic Protein Interactions of AMPA Receptor Subunits

The cytoplasmic C-terminal tails of GluR subunits are drawn to scale, with the sites of interaction with specific proteins depicted (single letter amino acid code). Little is known about GluR4 interactions.

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Upon overexpression, GluR1 subunits form homomeric receptors that have an electrophysiological property (inward rectification) which distinguishes them from endogenous heteromeric AMPA receptors in hippocampal pyramidal neurons. The appearance of inward rectification in excitatory postsynaptic currents (EPSCs) can be used to infer the delivery of functional GluR1 receptors to postsynaptic sites. Using this "electrophysiological tagging" approach, Malinow and colleagues showed that overexpression of GluR1 in neurons by Sindbis virus infection was insufficient for GluR1 incorporation into synapses. However, homomeric GluR1 receptors were "delivered" to synapses in response to NMDA receptor stimulation and activation of CaMKII (Hayashi et al., 2000). This recruitment required the C terminus of GluR1, which binds to the PDZ protein SAP97.

GluR4 behaves similarly to GluR1 in terms of NMDA receptor-dependent delivery to synapses; however, unlike GluR1, homomeric GluR4 does not require CaMKII activity (Zhu et al., 2000). GluR4 is expressed earlier in development than GluR1, thus these long-tailed GluR subunits may be involved in regulated synaptic delivery of AMPA receptors at different stages of brain ontogeny: GluR4 during maturation of synapses, and GluR1 during plasticity of mature synapses.

A major insight from Shi et al. (2001) was that unlike GluR1 and GluR4, the synaptic delivery of GluR2 is constitutive and independent of activity. For this experiment, the GluR2 ion channel had to be engineered such that it was inwardly rectifying and distinguishable electrophysiologically from endogenous AMPA receptors. Electrophysiologically-tagged GluR2 homomers accumulated in synapses, as judged by inward rectification. However, there was no change in the overall amplitude of EPSCs, implying that GluR2 receptors were being exchanged for existing endogenous synaptic AMPA receptors rather than being added to them. Consistent with this interpretation is that unlike GluR1 and GluR4, GluR2 homomeric receptors were not delivered to silent synapses (which lack AMPA receptors). The synaptic accumulation of GluR2 required its C-terminal PDZ binding sequence, suggesting that interaction with GRIP/ABP or PICK-1 is important. The cytoplasmic tail of GluR2 also binds to NSF (*N*-ethylmaleimide-sensitive factor), a hexameric ATPase involved in SNARE-mediated membrane fusion (Figure 1). The NSF binding site of GluR2 is also critical for synaptic incorporation of GluR2 receptors, though exactly how NSF acts to facilitate synaptic delivery of AMPA receptors remains elusive. Experiments with chimeric receptors and dominant interfering constructs showed that the cytoplasmic tails of GluR1 and GluR2 direct the distinctive trafficking behavior of these subunits: the long tail of GluR1 restricts the receptor from synapses in the absence of activity and mediates inducible delivery, whereas the short tail of GluR2 mediates constitutive delivery of AMPA receptors to synapses. In terms of synaptic delivery, GluR1 appears to act "dominantly" over GluR2 in heteromeric receptors, since coexpression of GluR1 prevents the constitutive synaptic incorporation of GluR2 (Shi et al., 2001). Importantly, the heteromeric GluR1/GluR2 receptor can be driven into synapses by CaMKII activation.

GluR3, often considered interchangeable with GluR2 because of sequence similarity, shows a distinct traf-

ficking behavior (Shi et al., 2001). GluR3 localizes in dendritic spines like GluR2, but by itself cannot incorporate into synapses based on electrophysiological tagging assays. However, GluR3 did not prevent the constitutive delivery of heteromeric GluR2/GluR3 receptors to synapses. The subunit-dependent trafficking behavior of AMPA receptors revealed in these studies has wider implications. The large families of subunits that encode most receptors and ion channels in neurons may have evolved to allow a more sophisticated control of the subcellular targeting of receptor/channels, rather than, or in addition to, an increased diversity of electrophysiological properties.

The electrophysiological tagging approach in virus-transfected brain slices is elegant in that it provides a functional assay of synaptic AMPA receptor delivery and allows a genetic dissection of the sequence determinants involved. However, it lacks the spatiotemporal resolution to uncover the precise cell biological mechanisms that underlie AMPA receptor trafficking to synapses. For instance, it is not clear whether the regulated "synaptic delivery" of electrophysiologically tagged AMPA receptors is mediated by exocytosis from intracellular pools directly into the postsynaptic membrane, or by lateral translocation from extrasynaptic regions on the neuronal surface, or both. Clearly, exocytosis of AMPA receptors is required at some point for synaptic delivery, since interfering with postsynaptic membrane fusion prevents synaptic potentiation and causes rundown of basal transmission (Lledo et al., 1998; Lüscher et al., 1999). However, the possibility that AMPA receptors are first inserted into nonsynaptic regions of the neuronal surface before translocation into synapses is raised by studies of the *Stargazer* mouse mutant (Chen et al., 2001). Stargazin-deficient cerebellar neurons lack surface AMPA receptors, and this phenotype is rescued by transfection of wild-type Stargazin. Importantly, however, a mutant Stargazin lacking its C terminus restores the surface but not the synaptic expression of AMPA receptors. This finding implies a two-step mechanism for AMPA receptor synaptic targeting, raising the possibility that surface translocation from nonsynaptic to synaptic sites may be a regulated step in synaptic delivery.

AMPA Receptor Internalization

Counterbalancing delivery is the removal of AMPA receptors from synapses. Internalization of AMPA receptors from the neuronal surface is emerging as a major means for removing synaptic AMPA receptors and depressing synaptic transmission (Carroll et al., 2001). AMPA receptor endocytosis is enhanced by factors that induce synaptic depression, including NMDA receptor stimulation and insulin (Lin et al., 2000; Ehlers, 2000; Beattie et al., 2000). Preventing AMPA receptor endocytosis blocks long-term synaptic depression in hippocampus and cerebellum (reviewed in Carroll et al., 2001). There has been substantial progress in elucidating the cell biological pathways of AMPA receptor internalization, which occurs via a dynamin-dependent mechanism. Even in basal conditions in culture, ~10%–20% of surface AMPA receptors are internalized from the surface in 10 min, this rate depending in part on endogenous synaptic activity in culture (Lin et al., 2000; Ehlers, 2000). The rapid rate of basal and regulated internalization appears specific for AMPA receptors, since NMDA

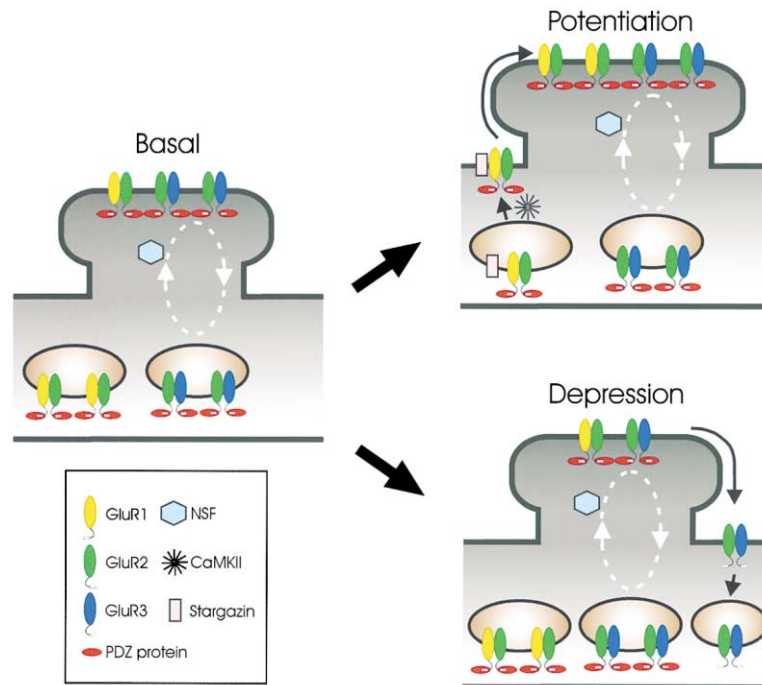


Figure 2. A Model of Postsynaptic AMPA Receptor Trafficking
See text.

receptors and other postsynaptic membrane proteins are relatively stable on the cell surface (Lin et al., 2000; Ehlers, 2000).

After endocytosis, most of the internalized AMPA receptors are recycled quickly to the neuronal surface and synaptic sites (Ehlers, 2000; Lin et al., 2000). NSF is likely involved in this continuous recycling process, since disruption of the interaction between NSF and GluR2 in neurons leads to rundown of EPSCs (Carroll et al., 2001 and references therein). Why would synapses undergo constitutive (and energetically wasteful) AMPA receptor cycling? In many metabolic pathways, “futile cycling” of substrates provides increased sensitivity of regulation, since small changes in the rate of one or both arms of the cycle result in larger changes in net flux. The cycling of AMPA receptors could offer a similar benefit of increased responsiveness of EPSC amplitude to small changes in delivery or removal rates.

Not all AMPA receptors need be recycled. A subset of AMPA receptors may be diverted after internalization to non-recycling endosomes or lysosomes under particular conditions (Ehlers, 2000; Lin et al., 2000). PDZ-mediated interactions may specify the intracellular sorting of AMPA receptors, as they do for other membrane receptors. Degradation of AMPA receptors after endocytosis is a possible mechanism for controlling synaptic AMPA receptor levels in the longer term.

Both GluR1- and GluR2-containing receptors are internalized in neurons, but the sequence determinants that control GluR endocytosis have not been examined in neurons. Studies in heterologous cells indicate that it is GluR2 rather than GluR1 that mediates insulin-stimulated endocytosis (Man et al., 2000; Lin et al., 2000). If borne out for activity-dependent internalization in neurons, the subunit-specific rules governing AMPA receptor endocytosis would be the opposite of those governing synaptic delivery.

A Model of AMPA Receptor Trafficking

The subunit-specific regulation of AMPA receptor delivery to synapses inspires the following model of AMPA receptor trafficking at a hippocampal synapse (Figure 2). In basal conditions, AMPA receptors (GluR1/GluR2 or GluR2/GluR3 heteromers) are concentrated in the postsynaptic membrane but also exist abundantly in endosomal compartments. Intracellular GluR2/GluR3 receptors undergo constant recycling with surface receptors in an activity-independent fashion. This exchange requires NSF and replaces existing synaptic AMPA receptors, thereby maintaining a constant level of AMPA receptors in the postsynaptic membrane. Meanwhile, intracellular GluR1/GluR2 heteromeric receptors lie dormant, held in check by the dominant subunit GluR1.

Upon NMDA receptor stimulation and CaMKII activation, GluR1 exocytosis is triggered, leading to surface targeting and synaptic delivery of GluR1/GluR2 heteromers. The site of surface insertion of AMPA receptors is unknown, but we propose that it occurs at extrasynaptic sites and is followed by rapid translocation into synapses. Either or both these steps require molecular interactions of the GluR1 C terminus, presumably with PDZ proteins. GluR1/GluR2-containing receptors can be recruited into synapses that lack AMPA receptors or that already have AMPA receptors, leading to unsilencing of silent synapses and enhancement of EPSCs. An important point made by Shi et al. (2001) is that the increased level of synaptic AMPA receptors can be maintained long-term by exchange with intracellular GluR2/GluR3 heteromers, thereby stabilizing synaptic “memory.”

In synaptic depression, the prevailing evidence suggests that GluR2- or GluR3-mediated endocytotic mechanisms are activated, perhaps involving phosphorylation of the GluR2/3 C terminus and consequent release from GRIP/ABP anchors (Carroll et al., 2001). Per-

haps GluR2 and/or GluR3 act as dominant subunits to control regulated endocytosis, in the same way that GluR1 appears to govern regulated delivery to synapses. It is unknown if GluR1/GluR2 and GluR2/GluR3 receptors "drift" outside of the synapse before being captured by the endocytotic machinery; however, this would facilitate the endocytosis of AMPA receptors without cointernalization of NMDA receptors and other synaptic membrane proteins.

Lynch and Baudry (1984) first proposed that activity-dependent changes in number or insertion of glutamate receptor to synaptic sites might underlie synaptic plasticity. Recent discoveries lend strong support to this idea; however, much remains to be learned about the mechanisms and significance of AMPA receptor trafficking. For instance, what are the kinetics of GluR exocytosis and synaptic accumulation? Where on the neuronal surface are AMPA receptors first inserted and where are they internalized? What are the precise molecular mechanisms that regulate AMPA receptor exocytosis and endocytosis? What are the molecules (Malinow's "slots") that determine the number of AMPA receptors in the postsynaptic membrane? Are there proteins that are cointernalized and coinserted with AMPA receptors? These are questions of general significance since regulated trafficking may be a universal mechanism for regulating the density of receptors in specific cell surface microdomains and for controlling cellular responsiveness to secreted factors.

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