35 Sweitzer, S.M. and Hinshaw, J.E. (1998) Dynamin undergoes a GTP-dependent conformational change causing vesiculation. *Cell* 93, 1021–1029
36 Stowell, M.H. *et al.* (1999) Nucleotide-dependent conformational changes in dynamin: evidence for a mechanochemical molecular spring. *Nat. Cell Biol.* 1, 27–32
37 Sever, S. *et al.* (1999) Impairment of dynamin's GAP domain stimulates receptor-mediated endocytosis. *Nature* 398, 481–486
38 Sever, S. *et al.* (2000) Garrotes, springs, ratchets, and whips: putting dynamin models to the test. *Traffic* 1, 385–392
39 Cremona, O. *et al.* (1999) Essential role of phosphoinositide metabolism in synaptic vesicle recycling. *Cell* 99, 179–188
40 Hughes, W.E. *et al.* (2000) Sac phosphatase domain proteins. *Biochem. J.* 350, 337–352
41 Cremona, O. and De Camilli, P. (2001) Phosphoinositides in membrane traffic at the synapse. *J. Cell Sci.* 114, 1041–1052
42 Cousin, M.A. (2000) Synaptic vesicle endocytosis: calcium works overtime in the nerve terminal. *Mol. Neurobiol.* 22, 115–128
43 Beattie, E.C. *et al.* (2000) Regulation of AMPA receptor endocytosis by a signaling mechanism shared with LTD. *Nat. Neurosci.* 3, 1291–1300
44 Lai, M.M. *et al.* (2000) The calcineurin binding protein Cain is a negative regulator of synaptic vesicle endocytosis. *J. Biol. Chem.* 275, 34017–34020
45 Lai, M.M. *et al.* (1999) The calcineurin–dynamin 1 complex as a calcium sensor for synaptic vesicle endocytosis. *J. Biol. Chem.* 274, 25963–25966
46 Cousin, M.A. and Robinson, P.J. (2000) Ca^{2+} inhibition of dynamin arrests synaptic vesicle recycling at the active zone. *J. Neurosci.* 20, 949–957
47 Lai, M.M. *et al.* (1998) Cain, a novel physiologic protein inhibitor of calcineurin. *J. Biol. Chem.* 273, 18325–18331
48 Artalejo, C.R. *et al.* (1996) Calmodulin is the divalent cation receptor for rapid endocytosis, but not exocytosis, in adrenal chromaffin cells. *Neuron* 16, 195–205
49 Engisch, K.L. and Nowycky, M.C. (1998) Compensatory and excess retrieval: two types of endocytosis following single step depolarizations in bovine adrenal chromaffin cells. *J. Physiol.* 506, 591–608
50 Kuromi, H. *et al.* (1997) An inhibitory role of calcineurin in endocytosis of synaptic vesicles at nerve terminals of *Drosophila* larvae. *Neurosci. Res.* 27, 101–113
51 Kuromi, H. and Kidokoro, Y. (1999) The optically determined size of exo/endo cycling vesicle pool correlates with the quantal content at the neuromuscular junction of *Drosophila* larvae. *J. Neurosci.* 19, 1557–1565
52 Powell, K.A. *et al.* (2000) Phosphorylation of dynamin I on Ser-795 by protein kinase C blocks its association with phospholipids. *J. Biol. Chem.* 275, 11610–11617
53 Earnest, S. *et al.* (1996) Phosphorylation of dynamin by ERK2 inhibits the dynamin-microtubule interaction. *FEBS Lett.* 396, 62–66
54 Rosales, J.L. *et al.* (2000) Cdk5/p25(nck5a) interaction with synaptic proteins in bovine brain. *J. Cell Biochem.* 78, 151–159
55 Floyd, S.R. *et al.* (2001) Amphiphysin binds the cdk5 regulatory subunit p35 and is phosphorylated by cdk5 and cdc2. *J. Biol. Chem.* 276, 8104–8110
56 Kariya, K. *et al.* (2000) Regulation of complex formation of POB1/epsin/adaptor protein complex 2 by mitotic phosphorylation. *J. Biol. Chem.* 275, 18399–18406
57 Wilde, A. and Brodsky, F.M. (1996) *In vivo* phosphorylation of adaptors regulates their interaction with clathrin. *J. Cell Biol.* 135, 635–645
58 Slepnev, V.I. *et al.* (1998) Role of phosphorylation in regulation of the assembly of endocytic coat complexes. *Science* 281, 821–824
59 Fowler, V.M. and Adam, E.J. (1992) Spectrin redistributes to the cytosol and is phosphorylated during mitosis in cultured cells. *J. Cell Biol.* 119, 1559–1572
60 Kim, J. *et al.* (1994) Phosphorylation, high ionic strength, and calmodulin reverse the binding of MARCKS to phospholipid vesicles. *J. Biol. Chem.* 269, 28214–28219
61 Hosaka, M. *et al.* (1999) A phospho-switch controls the dynamic association of synapsins with synaptic vesicles. *Neuron* 24, 377–387
62 Liu, J.P. *et al.* (1994) Dynamin I is a Ca^{2+}-sensitive phospholipid-binding protein with very high affinity for protein kinase C. *J. Biol. Chem.* 269, 21043–21050
63 Baron, V. *et al.* (1998) Dynamin associates with Src-homology collagen (Shc) and becomes tyrosine phosphorylated in response to insulin. *Endocrinology* 139, 3034–3037
64 Kranenburg, O. *et al.* (1999) Gi-mediated tyrosine phosphorylation of Grb2 (growth-factor-receptor-bound protein 2)-bound dynamin-II by lysophosphatidic acid. *Biochem. J.* 339, 11–14
65 Fingerhut, A. *et al.* (2000) Binding of AP2 to sorting signals is modulated by AP2 phosphorylation. *J. Biol. Chem.* 276, 5476–5492

Restless AMPA receptors: implications for synaptic transmission and plasticity

Christian Lüscher and Matthew Frerking

A central assumption in neurobiology holds that changes in the strength of individual synapses underlie changes in behavior. This concept is widely accepted in the case of learning and memory where LTP and LTD are the most compelling cellular models. It is therefore of great interest to understand, on a molecular level, how the brain regulates the strength of neuronal connections. We review a large body of evidence in support of the very straightforward regulation of synaptic strength by changing the number of postsynaptic receptors, and discuss the molecular machinery required for insertion and removal of AMPA receptors.

The hypothesis that insertion of AMPA receptors (AMPARs) underlies the increase of synaptic strength associated with LTP was put forward almost 20 years ago[1], but was largely ignored until the mid-1990s, resurfacing with quantal analysis of LTP (Ref. 2). However, only recently has it become the subject of direct experimental scrutiny.

The spark for the myriad of research published in the past few years in the field was experiments conducted independently by two groups[3,4], in which single connections between CA3 axons and CA1 pyramidal cells in acute hippocampal slices were functionally isolated and in some cases yielded only responses from NMDA receptors (NMDARs) and not AMPARs. Inducing LTP, however, caused the appearance of AMPAR mediated excitatory postsynaptic currents (EPSCs). This led to the proposal that a fraction of 'silent' synapses contain

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Table 1. AMPA receptor subunits: differences in structure and binding partners

Subunit	Splice variants[a]	PDZ domain recognition sites	NSF binding	Subunit contribution to endogenous receptors[d]
GluR1	–	Class 1 site[c]	No	Homomeric, w/ GluR2
GluR2	Short and long (s and l)[b]	Class 2 site	Yes	w/ GluR1 and GluR3
GluR3	–	Class 2 site	No	w/ GluR2
GluR4	Short and long (s and l)	Class 2 site (s only)	Yes (s only)	Mainly expressed <P10

[a]Splice variants listed are those in addition to the flip/flop splice site, which is present on all AMPAR subunits.
[b]Although GluR2 can be differentially spliced into short and long forms, the short form is predominantly expressed (>90%) and results presented are for the short form (reviewed in Ref. 53).
[c]Class 1 sites have a xS/TxV/L motif, class II have a XϕXϕ motif, where ϕ is a hydrophobic amino acid[54].
[d]Results presented are for endogenous receptors in hippocampal pyramidal neurons[32,55].

only NMDARs, and thus are functionally inactive at normal resting potential, but maintain the capability to undergo LTP, which would be expressed by AMPAR insertion. Although alternative explanations for these results have been proposed[5,6], synapses with NMDARs but not AMPARs have now been directly identified anatomically in cultured hippocampal neurons using immunofluorescence[7,8] and with immuno-gold preparations visualized by electron microscopy in hippocampal slices[9–11].

AMPARs move from the cytoplasm to the surface and back again

Constitutive recycling

If LTP expression is caused by postsynaptic AMPAR insertion, it must be the case that AMPARs can be inserted into and removed from the postsynaptic membrane on a relatively rapid time scale. To test this experimentally, CA1 pyramidal cells in acute hippocampal slices have been loaded with toxins that cleave vSNARE proteins or dominant-negative peptides that disrupt dynamin function, thus blocking exo- or endocytosis in the postsynaptic cell[12]. When exocytosis was blocked, EPSC amplitudes decreased over a time period of 30 min. Conversely, blocking endocytosis caused an increase in the EPSC amplitude, presumably as a result of an accumulation of AMPARs, with unabated exocytosis. Taken together these results suggest that AMPARs are constantly removed from and inserted into the synaptic membrane independent of activity, indicating a constitutive recycling of receptors. The existence of such a mobile pool of AMPARs is now confirmed by biochemical assays of receptor turnover and immunocytochemical methods in cultured hippocampal neurons and rat hippocampal slices[13]. Constitutive endocytosis of AMPARs has also been demonstrated in cultured hippocampal neurons[14] and the finding that this endocytosis is not accompanied by an eventual complete loss of synaptic AMPARs strongly implies constitutive recycling.

The available evidence suggests that the endocytotic arm of this recycling occurs via dynamin-dependent endocytosis of clathrin-coated vesicles[12], whereas the exocytotic arm requires some of the players in vesicle fusion that have been described in transmitter release, including SNAP (Ref. 15), synaptobrevin[12] and N-ethylmaleimide-sensitive fusion protein (NSF)[12,16–18]. An intriguing, but poorly understood, aspect of exocytosis is that GluR2 has been shown to specifically interact with NSF (see Table 1), which is enriched in the postsynaptic density (PSD) (for reviews see Refs 19,20). Disruption of this interaction leads to a decrease of synaptic responses (and concomitant loss of surface AMPAR immunostaining) similar in kinetics and magnitude to the decrease caused by the block of exocytosis. To date, the exact role of this interaction between the two proteins is still under investigation. Mice with the targeted disruption of GluR2 have a decreased ratio of AMPA:NMDA responses, corroborating that even on this longer time scale, the absence of GluR2 might also lead to a partial deficit of AMPAergic transmission[21].

Activity drives removal of AMPARs

In addition to the above described constitutive recycling, several forms of receptor activation strongly accelerate dynamin-dependent endocytosis of AMPARs (Ref. 22). NMDAR activation induces AMPAR internalization and recycling. This internalization requires the activation of protein phosphatases[13], one of which is the Ca^{2+}-dependent protein phosphatase calcineurin[23]. The crucial dephosphorylated target is still unknown. Internalization is accompanied by dephosphorylation of GluR1 at a PKA phosphorylation site[13]; however, dephosphorylation of a component of the endocytotic machinery might alternatively be required. Indeed, calcineurin is known to dephosphorylate several proteins involved in endocytosis, including dynamin[24,25].

AMPAR activation can also induce AMPAR internalization[26], but the mechanisms involved are controversial. Some evidence suggests that ligand binding by itself is a trigger for endocytosis[13,14], but it has also been reported that AMPAR-dependent internalization is caused by an AMPAR-dependent depolarization, which then induces Ca^{2+} influx through activation of voltage-gated Ca^{2+} channels[14,23,27], and would presumably act through the same mechanisms as NMDA-induced internalization. The ligand-dependent AMPAR internalization is not dependent on intracellular Ca^{2+} and is not accompanied by dephosphorylation of GluR1; notably, AMPARs internalized by this ligand-dependent mechanism are targeted to lysosomes rather than recycled[14]. Based on this, it has been suggested that the phosphorylation state of GluR1 at the PKA site might regulate the sorting of internalized receptors[13] (see Fig. 1).

In addition to glutamate, insulin, which is known to promote endocytosis in many cells, has also been found to enhance AMPAR internalization[28]. The mechanisms underlying this internalization remain

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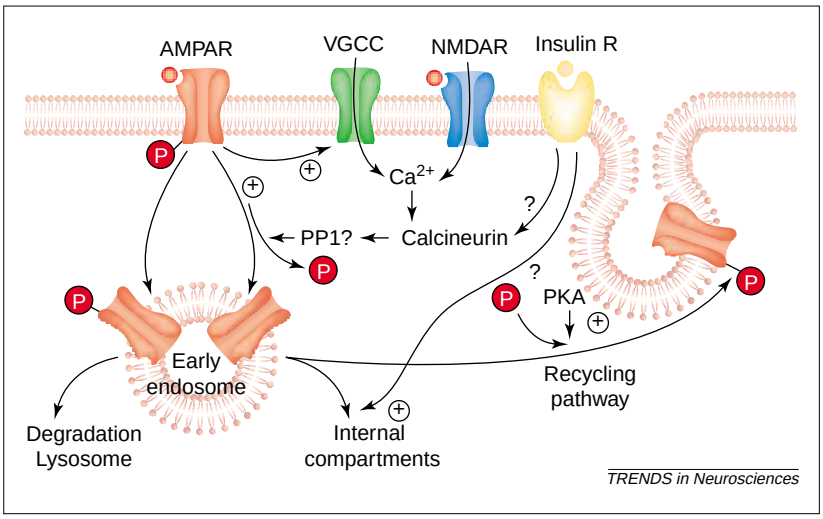

Fig. 1. Model of activity-driven internalization of AMPARs highlighting some unresolved issues. Glutamate (red dots) and insulin (yellow dot) can drive removal of AMPARs from the surface. Increased intracellular Ca^{2+} [via NMDAR or voltage-gated Ca^{2+} channel (VGCC)-activation] plays a crucial role in mediating this process, by activating the phosphatase calcineurin. However, some evidence suggests that ligand binding might be sufficient to drive removal on its own. Dephosphorylation of a PKA site near the C terminus of the GluR1 subunit targets internalized AMPARs to the recycling pathway. Conversely, ligand-dependent internalization of AMPARs will not lead to dephosphorylation and as a consequence, the receptors will be degraded in lysosomes. The molecular mechanism as well as the physiological role of insulin-driven internalization remains elusive.

largely unknown. Calcineurin could possibly be involved[14], but this has been contested[23]. Curiously, AMPARs internalized by insulin are initially targeted to early endosomes, but are then sorted into a separate, non-lysosomal internal compartment[14]. The insulin-induced internalization also requires the C-terminal tail of GluR2, but not GluR1, although the PDZ (PSD-95, Discs-large, ZO-1) recognition sequence at the C-terminal (see Table 1 and Box 1) is apparently not required[14]. Considerable work remains to elucidate how receptors are sent into this distinct sorting path, and to determine the physiological significance of this signaling cascade.

Insertion of AMPARs

Exocytosis of AMPARs is more difficult to investigate, partly because most stimulation protocols applied to cultured neurons lead to AMPAR endocytosis. Recently, using brief application of glycine to stimulate NMDARs of cultured hippocampal neurons[29] it was possible to reliably increase surface expression of AMPARs and synaptic efficacy. Both results were blocked by NMDAR antagonists and postsynaptically applied tetanus toxin. In organotypic slice cultures overexpressing GluR1, activation of NMDARs was sufficient to translocate this subunit into spines[30] and drive the synaptic insertion of AMPARs made exclusively of GluR1 subunits. It was found that perfusion of neurons with activated Ca^{2+}–calmodulin kinase type II (CaMKII) was sufficient to drive receptor insertion. Subcellular fractionation assays of receptor movement similarly suggest that NMDAR activation can result in AMPAR insertion[31]. Overexpression of different AMPAR subunits in CA1 neurons of organotypic hippocampal slice cultures indicates that receptors made of different subunits participate preferentially in various forms of AMPAR insertion. Although GluR2- and GluR3-containing receptors make up the bulk of constitutively recycling AMPARs, GluR1 dominates if receptors are inserted after appropriate NMDAR activation[32,33]. It is interesting to note that subunit-specificity of these interactions might vary from synapse to synapse. At the cerebellar parallel fiber-stellate cell synapses, high-frequency stimulation leads to insertion of GluR2-containing AMPARs and removal of GluR2-lacking AMPARs (Ref. 34).

Surface or synapse?

Variation in subunit composition could also explain differences between synaptic and extrasynaptic AMPARs. Knockout mice lacking GluR1 have a normal AMPAR complement as assessed by synaptic currents, but massive deficits in AMPAR responses to an exogenous agonist[35]. This indicates that under normal conditions, the bulk of surface GluR1 is not located at synapses, but is presumably at extrasynaptic sites. This raises the possibility that distinct mechanisms would be responsible for surface insertion and synaptic targeting. Additional support for this idea comes from the analysis of the stargazer mutant mouse. Stargazin mutants have a complete lack of AMPAR surface expression in cerebellar granule cells[36,37], and it appears that stargazin, through a direct interaction with AMPARs and PDZ-containing tethers, is directly involved in receptor targeting[38]. The stargazin protein has a PDZ-binding domain, and expression of a truncated form of stargazin lacking the C-terminal PDZ interaction site rescues responses to extrasynaptic AMPAR activation, but not spontaneous EPSCs (and therefore synaptic AMPARs). This dissociation suggests distinct pathways for surface insertion and synaptic targeting, with an, as yet, undetermined region of stargazin required for trafficking of AMPARs to the surface, and stargazin-PDZ interactions required for synaptic expression of AMPARs.

When do AMPARs take a break?

The numerous results demonstrating AMPAR movement raise the question of when, or whether, AMPARs ever stop moving. Is there a population of AMPARs that under normal conditions are stably held at the synapse? One suggestion that such a stable population exists is that the rundown of AMPAR-mediated EPSC amplitude induced by inhibitors of exocytosis is incomplete[12]. Another suggestion of such a stable population is that the glutamate-induced removal of AMPARs from the surface is not accompanied by an increase in the rate of endocytosis[27], suggesting that internalization is rate-limited by release of AMPARs from anchors at the synapse.

Tethering of AMPARs at synapses might ultimately be accomplished by interactions with the cytoskeleton;

Box 1. Are other receptors restless?

Recent attention has been focused on AMPA receptor movement and localization, but much research has also been carried out on other receptors. We briefly outline the ins-and-outs of two other major ionotropic receptors in the CNS: NMDA receptors and $GABA_A$ receptors.

NMDA receptors

It is clear that few of the mechanisms involved in AMPA receptor (AMPAR) trafficking apply to NMDA receptors (NMDARs); indeed, stable NMDAR EPSCs are frequently used as a control when manipulations that disrupt trafficking cause changes in AMPAR EPSCs. However, a recent study found that the subunit composition of synaptic NMDARs in visual cortex can change in an activity-dependent fashion over a few hours, suggesting that regulated receptor insertion can occur for NMDARs (Ref. a), and recent evidence suggests that protein kinase C (PKC) activity can trigger NMDAR insertion[b]. The mechanisms by which the NMDAR number at the synapse is regulated are unknown. Surprisingly, however, the widely examined interaction between PSD-95 and NMDARs seems not to be involved, because mice with a truncated PSD-95 that does not localize at synapses have normal synaptic NMDAR responses[c] and disruption of PSD-95 localization to the postsynaptic density does not affect synaptic clustering of NMDARs (Ref. c). Presumably other proteins regulate NMDAR number at the synapse; α-actinin is known to bind to NMDARs (Ref. d) and might stabilize NMDARs at the synapse, and PDZ-domain-interacting proteins other than PSD-95 might regulate synaptic expression of NMDARs.

$GABA_A$ receptors

It has been known for some time that $GABA_A$ receptor agonists can cause $GABA_A$ receptor internalization in neurons, and an agonist-independent receptor internalization that is activated by phorbol esters has been shown in heterologous cells (reviewed in Ref. e). Rapid receptor insertion at GABAergic synapses in response to insulin[f] and kindling-induced epilepsy[g] has also been reported. Moreover, blockade of postsynaptic endocytosis increases synaptic $GABA_A$ responses, indicating that constitutive endocytosis of these receptors regulates GABAergic synaptic strength[h]. The machinery for regulated $GABA_A$ receptor insertion and removal from the synapses therefore seems to be present, but little is known about the nature of this machinery. A probable player in these phenomena is gephyrin, a microtubule-binding protein that was first shown to cluster synaptic glycine receptors and subsequently also $GABA_A$ receptors[i]. However, a strong direct interaction between $GABA_A$ receptors and gephyrin has not been found[j], suggesting that other linker proteins might regulate $GABA_A$ receptor number at the synapse.

References

a Quinlan, E. *et al.* (1999) Rapid, experience-dependent expression of synaptic NMDA receptors in visual cortex *in vivo*. *Nat. Neurosci.* 2, 352–357
b Lan, J-Y. *et al.* (2001) Protein kinase C modulates NMDA receptor trafficking and gating. *Nat. Neurosci.* 4, 382–390
c Migaud, M. *et al.* (1998) Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. *Nature* 396, 433–439
d Wyszynski, M. *et al.* (1997) Competitive binding of alpha-actinin and calmodulin to the NMDA receptor. *Nature* 385, 439–442
e Barnes, E. (2000) Intracellular trafficking of $GABA_A$ receptors. *Life Sci.* 66, 1063–1070
f Wan, Q. *et al.* (1997) Recruitment of functional $GABA_A$ receptors to postsynaptic domains by insulin. *Nature* 388, 686–690
g Nusser, Z. *et al.* (1998) Increased number of synaptic $GABA_A$ receptors underlies potentiation at hippocampal inhibitory synapses. *Nature* 395, 172–177
h Kittler, J. *et al.* (2000) Constitutive endocytosis of $GABA_A$ receptors by an association with the adaptin AP2 complex modulates inhibitory synaptic currents in hippocampal neurons. *J. Neurosci.* 20, 7972–7977
i Kneussel, M. *et al.* (1999) Loss of postsynaptic $GABA_A$ receptor clustering in gephyrin-deficient mice. *J. Neurosci.* 19, 9289–9297
j Kirsch, J. *et al.* (1995) Targeting of glycine receptor subunits to gephyrin-rich domains in transfected human embryonic kidney cells. *Mol. Cell Neurosci.* 6, 450–461

indeed, GluR1 is reported to bind directly to the membrane cytoskeletal protein 4.1 (Ref. 39). Depolymerization of actin leads to a loss of AMPARs from the surface[27,40] and a decrease in synaptic efficacy[41]. Conversely, stabilization of actin has been reported to prevent glutamate-induced AMPAR internalization[27]; however, this result is controversial[23], and interpretation is further complicated by the observation that actin stabilization also interferes to some degree with the endocytotic process[27].

PDZ domain-containing proteins have also been described – glutamate receptor-interacting protein (GRIP), AMPAR binding protein (ABP), and protein interacting with C kinase (PICK1) – that bind to GluR2 and GluR3 through their class II PDZ domain recognition site, and could be involved in regulating the number of AMPARs at the synapse. Removal of the PDZ binding site from GluR2 reduces the accumulation of AMPAR levels at the synapse[42]. Moreover, disruption of the interaction between GluR2 and GluR3 and PDZ domains has been reported to block LTD in the hippocampus[43] and cerebellum[44]. These effects appear to be regulated by protein kinase C (PKC), which reduces binding of GluR2 to GRIP and ABP, but not PICK1, by phosphorylating AMPARs at a serine residue (Ser-880) near the C-terminal[45]. However, disruptions of the interaction between AMPARs and PDZ domains have little consistent effect on baseline synaptic transmission[43]. Moreover, the GluR2–PICK1 interaction appears to be required for cerebellar LTD (Ref. 44), but hippocampal LTD requires the GluR2–GRIP/ABP interaction[43]. Given these complexities, a comprehensive model seems far off; nevertheless, the evidence is strong for regulation of AMPAR localization by specific protein–protein interactions at the PDZ binding site.

Do AMPARs move during synaptic plasticity?

LTP

Triggered by the silent synapse hypothesis, the main drive for investigating AMPAR recycling derives from the desire to understand the cellular mechanism of synaptic plasticity, and, in particular, LTP and LTD.

If the enhanced synaptic efficacy associated with LTP is the result of an increase in the number of synaptic AMPARs, one has to postulate a mechanism, which delivers more receptors to the synapse. This could be achieved by a transient increase of the rate of exocytosis. Alternatively, in the presence of constant vesicle turnover, vesicles could be filled with more AMPARs, causing a larger number to be delivered to the surface with each fusion event. Both of these models predict exocytosis would be essential for LTP. Indeed cleavage of postsynaptic v-SNAREs by introducing either botulinum toxin or tetanus toxin into CA1 neurons prevented LTP expression[15]. In these experiments, the effects of these toxins on constitutive cycling were not observed. However, unlike studies directly examining constitutive cycling, these experiments were carried out with sharp electrodes. This has the technical advantage that wash-out of LTP seen with patch-clamp recording is prevented, but also has the limitation that considerable time (10–15 min) is required for the input resistance of the cell to recover after impalement by the electrode. The synaptic rundown caused by toxins that block exocytosis occurs on a 10–20 min time-scale, and is expected to have largely occurred before stabilization of sharp electrode recordings. Because of these technical considerations, the effects of these agents on constitutive recycling and LTP induction have not yet been examined by the same methods in the same cells. Nevertheless, by monitoring fluorescence of the styryl dye FM1-43 (Ref. 46) incorporated into postsynaptic membrane organelles, it was shown that high-frequency stimulation similar to the kind used to induce LTP led to Ca^{2+}-dependent exocytosis. Evidence that this postsynaptic exocytosis delivers AMPARs comes from experiments in which transfection of CA1 neurons in organotypic slice cultures with a green fluorescent protein (GFP)–GluR1 fusion protein and application of an LTP induction protocol triggers the translocation of this protein from dendritic shafts to the spines[30]. Using rectification as an electrophysiological tag, the authors could show that GluR1 homomeric receptors were indeed synaptically inserted[47]. LTP-induced AMPAR insertion appears to require the GluR1 subunit, as mice deficient in GluR1 lack LTP (Ref. 35), but can be rescued by transfection of the GluR1 subunit into CA1 pyramidal cells of organotypic cultures made from $GluR1^{-/-}$ mice[48]. Experiments with mutant GluR1 subunits determined that a PDZ interaction site located at the C terminal was needed for synaptic insertion[47].

LTD

If LTD is a functional reversal of LTP, and if LTP is the result of receptor insertion, then LTD might be expected to be caused by receptor internalization. Indeed, blockade of postsynaptic endocytosis using peptides that interfere with dynamin function prevents the induction of LTD (Ref. 12). In cultured hippocampal neurons, where changes in the synaptic AMPARs can be directly visualized, LTD induction led to decreases in synaptic efficacy that were associated with a loss of synaptic GluR1 puncta but not NMDAR1 puncta[49]. The pool of AMPARs internalized by LTD appears to overlap to a large extent with the constitutively recycling pool, as the rundown of EPSCs in the presence of botulinum toxin occludes subsequent LTD (Ref. 12). Furthermore, disrupting the NSF–GluR2 interaction also occluded LTD (Refs 12,50). However, LTD is also blocked by peptides that prevent the interaction between GluR2 and PDZ domains (see above), even though these peptides only have sporadic effects on baseline transmission. This result could indicate that some of the receptors internalized by LTD come from a stabilized pool, or alternatively that AMPAR–PDZ interactions do not exclude AMPARs from the recycling pool. It is interesting to note that in cerebellar LTD (an NMDAR-independent process), interference with the clathrin endocytotic complex or PDZ-domain interactions also block changes in synaptic efficacy[44,51]. This suggests that the dynamic modulation of the number of postsynaptic receptors is a mechanism to regulate synaptic strength shared by several forms of long-term synaptic plasticity.

Conclusion and unresolved issues

In conclusion, a substantial body of evidence supports the idea that AMPA receptors are dynamic components of the postsynaptic density. It is clear that at least some AMPARs are mobile, even under normal conditions, and that changes in AMPAR traffic can be induced by exogenous manipulations. Long-lasting forms of synaptic plasticity also appear to cause changes in the synaptic complement of AMPARs, although the available evidence does not preclude additional changes through other mechanisms (e.g. conductance change[52]). Preliminary insights have also been made in several areas, including how receptors are sorted intracellularly, what signals determine synaptic localization on the surface, and what proteins interact with AMPARs to allow trafficking to occur.

However, several questions remain. If surface AMPARs are constantly being exchanged with an intracellular pool, where are the intracellular AMPAR-containing vesicles? A pool of AMPARs in dendritic spines has been reported, but only at synapses that already have a pronounced synaptic AMPAR population[11]. One possibility is that these vesicles are located on dendrites near the spine for weaker synapses, but the relative paucity of these vesicles is troubling and a comprehensive, high-resolution search is warranted. Another issue to be resolved is whether there are distinct pools of AMPARs that recycle, or that are moved during synaptic plasticity, or that interact with proposed tethers. If such a distinction can be made, the features of AMPARs that restrict them to a given pool will also be of interest. Current evidence suggests that subunit composition might be such a

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feature, but the rules that regulate endogenous receptors, most of which contain multiple subunit types, are not yet elucidated. Another obvious possibility with some support is that phosphorylation and dephosphorylation events represent the cue that allows switching between pools. Although the answers to these questions remain obscure, one thing is clear – AMPA receptors don't get much rest, and neither will the people who study them.

References

1 Lynch, G. and Baudry, M. (1984) The biochemistry of memory: a new and specific hypothesis. *Science* 224, 1057–1063
2 Kullmann, D.M. (1994) Amplitude fluctuations of dual-component EPSCs in hippocampal pyramidal cells: implications for long-term potentiation. *Neuron* 12, 1111–1120
3 Liao, D.Z. *et al.* (1995) Activation of postsynaptically silent synapses during pairing induced LTP in CA1 region of hippocampal slice. *Nature* 375, 400–404
4 Isaac, J.T.R. *et al.* (1995) Evidence for silent synapses. Implications for the expression of LTP. *Neuron* 15, 427–434
5 Choi, S. *et al.* (2000) Postfusional regulation of cleft glutamate concentration during LTP at 'silent synapses'. *Nat. Neurosci.* 3, 330–336
6 Kullmann, D.M. *et al.* (1996) LTP of AMPA and NMDA receptor-mediated signals: evidence for presynaptic expression and extrasynaptic glutamate spill-over. *Neuron* 17, 461–474
7 Liao, D. *et al.* (1999) Regulation of morphological postsynaptic silent synapses in developing hippocampal neurons. *Nat. Neurosci.* 2, 37–43
8 Gomperts, S.N. *et al.* (2000) Distinct roles for ionotropic and metabotropic glutamate receptors in the maturation of excitatory synapses. *J. Neurosci.* 20, 2229–2237
9 Petralia, R.S. *et al.* (1999) Selective acquisition of AMPA receptors over postnatal development suggests a molecular basis for silent synapses. *Nat. Neurosci.* 2, 31–36
10 Takumi, Y. *et al.* (1999) Different modes of expression of AMPA and NMDA receptors in hippocampal synapses. *Nat. Neurosci.* 2, 618–624
11 Nusser, Z. *et al.* (1998) Cell type and pathway dependence of synaptic AMPA receptor number and variability in the hippocampus. *Neuron* 21, 545–559
12 Lüscher, C. *et al.* (1999) Role of AMPA receptor cycling in synaptic transmission and plasticity. *Neuron* 24, 649–658
13 Ehlers, M.D. (2000) Reinsertion or degradation of AMPA receptors determined by activity-dependent endocytic sorting. *Neuron* 28, 511–525
14 Lin, J.W. *et al.* (2000) Distinct molecular mechanisms and divergent endocytotic pathways of AMPA receptor internalization. *Nat. Neurosci.* 3, 1282–1290
15 Lledo, P.M. *et al.* (1998) Postsynaptic membrane fusion and long-term potentiation. *Science* 279, 399–403
16 Nishimune, A. *et al.* (1998) NSF binding to GluR2 regulates synaptic transmission. *Neuron* 21, 87–97
17 Osten, P. *et al.* (1998) The AMPA receptor GluR2 C terminus can mediate a reversible, ATP-dependent interaction with NSF and α- and β-SNAPs. *Neuron* 21, 99–110
18 Song, I. *et al.* (1998) Interaction of the N-ethylmaleimide-sensitive factor with AMPA receptors. *Neuron* 21, 393–400
19 Lin, J.W. and Sheng, M. (1998) NSF and AMPA receptors get physical. *Neuron* 21, 267–270
20 Jahn, R. (1998) Synaptic transmission: two players team up for a new tune. *Curr. Biol.* 8, R856–R858
21 Jia, Z. *et al.* (1996) Enhanced LTP in mice deficient in the AMPA receptor GluR2. *Neuron* 17, 945–956
22 Carroll, R.C. *et al.* (1999) Dynamin-dependent endocytosis of ionotropic glutamate receptors. *Proc. Natl. Acad. Sci. U. S. A.* 96, 14112–14117
23 Beattie, E.C. *et al.* (2000) Regulation of AMPA receptor endocytosis by a signaling mechanism shared with LTD. *Nat. Neurosci.* 3, 1291–1300
24 Liu, J.P. *et al.* (1994) Calcineurin inhibition of dynamin I GTPase activity coupled to nerve terminal depolarization. *Science* 265, 970–973
25 Marks, B. and McMahon, H.T. (1998) Calcium triggers calcineurin-dependent synaptic vesicle recycling in mammalian nerve terminals. *Curr. Biol.* 8, 740–749
26 Lissin, D.V. *et al.* (1999) Rapid, activation-induced redistribution of ionotropic glutamate receptors in cultured hippocampal neurons. *J. Neurosci.* 19, 1263–1272
27 Zhou, Q. *et al.* (2001) Contribution of cytoskeleton to the internalization of AMPA receptors. *Proc. Natl. Acad. Sci. U. S. A.* 98, 1261–1266
28 Man, H-Y. *et al.* (2000) Regulation of AMPA receptor-mediated synaptic transmission by clathrin-dependent receptor internalization. *Neuron* 25, 649–662
29 Lu, W. *et al.* (2001) Activation of synaptic NMDA receptors induces membrane insertion of new AMPA receptors and ltp in cultured hippocampal neurons. *Neuron* 29, 243–254
30 Shi, S.H. *et al.* (1999) Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. *Science* 284, 1811–1816
31 Broutman, G. and Baudry, M. (2001) Involvement of the secretory pathway for AMPA receptors in NMDA-induced potentiation in hippocampus. *J. Neurosci.* 21, 27–34
32 Zhu, J.J. *et al.* (2000) Postnatal synaptic potentiation: delivery of GluR4-containing AMPA receptors by spontaneous activity. *Nat. Neurosci.* 3, 1098–1106
33 Shi, S. *et al.* (2001) Subunit-specific rules governing AMPA receptor trafficking to synapses in hippocampal pyramidal neurons. *Cell* 105, 331–343
34 Liu, S.Q. and Cull-Candy, S.G. (2000) Synaptic activity at calcium-permeable AMPA receptors induces a switch in receptor subtype. *Nature* 405, 454–458
35 Zamanillo, D. *et al.* (1999) Importance of AMPA receptors for hippocampal synaptic plasticity but not for spatial learning. *Science* 284, 1805–1811
36 Hashimoto, K. *et al.* (1999) Impairment of AMPA receptor function in cerebellar granule cells of ataxic mutant mouse stargazer. *J. Neurosci.* 19, 6027–6036
37 Chen, L. *et al.* (1999) Impaired cerebellar synapse maturation in waggler, a mutant mouse with a disrupted neuronal calcium channel gamma subunit. *Proc. Natl. Acad. Sci. U. S. A.* 96, 12132–12137
38 Chen, L. *et al.* (2000) Stargazing regulates synaptic targeting of AMPA receptors by two distinct mechanisms. *Nature* 408, 936–943
39 Shen, L. *et al.* (2000) Regulation of AMPA receptor GluR1 subunit surface expression by a 4.1N-linked actin cytoskeletal association. *J. Neurosci.* 20, 7932–7940
40 Allison, D.W. *et al.* (1998) Role of actin in anchoring postsynaptic receptors in cultured hippocampal neurons: differential attachment of NMDA versus AMPA receptors. *J. Neurosci.* 18, 2423–2436
41 Kim, C.H. and Lisman, J.E. (1999) A role of actin filament in synaptic transmission and long-term potentiation. *J. Neurosci.* 19, 4314–4324
42 Osten, P. *et al.* (2000) Mutagenesis reveals a role for ABP/GRIP binding to GluR2 in synaptic surface accumulation of the AMPA receptor. *Neuron* 27, 313–325
43 Daw, M.I. *et al.* (2000) PDZ proteins interacting with C-terminal GluR2/3 are involved in a PKC-dependent regulation of AMPA receptors at hippocampal synapses. *Neuron* 28, 873–886
44 Xia, J. *et al.* (2000) Cerebellar long-term depression requires PKC-regulated interactions between GluR2/3 and PDZ domain-containing proteins. *Neuron* 28, 499–510
45 Chung, H.J. *et al.* (2000) Phosphorylation of the AMPA receptor subunit GluR2 differentially regulates its interaction with PDZ domain-containing proteins. *J. Neurosci.* 20, 7258–7267
46 Maletic-Savatic, M. and Malinow, R. (1998) Calcium-evoked dendritic exocytosis in cultured hippocampal neurons. Part I: trans-Golgi network-derived organelles undergo regulated exocytosis. *J. Neurosci.* 18, 6803–6813
47 Hayashi, Y. *et al.* (2000) Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. *Science* 287, 2262–2267
48 Mack, V. *et al.* (2001) Conditional restoration of hippocampal synaptic potentiation in Glur-A-deficient mice. *Science* 292, 2501–2504
49 Carroll, R.C. *et al.* (1999) Rapid redistribution of glutamate receptors contributes to long-term depression in hippocampal cultures. *Nat. Neurosci.* 2, 454–460
50 Lüthi, A. *et al.* (1999) Hippocampal LTD expression involves a pool of AMPARs regulated by the NSF-GluR2 interaction. *Neuron* 24, 389–399
51 Wang, Y.T. and Linden, D.J. (2000) Expression of cerebellar long-term depression requires postsynaptic clathrin-mediated endocytosis. *Neuron* 25, 635–647
52 Benke, T.A. *et al.* (1998) Modulation of AMPA receptor unitary conductance by synaptic activity. *Nature* 393, 793–797
53 Braithwaite, S.P. *et al.* (2000) Interactions between AMPA receptors and intracellular proteins. *Neuropharmacology* 39, 919–930
54 Sheng, M. and Sala, C. (2001) Pdz domains and the organization of supramolecular complexes. *Annu. Rev. Neurosci.* 24, 1–29
55 Wenthold, R.J. *et al.* (1996) Evidence for multiple AMPA receptor complexes in hippocampal CA1/CA2 neurons. *J Neurosci* 16, 1982–1989