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Reconciling Endosomes Supply AMPA Receptors for LTP

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Long-term potentiation (LTP) of synaptic strength, the most established cellular model of information storage in the brain, is expressed by an increase in the number of postsynaptic AMPA receptors. However, the source of AMPA receptors mobilized during LTP is unknown. We report that AMPA receptors are transported from recycling endosomes to the plasma membrane for LTP. Stimuli that triggered LTP promoted not only AMPA receptor insertion but also generalized recycling of cargo and membrane from endocytic compartments. Thus, recycling endosomes supply AMPA receptors for LTP and provide a mechanistic link between synaptic potentiation and membrane remodeling during synapse modification.

Information storage and processing in the brain involve modification of synaptic strength. This process is exemplified by long-term potentiation (LTP) in the hippocampus (1, 2), the dominant cellular model for learning and memory. LTP-inducing stimuli increase the number of functional AMPA-type glutamate receptors at the postsynaptic membrane (3–8), leading to an increase in AMPA receptor-mediated transmission at excitatory synapses (9). However, the source of AMPA receptors mobilized for LTP is unknown.

AMPA receptors inserted during LTP are thought to originate from an intracellular reserve pool (10, 11). In hippocampal dendrites, AMPA receptors undergo continuous cycling into and out of the postsynaptic membrane (12, 13). Upon internalization, AMPA receptors are sorted in early endosomes either to a specialized recycling endosome compartment for reinsertion to the plasma membrane or to late endosomes and lysosomes for degradation (fig. S1) (13, 14). We hypothesized that dendritic endosomes may contain a reserve pool of AMPA receptors available for modifying synaptic strength.

To test whether transport from recycling endosomes to the plasma membrane was required to maintain the supply of AMPA receptors at the dendritic plasma membrane, we took advantage of a mutant version of the Eps15-homology domain protein EHD1/Rme1 (Rme1-G429R) as well as a constitutively inactive guanosine diphosphate–bound form of the small guanosine triphosphatase (GTPase) Rab11a (Rab11a-S25N). Both of these mutants selectively impair endocytic recycling by preventing trafficking from recycling endosomes to the plasma membrane (fig. S1) (15, 16). To detect AMPA receptors at the cell surface, we performed live-cell immunocytochemistry on hippocampal neurons with the use of antibodies directed against extracellular epitopes of the AMPA receptor subunit GluR1. Upon blockade of endocytic recycling, the surface expression of AMPA receptors was markedly reduced (Fig. 1A). Conversely, expression of wild-type Rme1 to augment recycling caused a corresponding increase in surface AMPA receptors (Fig. 1A).

To determine whether the reduction in surface AMPA receptors in neurons expressing (red). Arrows indicate colocalization. Scale bar, 2 μm. (E) Endogenous NMDA (NMDAR, NR1) and AMPA receptors (AMPA, GluR1) were visualized by immunocytochemistry on neurons expressing GFP, Rme1-G429R, or Rab11a-S25N. Arrowheads indicate synapses containing both NR1 and GluR1. Arrows indicate synapses containing NR1 without detectable GluR1. Scale bar, 2 μm. (F) Average pixel intensity ratios of GluR1 to NR1 on individual synapses were measured. Values of n are 79, 48, and 88 synapses on four to six neurons each. **P < 0.001 relative to GFP, t test. Error bars indicate SEM. AFU, arbitrary fluorescence units.

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Rme1-G429R and Rab11a-S25N was due to decreased reinsertion of internalized receptors, we monitored AMPA receptor endocytosis and recycling by using live-cell antibody uptake assays and quantitative fluorescence microscopy (13, 17). In neurons expressing Rme1-G429R or Rab11a-S25N, endocytosis of recombinant AMPA receptors containing an N-terminal hemagglutinin (HA) epitope tag immediately followed by a thrombin protease cleavage site (HA/T-GluR1) was indistinguishable from that in green fluorescent protein (GFP)-expressing control neurons (Fig. 1, B and C). After endocytosis, the majority of internalized AMPA receptors recycled within 1 hour in control neurons (Fig. 1, B and C). In contrast, internalized AMPA receptors were retained intracellularly and failed to cycle back to the plasma membrane in neurons expressing Rme1-G429R or Rab11a-S25N (Fig. 1, B and C). Total AMPA receptor expression levels were unchanged by the expression of Rme1-G429R or Rab11a-S25N (fig. S2). Thus, transport from recycling endosomes maintains surface AMPA receptors by regulating their recycling to the postsynaptic membrane.

Expression of Rme1-G429R caused a notable redistribution of the total pool of AMPA receptors from dendritic spines to endosomal compartments in the dendritic shaft (fig. S3). In further experiments, we simultaneously monitored the endocytic trafficking of endogenous AMPA receptors and transferrin receptors (TfRs) originating from the cell surface (17) and found that expression of Rme1-G429R resulted in the trapping of both endocytic cargos in recycling endosomes without affecting their endocytosis (Fig. 1D). To test whether the redistribution from spines to endosomes was selective for AMPA receptors, we double-labeled hippocampal neurons expressing Rme1-G429R or Rab11a-S25N with antibodies against the AMPA receptor subunit GluR1 and the N-methyl-D-aspartate (NMDA) receptor subunit NR1. There was a marked decrease in the average AMPA receptor-to-NMDA receptor fluorescence intensity ratio at hippocampal synapses (Fig. 1, E and F), indicating a selective loss of AMPA receptors. Thus, blocking membrane trafficking out of recycling endosomes trapped AMPA receptors in TfR-positive recycling compartments within dendrites, and endocytic recycling was required to maintain AMPA receptors, but not NMDA receptors, at excitatory synapses.

We next tested whether AMPA receptors recruited during LTP use the transport pathway from recycling endosomes to arrive at synapses. We used a cell culture model of LTP employing glycine stimulation to activate synaptic NMDA receptors (6, 18, 19) together with selective detection of newly inserted AMPA receptors using HA/T-GluR1 (6, 11, 17, 19, 20). As expected, 20 to 25 min after a brief application of glycine (200 μM for 3 min), newly inserted AMPA receptors were significantly increased compared to unstimulated control cells (Fig. 2, A and B). This increase was blocked by coapplication of the NMDA receptor antagonist D-AP5 (Fig. 2, A and B) (6, 19). Hippocampal neurons expressing Rme1-G429R and Rab11a-S25N to prevent transport from recycling endosomes failed to exhibit a detectable increase in AMPA receptor insertion upon glycine stimulation (Fig. 2B). In contrast, neurons expressing wild-type versions of Rme1 and Rab11a exhibited robust glycine-induced AMPA receptor insertion (Fig. 2B). Expression of Rab6a-T27N, a dominant negative version of the Golgi-associated Rab family GTPase Rab6a (21–23), had no effect on glycine-induced AMPA receptor insertion (Fig. 2B). Expression of a soluble fragment of the recycling endosome SNARE [SNAP (soluble N-ethylmaleimide–sensitive factor attachment protein) receptor] protein syntaxin 13 (Syn13ATM) (24) that forms cognate SNARE complexes but blocks membrane fusion due to its inability to bind membranes (25) completely abolished glycine-induced AMPA receptor insertion (Fig. 2B). Expression of a soluble fragment of the late endosome SNARE protein syntaxin 7 (25) had no effect on LTP-induced AMPA receptor insertion (Fig. 2B). Together, these results provide strong evidence that recycling endosomes supply newly inserted AMPA receptors during LTP.

To determine whether AMPA receptors inserted during LTP derive originally from
the plasma membrane or are newly synthesized, we tested the effect of protein synthesis inhibition. Inhibiting protein synthesis in hippocampal neurons (20 \( \mu M \) anisomycin for 4 hours) had no effect on glycine-induced insertion of HA/T-GluR1 (Gly/basal insertion: untreated, 156 ± 16% and n = 21; anisomycin, 144 ± 13% and n = 21; P = 0.63), consistent with the notion that mobilized AMPA receptors were not newly synthesized. Next, we tested whether previously endocytosed AMPA receptors were delivered to the plasma membrane in response to LTP-inducing stimuli. With use of live-cell antibody feeding, we visualized internalized HA/T-GluR1 receptors and followed the loss of intracellular HA/T-GluR1 fluorescence as a direct measure of AMPA receptor recycling back to the plasma membrane (17). Under these conditions, internalized AMPA receptors recycled to the plasma membrane, and this recycling was enhanced twofold by glycine stimulation (Fig. 2C, top). The loss of internalized receptors was not due to degradation, because the sum total of intracellular plus recycled AMPA receptors was constant under all conditions (Fig. 2C, bottom). After the standard 5-min thrombin treatment (19) (Fig. 2A), glycine stimulation caused a robust insertion of HA/T-GluR1 at the dendritic plasma membrane (Fig. 2, D and E). In contrast, after prolonged thrombin incubation (4 hours at 37°C) to remove HA tags from all HA/T-GluR1 receptors cycling to the plasma membrane during this time period, glycine-induced insertion of HA/T-GluR1 was undetectable (Fig. 2, D and E). Thus, AMPA receptors inserted during LTP derived from a pool that was present at the plasma membrane in the previous 4 hours. Prolonged thrombin treatment had no effect on cell viability (Fig. S4), the internalization or recycling of transferrin (Tf) (fig. S4), or ongoing basal insertion of HA/T-GluR1 (Fig. 2, D and E). In neurons expressing Rme1-G429R, Rab11a-S25N, or Syntaxin13TM, depletion of the cycling pool of HA/T-GluR1 had no effect on AMPA receptor insertion (Fig. 2E), establishing the specificity of these reagents for disrupting endocytic recycling but not de novo insertion of newly synthesized receptors. Thus, AMPA receptors mobilized during LTP-inducing stimuli derive from a recycled pool.

To investigate the physiological role of recycling endosome transport in synaptic plasticity, we used whole-cell patch-clamp recordings to measure LTP at the Schaffer collateral–CA1 synapse in hippocampal slices. To interfere with endocytic recycling, we infected CA1 pyramidal neurons with recombinant Sindbis virus to express GFP, GFP-Rme1-G429R, or GFP-Rab11a-S25N before electrophysiological measurements. In control neurons expressing GFP, brief high-frequency afferent stimulation (HFS) elicited robust LTP (Fig. 3A). When transport from recycling endosomes to the plasma membrane was blocked by expressing Rme1-G429R or Rab11a-S25N, LTP was absent (Fig. 3A). This effect was not due to generalized changes in synaptic function because no differences between neurons transfected with different constructs were seen in the sizes of evoked excitatory postsynaptic currents (EPSCs) (for Rme1-G429R, 39.3 ± 10.9 pA and n = 8; P = 0.45; for Rab11a-S25N, 34.1 ± 9.1 pA and n = 7; P = 0.83; for control GFP, 32.4 ± 3.4 pA and n = 17), paired-pulse facilitation (for GFP, 2.24 ± 0.21 and n = 11; for Rme1-G429R, 2.65 ± 0.26 and n = 12; P = 0.26), posttetanic potentiation (PTP) (fig. S5), or the depolarization envelope in response to HFS (for GFP, 0.68 ± 0.07 V s and n = 4; for Rme1-G429R, 0.70 ± 0.07 V s and n = 4; P = 0.84). Disruption of endocytic recycling by expressing Rme1-G429R also abolished LTP induced by pairing postsynaptic depolarization with afferent stimulation (fig. S6). Expression of GFP-Rab6a-T27N to disrupt vesicular transport at the level of the Golgi had no effect on LTP (fig. S7). NMDA receptor–mediated synaptic currents in neurons expressing Rme1-G429R did not differ from those in neighboring uninfected neurons within the same slice (for Rme1-G429R, 30.1 ± 4.3 pA and n = 6; for control, 23.6 ± 3.7 pA and n = 6; P = 0.36), indicating that the absence of LTP could not be accounted for by a decrease in functional NMDA receptors and suggesting the idea that LTP-inducing stimuli promoted the recycling of AMPA receptors but not NMDA receptors.

To rule out effects of chronic disruption of endocytic recycling, we delivered purified syntaxin13TM or syntaxin7TM fusion proteins intracellularly by inclusion in the pipette solution used to record CA1 cells in acutely prepared hippocampal slices. After brief HFS, CA1 neurons filled with syntaxin7TM exhibited robust LTP (Fig. 3B). In contrast, LTP was blocked in those neurons filled with syntaxin13TM (Fig. 3B). Perfusion of syntaxin13TM in the absence of HFS had no effect on basal EPSC amplitudes over the 30-min course of the experiment (Fig. 3B), and neither syntaxin13TM nor syntaxin7TM infusion altered PTP (fig. S5). The rapidity of action of syntaxin13TM (10 to 13 min) indicated that even a very brief block of endocytic recycling was sufficient to prevent LTP.

Given our finding that AMPA receptors come from recycling endosomes for LTP (Figs. 2 and 3), we hypothesized that LTP is accompanied by an overall increase in membrane trafficking from recycling endosomes to the plasma membrane, providing a potential source of membrane and other proteins for synapse and spine remodeling (26, 27).
To test this hypothesis, we measured the effect of LTP-inducing stimuli on overall endocytic recycling in hippocampal neurons by using the classic constitutive recycling cargo Tf (15). Hippocampal neurons exhibited robust uptake of fluorescent Alexa 568–Tf (Alx-Tf; Molecular Probes, Eugene, OR) into recycling endosomes at steady state (Fig. 4, A and B; 60-min saturation). A subsequent 25-min period of further incubation in excess unlabeled Tf revealed a loss of intracellular Alx-Tf, reflecting ongoing basal recycling (Fig. 4, A and B). Brief application of glycine sufficient to induce LTP (6, 18) accelerated the recycling of intracellular Alx-Tf (Fig. 4, A and B). Recycling was enhanced roughly twofold (Fig. 4, A and B), an effect quantitatively similar to observed changes in AMPA receptor insertion (Fig. 2) and AMPA receptor-mediated EPSCs (Fig. 3). The glycine-induced increase in Tf recycling was prevented by coapplication of D-AP5 (Fig. 4, A and B), indicating a requirement for NMDA receptor activation. The enhanced Tf recycling triggered by glycine was absent in neurons expressing Rme1-G429R (Fig. 4B). In addition, astrocytes in the same mixed cultures displayed robust endocytosis and recycling of Alx-Tf, but the recycling was unaffected by glycine stimulation (Fig. 4C). Thus, LTP-inducing stimuli enhance overall transport from recycling endosomes to the neuronal plasma membrane, and membrane trafficking events associated with LTP are not limited to AMPA receptors.

We have demonstrated that transport of AMPA receptors between the plasma membrane and recycling compartments maintains AMPA receptors in a dynamic pool that is subject to rapid mobilization in response to NMDA receptor activation (fig. S8). Such kinetic retention or kinetic mobilization from recycling endosomes provides a powerful mechanism for tightly tuning the abundance of postsynaptic AMPA receptors by shifting the steady-state equilibrium between intracellular compartments and the postsynaptic membrane. Our results suggest that mechanisms linking LTP induction and expression may converge on the recycling endosome, providing a potential framework for clarifying and merging the diverse molecular mechanisms for LTP. Several effectors of endosome fusion and vesicular transport are Ca2+-sensitive (28, 29) or targets of calcium/calmodulin-dependent protein kinase II (30), two primary upstream signals for LTP (2). Certain endosomal proteins are restricted to endosomes that senses and responds to NMDA receptor–mediated Ca2+ influx during LTP as well as the regulatory proteins within endosomes that associate directly or indirectly with AMPA receptors.

Stimulus-dependent recycling of endocytic cargo provides an attractive cellular mechanism linking the insertion of AMPA receptors to morphological changes in synapse structure during LTP. We have shown that, in response to LTP-inducing stimuli, overall transport from recycling endosomes to the neuronal plasma membrane is enhanced. This enhanced recycling provides more AMPA receptors and ensures enhanced synaptic efficacy, but also supplies additional lipid membrane and other yet-unknown proteins that could mediate spine growth, post-synaptic density expansion, and more globally alter messenger release and more globally alter postsynaptic composition. By coupling synaptic potentiation with membrane remodeling, LTP-induced transport from recycling endosomes to the plasma membrane provides an appealing unifying mechanism for activity-dependent synapse modification.

**References and Notes**

17. Materials and methods are available as supporting material on Science Online.

![Fig. 4](https://www.sciencemag.org/cgi/content/full/305/5692/1972/DC1)

**Supporting Online Material**

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**Materials and Methods**

Figs. S1 to S8

**References**

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