Research News

A recipe for ridding synapses of the ubiquitous AMPA receptor

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Getting AMPA receptors into and out of synapses represents an important mechanism for changing synaptic strength, but the signals that target AMPA receptors for removal from the synaptic membrane are incompletely understood. A recent study in *Ceanorhabditis elegans* suggests that ubiquitination of AMPA receptors is one important signal that targets these receptors for endocytosis.

Plastic changes in the nervous system are stored, in part, through changes in the strengths of synaptic connections. Recent work has suggested that changes in the number of glutamate receptors clustered at synaptic sites play an important role in several forms of long-lasting activity-dependent synaptic plasticity, including long-term potentiation (LTP), long-term depression (LTD) and synaptic scaling [1–4]. These observations have launched a wave of studies on the mechanisms by which glutamate receptors – in particular, ionotropic AMPA receptors – move into and out of the postsynaptic membrane, and how this process is regulated by activity [4]. Synaptic receptors, like all integral membrane proteins, turn over in the membrane with a half-life determined by the rates of exocytosis and endocytosis of receptor-containing vesicles. For AMPA receptors, this turnover appears to be rapid – in the order of minutes to hours [4]. One mechanism by which activity could influence the accumulation of synaptic AMPA receptors would, therefore, be to alter the rates of receptor exocytosis or endocytosis. A second mechanism would be to induce a one-time regulated insertion or removal of receptors [3]. Both are likely to be important plasticity mechanisms at glutamatergic synapses, but the signals that target AMPA receptors for insertion or removal are incompletely understood. In a recent paper, Burbea *et al.* used an elegant set of genetic manipulations in the nematode *Caenorhabditis elegans* to show that ubiquitination is a key process regulating AMPA-receptor endocytosis [5].

Tagging receptors for endocytosis

Although many insights have been gained into the processes governing AMPA-receptor turnover, it is still unclear how synaptic receptors are targeted for removal from the postsynaptic membrane. Plasticity mechanisms that involve a loss of synaptic AMPA receptors, such as some forms of LTD, appear to rely on the same endocytotic machinery that has been well characterized in presynaptic vesicle endocytosis [6,7]. Vesicle endocytosis depends on cytoplasmic-coat proteins such as clathrin, which assemble into a spherical shell and slowly pinch the vesicle off from the surrounding membrane. Once removed from the membrane via endocytosis, AMPA receptors can be sorted for reinsertion into the plasma membrane or for degradation, and this sorting process is itself regulated [8–10].

'ubiquitination is a key process regulating AMPA-receptor endocytosis'

Most forms of LTD involve modest (20–30%) reductions in synaptic strength, suggesting that not all synaptic receptors are eligible for regulated removal from the synaptic membrane. Similarly, constitutive endocytosis removes only a small fraction of total synaptic receptors per unit time. This raises the possibility that some AMPA receptors are tagged for removal, either as a function of time in the membrane (constitutive cycling) or in response to signals mediating regulated endocytosis. Ubiquitination is a general mechanism for targeting proteins for degradation, and for regulating the intracellular trafficking of some proteins [11]. This process involves the addition of ubiquitin to lysine groups in the target protein. Ubiquitin must first be activated by an enzyme (E1), and is then passed on to one of a large group of ubiquitin conjugating enzymes (E2), which ubiquitinate the protein either alone or in concert with a ubiquitin–protein ligase (E3) [11]. Proteins can be either mono-ubiquitinated or poly-ubiquitinated; the latter appears to be necessary for ubiquitin-mediated degradation. The experiments of Burbea *et al.* suggest that, in addition to targeting proteins for degradation, ubiquitination represents an important mechanism for targeting AMPA receptors for removal from the synaptic membrane.

Ubiquitin regulates AMPA-receptor abundance

Using transgenic *C. elegans* in which one AMPA-receptor subtype, GLR-1, is fused to green-fluorescent protein (GFP), Burbea *et al.* were able to visualize the number and intensity of synaptic GLR-1 puncta in one population of interneurons in the ventral nerve cord. A genetic screen for increased abundance of GLR-1 pulled out a mutation in AP180, a clathrin-adaptor protein necessary for endocytosis. This mutation increased the intensity of GLR-1-GFP puncta, and this increase was prevented by selectively expressing a construct encoding wild-type AP180 in this population of interneurons. This suggests that the amount of synaptically localized GLR-1 is regulated by clathrin-mediated endocytosis, as has been suggested for AMPA receptors in the mammalian CNS. Because the GFP signal arises from both surface and internalized receptors, the increase in fluorescence when endocytosis is blocked suggests that a significant fraction of internalized receptors are normally broken down.

In a second series of experiments, Burbea *et al.* found that GLR-1 accumulation at synaptic sites could also be regulated by ubiquitination. Increasing the pool of ubiquitin (which is rate-limiting for ubiquitination) by expression of a ubiquitin transgene

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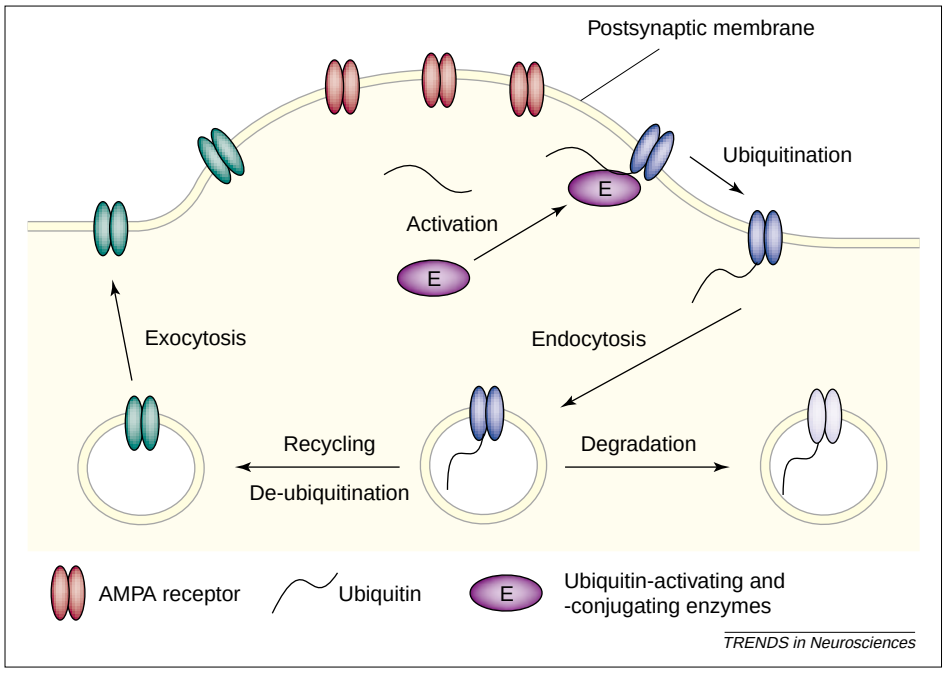

Fig. 1. Model for the role of ubiquitination in AMPA-receptor endocytosis. AMPA receptors are continuously cycling into (green) and out of (blue) the membrane, through exocytosis and endocytosis, respectively. Once ubiquitinated by ubiquitin-activating and ubiquitin-conjugating enzymes (E), AMPA-receptors are taken back into the cell by endocytosis, and are either degraded or de-ubiquitinated and recycled back to the synaptic membrane.

decreased the abundance of synaptic GLR-1. This appears to be a direct (rather than a secondary) consequence of ubiquitination of GLR-1, because mutation of a specific ubiquitination sequence in GLR-1 increased GLR-1 abundance and blocked the effects of the ubiquitin over-production. Interestingly, the ubiquitin-encoding transgene also reduced the number of GLR-1 puncta, an effect that was not directly mediated by ubiquitination of GLR-1. Ubiquitination is, thus, likely to regulate synaptic function in several important ways by targeting multiple postsynaptic proteins.

Taken together, these data suggest that the abundance of GLR-1 at synapses is regulated both by clathrin-mediated endocytosis and by ubiquitination. Are these separate mechanisms, or are they sequential steps in the same regulatory process? Mutations in AP180 that prevent endocytosis and mutations in GLR-1 that prevent ubiquitination both increase abundance of GLR-1 at synapses. If these two processes act in parallel, these effects should have been additive. In fact they were not, suggesting that they are two sequential steps in the same regulatory cascade. Additionally, the AP180 mutation prevented the decrease in GLR-1 abundance that was produced by over-production of ubiquitin, suggesting that ubiquitination of GLR-1 precedes endocytosis. The simplest explanation for these results is that ubiquitination of GLR-1 leads to clathrin-mediated endocytosis and subsequent degradation of a fraction of GLR-1 (Fig. 1). It is not yet clear whether AMPA receptors are mono- or poly-ubiquitinated, and whether, once the receptors are pulled from the membrane, ubiquitination influences the proportion of receptors that are degraded rather than recycled back to the synaptic membrane.

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Because the process of ubiquitination itself has many potential sites for regulation [11], this study opens up a new and possibly very fruitful arena in which to study AMPA-receptor trafficking. Many signals have been shown to alter surface density of AMPA receptors, including activation of AMPA or NMDA receptors by agonists [9,10,12,13], alterations in the overall level of activity of the neuron [1,2] or exposure to hormones such as insulin [7]. Which of these processes might be mediated by ubiquitination is still an open question. Likewise, whether this mechanism will prove to be important in synapse-specific forms of plasticity such as LTP and LTD [4], or homeostatic forms that regulate total synaptic strength such as synaptic scaling [14], remains to be seen. The search for regulated intermediaries in the ubiquitiation of AMPA receptors should prove fun, and is likely to generate important insights into the mechanisms of synaptic plasticity and synapse stability.

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