This Week in The Journal

Two Calcium Sensors for Endocytosis at Synapses

Ying-Hui Jin, Xin-Sheng Wu, Bo Shi, Zhen Zhang, Xiaoli Guo, et al.

(see pages 9478–9490)

When synaptic vesicles fuse with the plasma membrane, vesicular membranes and associated proteins are inserted into the presynaptic active zone. This increases membrane surface area and thus capacitance, and it disrupts active zone structure, limiting the docking of new vesicles. Fortunately, synaptic membranes and proteins are quickly endocytosed and recycled to replenish vesicular stores and restore active zone integrity.

Endocytosis occurs through multiple mechanisms at presynaptic sites, resulting in fast or slow retrieval of single vesicles or bulk retrieval of components of several vesicles into large endocytic structures. The endocytic mechanism engaged at calyx of Held synapses varies with calcium levels, and all forms require the calcium-binding protein calmodulin (Wu et al., 2009, *Nat Neurosci* 12:1003). Jin, Wu, Shi et al. now report that protein kinase C (PKC) also contributes to endocytosis.

As expected, depolarization of the calyx of Held caused capacitance to increase as vesicles fused, and to return to baseline as endocytosis occurred. The rate of capacitance decay was significantly slower than normal in cells lacking PKC α or PKC β , but was faster than normal in the presence of a PKC enhancer. Moreover, the slowing occurred regardless of whether the stimulation protocol induced rapid or slow endocytosis in wild-type calyces. Sequential spikes in a train produce progressively smaller-capacitance increases as vesicles are depleted, and this effect was greater in calyces lacking PKC than in controls, suggesting that PKC-mediated endocytosis is necessary for restoration of the readily releasable vesicle pool.

Knocking out PKC α or PKC β also slowed endocytosis after spike trains in hippocampal cultures, as indicated by the slower decay of fluorescence by a pH-sensitive molecule. Importantly, endocytosis was rescued by reintroducing wild-type PKC into neurons, but not by expressing PKC lacking the calcium-binding domain.

Knocking out calmodulin also reduced endocytosis in hippocampal synapses, and this effect was rescued by reintroducing wild-type calmodulin, but not calmodulin lacking the calcium-binding domain. Electron microscopy provided additional evidence that knocking out PKC or calmodulin reduced vesicular and bulk endocytosis.

These results suggest that both PKC and calmodulin contribute to calcium-dependent endocytosis at multiple synapse types. Future work should determine how these calcium sensors regulate membrane retrieval and whether they have distinct or redundant roles.

After neurons were depolarized with KCl, the amount of endocytosis (indicated by internalized horseradish peroxidase, dark label) was greater in wild-type nerve terminals (top) than in those lacking PKC α (bottom). See Jin, Wu, Shi, et al. for details.

ABCA1 Trafficking, ApoE Lipidation, and A β Clearance

Varun Rawat, Shaowei Wang, Jian Sima, Roni Bar, Ori Liraz, et al.

(see pages 9611–9622)

The strongest genetic risk factor for late-onset Alzheimer's disease (AD) is possession of the $\epsilon 4$ allele of apolipoprotein E (ApoE), the main transporter of phospholipids and cholesterol in the brain. Astrocytes and other cells secrete ApoE, which is then loaded with lipids by membrane-associated ATP-binding cassette transporters, including ABCA1. The lipidated ApoE then binds to receptors on other

cells, including neurons, to deliver its cargo.

The ApoE $\epsilon 4$ allele (ApoE4) binds less lipid than the more common $\epsilon 3$ allele, and thus is less efficient at transferring cholesterol and other lipids from astrocytes to neurons. The expression of ApoE4 leads to reduced export of cholesterol from the brain, impaired glucose metabolism, sequestration of several membrane proteins—including glutamate and insulin receptors—in neuronal endosomes, and greater accumulation of A β peptides in AD. All these effects may contribute to cognitive impairment and neurodegeneration, but how the effects are related is unclear. Notably, however, overexpressing ABCA1 reduces, and reducing ABCA1 increases A β deposition in mice expressing human ApoE4, suggesting that poor lipidation of ApoE4 contributes to AD risk.

Rawat, Wang, et al. suggest that ApoE4 is poorly lipidated partly because it promotes the expression of ARF6, a GTPase that increases transfer of ABCA1 to late endosomes and thus into the protein degradation pathway. ARF6 levels were higher in astrocytes from mice expressing human ApoE4 than in ApoE3-expressing astrocytes. Likely as a consequence, membrane ABCA1 levels were significantly lower in ApoE4-expressing astrocytes than in ApoE3-expressing astrocytes. As expected, cholesterol efflux from ApoE4-expressing astrocytes was lower than that from ApoE3-expressing cells, but knocking out ARF6 increased cholesterol efflux. Intriguingly, an ABCA1 agonist decreased ARF6 expression, increased levels of ABCA1 in recycling endosomes and on the plasma membrane, and increased cholesterol efflux in ApoE4-treated cells. The agonist also increased β -amyloid clearance by immortalized ApoE4-expressing astrocytes.

These results suggest that poor lipidation of ApoE4 stems partly from ApoE4-dependent upregulation of ARF6, which diverts ABCA1 from recycling to late endosomes. This reduces ABCA1 surface expression, reducing the transfer of lipids to ApoE4. More work is needed to elucidate the causal links between these effects, but, regardless of the mechanism, increasing ABCA1-mediated lipidation of ApoE4 may attenuate AD risk in ApoE4 carriers.

This Week in The Journal was written by Teresa Esch, Ph.D.
<https://doi.org/10.1523/JNEUROSCI.twij.39.48.2019>