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Calcium: An Insignificant Thing

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Abstract

Fusion of synaptic vesicles in response to calcium influx requires precise localization of voltage-gated calcium channels. A study in this issue of *Nature Neuroscience* identifies a previously uncharacterized protein that mediates trafficking of CaV2 calcium channels in *C. elegans*.

In the presynaptic terminal a puff of calcium is an insignificant thing, a scintilla painted on the dark ceiling of the synaptic bouton. First visualized in 1992 by Rudolfo Llinas and colleagues in the synaptic terminals of the giant squid, these intracellular calcium increases are transient and highly local – confined to ‘microdomains’¹. The portals for extracellular calcium are voltage-gated calcium channels, usually of the CaV2 class, clustered at the active zone. Depolarization of the membrane opens the pore and a surge of calcium reaching 100 μ M flows into the cell². But this rise in calcium probably only extends 20 nm or so before dissipating; calcium diffusion is limited by the action of internal buffers which are very fast acting^{3,4}. The calcium sensor involved in fusion of synaptic vesicles with the membrane has a low affinity for calcium – it requires every bit of that 100 μ M for effective release of neurotransmitter⁵. If the calcium channel is not near the synaptic vesicle there will be no neurotransmission. So, where are the channels? Who docks them there? Who pilots the tug? There must be escorts that regulate the synthesis, transport and localization of voltage-gated calcium channels to these sites. In this issue, Saheki & Bargmann⁶ have labeled the calcium channels with GFP and localized them to nematode synapses. They then use a simple *in vivo* visual genetic screen to identify the proteins required to transport and localize calcium channels to presynaptic sites in *C. elegans*, and propose a mechanism of calcium channel trafficking.

There is a long and difficult history for studies of calcium channel localization. For example, one Sisyphean study used a combination of electrophysiology and electron microscopy². This study used electrophysiology on isolated hair cells and determined that there are approximately 1800 calcium channels in 20 discrete clusters. It also detailed an almost identical number of intramembrane particles by freeze-fracture electron microscopy and number of active zones by serial-section electron microscopy; from these results the authors concluded that calcium channels are positioned within 100 nm of the presynaptic active zone. But these are really difficult experiments and in the end indirect. For studies of the mechanism and dynamics of CaV channel trafficking, it would be nice to just be able to see the channels directly in living cells.

To visualize calcium channel localization, Saheki & Bargmann⁶ tagged a functional CaV2 channel with GFP and expressed it in a pair of neurons that make synapses along their axons in stereotyped positions. In these experiments, the tagged calcium channel specifically localizes to presynaptic active zones. Importantly, this pattern can be observed in living worms by epifluorescence. And herein is the beautiful bit – the authors can screen for mutants in which CaV2 channel localization is disrupted. It is not a particularly easy screen as worm screens go;

it requires that every worm be mounted on a fluorescence microscope and scored for mislocalization. Nevertheless, hard screens can pay off; the authors isolated mutants with mislocalized CaV2 channels and identify two proteins that are necessary for correct CaV2 transport: a novel protein, calcium channel localization factor 1 (CALF-1), and an $\alpha_2\delta$ subunit.

CALF-1 is a small protein, composed of a single transmembrane domain and a cytosolic tail, that resides in the endoplasmatic reticulum (ER). The authors found that the primary function of CALF-1 is in calcium channel biogenesis; in the absence of CALF-1, CaV2 channels are retained in the ER while other active zone and synaptic vesicle components were properly localized. ER retention is not a developmental defect since expression of CALF-1 induced in *calf-1* mutant adults promotes rapid exit of functional CaV2 channels from the ER and transport to synaptic sites. How does CALF-1 promote CaV2 exit from the ER? For most ion channels, ER retention motifs are contained in the channels themselves. After channel assembly and maturation, outfitter proteins mask the retention signal and allow channels to exit the ER⁷. In this case however, it is not the CaV2 channel itself but the accessory protein CALF-1 that has the ER retention motif: the cytosolic tail of CALF-1 contains multiple arginine-x-arginine (RXR) ER retention motifs embedded in basic and proline rich regions.

In their genetic screen, the authors also isolated new mutant alleles of the $\alpha_2\delta$ subunit UNC-36. Interestingly, the $\alpha_2\delta$ subunit appears to have related CaV2 trafficking functions to CALF-1. $\alpha_2\delta$ subunits are accessory subunits to CaV channels that, in mammalian systems at least, increase the number of functional CaV channels in the cell membrane⁷. $\alpha_2\delta$ subunits are mainly extracellular, with the α_2 subunits tethered to the extracellular face of the membrane by the δ subunit. *unc-36* mutants are uncoordinated, similar to CaV2 mutants, and GFP-tagged CaV2 is no longer detectable at presynaptic sites.

Is UNC-36 mainly involved in trafficking or does it also have a functional role? One experiment in particular demonstrates that $\alpha_2\delta$ has a functional role in nematodes: In $\alpha_2\delta$ mutants, overexpression of the CALF-1 protein partially restores CaV2 channel localization to synapses. However, locomotion is not restored, arguing for a role of $\alpha_2\delta$ in both channel function and trafficking. These results are consistent with data from mammalian and *Drosophila* studies though the effects in *C. elegans* are more severe. In mammalian cell culture, $\alpha_2\delta$ promotes CaV channel surface expression and alter subtle functional properties of calcium currents⁷. In flies, the $\alpha_2\delta$ mutant *straightjacket* has reduced neuronal transmission due to a reduction in CaV2 channels at the synapse^{9,10}. These studies underscore an important point, $\alpha_2\delta$ proteins are bonafide subunits of the calcium channel complex and assembly of these subunits is likely to be permissive for trafficking, whereas CALF-1 is more likely to be specifically involved in trafficking the complex.

Based on their findings, Saheki and Bargmann propose that the $\alpha_2\delta$ subunits and CALF-1 promote exit from the ER. Three possible processes come to mind: folding, a checkpoint for assembly, or formation of transport vesicles (Fig. 1)⁸. In the first model, CALF-1 functions as a chaperone for protein folding or promotes assembly of the subunits of the calcium channel complex. Failure to assemble the complex blocks exit of these proteins from the ER. In the second model, CALF-1 functions as a checkpoint protein, like a licensing factor, that allows exit of the fully assembled complex. In the third model, CALF-1 is interacting with the calcium channel at the ER exit site for the formation of transport vesicles – for example in the recruitment of coat proteins. The authors do not favor a particular mechanism; however they exclude the model that the ER retention motif of CALF-1 acts as a specific brake for an unassembled complex. First, loss of CALF-1 or elimination of the ER retention motif does not lead to constitutive exit of the calcium channel. Second, substitution of the cytosolic tail of CALF-1 with the ER retention motif from the adrenergic receptor partially rescues channel trafficking. Thus, the ER retention motif probably functions to return CALF-1 to the ER rather

than playing a specific role in calcium channel trafficking. Although CALF-1 does not have any obvious homologs outside of nematodes, the authors note that gamma subunits of CaV channels in mammals share similarities, that is, the RXR motifs and a proline-rich region, with CALF-1. It will be interesting to determine if mammalian gamma subunits could have similar biogenesis roles for CaV channels.

Saheki and Bargmann's paper brings a number of questions to mind. For example, how do neurons regulate the number of CaV2 channels at synapses? At mammalian synapses it has been proposed that there are a certain number of "slots" for each type of CaV2 channels¹¹. In Saheki and Bargmann's study, calcium channels at individual synapses are visible under conventional fluorescence microscope. Such a bright signal suggests a significant number of channels per synapse; however not all of the tagged channels are necessarily inserted into the membrane. Previous experiments suggest that there may be very few calcium channels at synapses in *C. elegans*: it has been estimated that there are less than two CaV channels per synapse at one type of sensory neuron.¹² If quantitative studies bear these numbers out, calcium channels really do look like an insignificant component of the active zone, at least numerically speaking. But Napoleon once said, "There are times when the most insignificant thing can decide the outcome of a battle." It is possible that the placement of just a single channel determines whether a particular synapse fires or remains silent. The tiny puff of calcium from a channel is not to be dismissed lightly -- all neurotransmission hinges on its function. We are now closer to understanding how that speck positioned itself to play such a prominent role.

References

1. Llinás R, Sugimori M, Silver RB. Microdomains of high calcium concentration in a presynaptic terminal. *Science* 1992;256:677–679. [PubMed: 1350109]
2. Roberts WM, Jacobs RA, Hudspeth AJ. Colocalization of ion channels involved in frequency selectivity and synaptic transmission at presynaptic active zones of hair cells. *J Neurosci* 1990;10:3664–3684. [PubMed: 1700083]
3. Yamada W, Zucker R. Time course of transmitter release calculated from simulations of a calcium diffusion model. *Biophysical Journal* 1992;61:671–682. [PubMed: 1354503]
4. Neher E. Vesicle pools and Ca²⁺ microdomains: new tools for understanding their roles in neurotransmitter release. *Neuron* 1998;20:389–399. [PubMed: 9539117]
5. Heidelberger R, et al. Calcium dependence of the rate of exocytosis in a synaptic terminal. *Nature* 1994;371:513–515. [PubMed: 7935764]
6. Saheki Y, Bargmann CI. CaV2 presynaptic calcium channel traffic requires the ER protein CALF-1 and the alpha2-delta subunit. 2009
7. Jarvis SE, Zamponi GW. Trafficking and regulation of neuronal voltage-gated calcium channels. *Current Opinion in Cell Biology* 2007;19:474–482. [PubMed: 17624753]
8. Herrmann JM, Malkus P, Schekman R. Out of the ER--outfitters, escorts and guides. *Trends Cell Biol* 1999;9:5–7. [PubMed: 10087610]
9. Ly CV, et al. straightjacket is required for the synaptic stabilization of cacophony, a voltage-gated calcium channel {alpha}1 subunit. *J Cell Biol* 2008;181:157–170. [PubMed: 18391075]
10. Dickman DK, Kurshan PT, Schwarz TL. Mutations in a *Drosophila* {alpha}2{delta} Voltage-Gated Calcium Channel Subunit Reveal a Crucial Synaptic Function. *J Neurosci* 2008;28:31–38. [PubMed: 18171920]
11. Cao Y, et al. Presynaptic Ca²⁺ Channels Compete for Channel Type-Preferring Slots in Altered Neurotransmission Arising from Ca²⁺ Channelopathy. *Neuron* 2004;43:387–400. [PubMed: 15294146]
12. Goodman MB, et al. Active currents regulate sensitivity and dynamic range in *C. elegans* neurons. *Neuron* 1998;20:763–772. [PubMed: 9581767]

Figure 1. CaV2 calcium channel trafficking in *C.elegans*

Saheki and Bargmann⁶ have identified two proteins that are necessary for CaV2 channel transport and localization to presynaptic sites: a novel protein CALF-1 and the $\alpha 2\delta$ subunit, UNC-36. CaV2 channels are retained in the endoplasmic reticulum (ER) in the absence of either CALF-1 or UNC-36. There are at least three possible models for the function of CALF-1. (1) CALF-1 permanently resides in the ER and acts as a specific chaperone for CaV2. Chaperone functions include channel folding and subunit assembly. (2) CALF-1 serves as an ER checkpoint that monitors the assembly of CaV2 channels. At the checkpoint only assembled channels are allowed to exit the ER. Although not shown in the figure, it is possible that a fully assembled CaV2 channel occludes the RXR motif and that a CALF-1/CaV2 complex is

incorporated into the transport vesicle. (3) CALF-1 stimulates CaV2 channel export from the ER by concentrating CaV2 channels at ER export sites and by recruiting coat proteins necessary to form transport vesicles. In this model, the main function of the RXR motif is to retrieve CALF-1 to the ER.