



## **Sorting and trafficking of voltage gated Ca<sup>2+</sup> channels in neurons: The C-terminus and the dynein motor complex.**

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Voltage gated Ca<sup>2+</sup> channels regulate a wide variety of physiological functions including excitation-contraction coupling in muscle and hormone secretion in glands. In neurons Ca<sup>2+</sup> channels determine the plateau potentials in dendrites, excitation-transcription coupling at the cell body, and excitation transmission coupling at the presynaptic terminal. Various types of Ca<sup>2+</sup> channels exist in order to perform these tasks including L-, N-, P/Q-, R- and T-type channels. These channel types have specific biophysical characteristics and are found in certain subcellular compartments. For example N-, P/Q- and R-type channels are found at the presynaptic terminals such as the Calyx of Held, but are localized differently relative to the transmitter release machinery. L-type channels play an important role in transmitter release early in development, but are not found at the presynaptic terminals of most mature neurons. T-type channels are only localized to the soma and dendrites where they are involved in pace-making and oscillation, and have not been detected presynaptically (for a review on Ca<sup>2+</sup> channel distribution see (Herlitze & Mark, 2005)). The different physiological functions of the various channel types predict that specific sorting and transport mechanisms exist to transport the channels to their subcellular site of action and then precisely anchor the channel complex at the somas, dendritic spines or presynaptic terminals. While Ca<sup>2+</sup> channel targeting has been studied extensively in heterologous expression systems little is known about the targeting of Ca<sup>2+</sup> channels in neurons and in particular the underlying mechanism and molecules involved in dendritic versus axonal targeting. The recent paper by Lai and coworkers published in April in Nature Neuroscience (Lai, Wang et al. 2005) shines new light onto the trafficking of neuronal Ca<sup>2+</sup> channels by describing the first motor protein which directly interacts specifically with the Ca<sup>2+</sup> channels pore-forming subunits of the Cav2 family.

Ca<sup>2+</sup> channels consist of several subunits: a pore-forming  $\alpha_1$  subunit and several ancillary subunits such as the  $\beta$  subunit,  $\alpha_2\delta$  and  $\gamma$  subunit. To elucidate the role of each of the subunit for channel complex transport to the plasma membrane, Ca<sup>2+</sup> channel targeting has extensively been studied in HEK293, COS-7, MDCK

cells and *Xenopus* oocytes by coexpressing various recombinant channel subunit combinations. Most studies agree that at least in vertebrate systems, the Ca<sup>2+</sup> channel's  $\beta$  subunit is necessary for transport of the  $\alpha_1$  subunit to the plasma membrane (but see also Spafford, Van Minnen et al. 2004). The idea is that the  $\beta$  subunit is shielding an ER retention signal on the  $\alpha_1$  subunit, thus allowing the  $\alpha_1/\beta$  complex to exit the ER.  $\beta$  subunit mediated plasma membrane targeting of L- and N-type channels can be regulated by phosphorylation via PI3kinase/Akt/PKB (Viard, Butcher et al. 2004). The role of  $\alpha_2\delta$  subunits on targeting has also been studied but not as extensively. A recent study by Gerald Zamponi's group comparing the effects of  $\beta$  and  $\alpha_2\delta$  subunits on  $\alpha_1$  mediated currents suggest a more important role of  $\alpha_2\delta$  subunits for surface expression of the channel complex in comparison to  $\beta$  subunits (Yasuda, Chen et al. 2004). Various other proteins have also been suggested to regulate the expression of the Ca<sup>2+</sup> channel complex. Down regulation of channel expression is mediated by the small GTPases, kir/gem as well as the members of the TARP family,  $\gamma_5$  and  $\gamma_7$ . The downregulation of channel expression probably involves different mechanisms. For kir/gem it has been suggested that the small GTPases directly interact with  $\beta$  subunits, thereby antagonizing the interaction of  $\beta$  with  $\alpha_1$ . Upregulation of channel expression is mediated by AKAP79. The PKA anchoring protein selectively targets L-type channels but not N/P/Q/R-type channels to the plasma membrane. L-type channel targeting may also involve the transport of the whole signaling cascade complex. Multi-protein L-type channel complexes including channels and the complete second messenger signaling cascade (GPCR, G protein, AC, PK and Phosphatase) have been identified somatodendritically in neurons supporting the role of physiological protein units found in subcellular compartments (Davare, Avdonin et al. 2001). For review on mechanisms of Ca<sup>2+</sup> channel targeting see (Herlitze, Xie et al. 2003).

Only a few studies have addressed mechanisms of Ca<sup>2+</sup> channel targeting in polarized neuronal cultures. N-type channels require the interaction between the proteins of the tripartite complex MINT and CASK to sufficiently target or cluster to presynaptic sites (see review Herlitze, Xie et al. 2003). The laminin Ca<sup>2+</sup> channel interaction was suggested to play a role in the formation and organization of the active zones of presynaptic nerve terminals (Nishimune, Sanes et al. 2004). Since presynaptic Ca<sup>2+</sup> channels interact with the proteins of the vesicle release machinery via the so called synprint site, it has been suggested that the interaction between these proteins is also involved in channel targeting. This seems to be the case at least in vertebrate neurons but has yet to be mentioned that synaptic targeting of Ca<sup>2+</sup> channels without the synprint site occurs in invertebrates (Spafford, Munno et al. 2003). Besides the role of  $\alpha_1$  in channel complex targeting, the Ca<sup>2+</sup> channel  $\beta_4$  subunits by itself contains structural information in its N- and C-termini which allow transport and clustering of the  $\beta_4$  subunit to and at presynaptic sites (Wittmann, Mark et al. 2000).

Until last month no protein had been identified which directly linked the Ca<sup>2+</sup> channels to the trafficking motor machinery in neurons or other cells. So far studies have shown that Ca<sup>2+</sup> channels are found in 80

nm dense core vesicles within pleiomorphic structures of developing neurons in culture suggesting that  $\text{Ca}^{2+}$  channels are part of preformed presynaptic sites (Waites, Craig et al. 2005). How  $\text{Ca}^{2+}$  channels within these vesicles were transported was unclear but suggested that microtubule-based motor proteins would be involved in the transport process.

The current paper by Ji-fang Zhang's group published in Nature Neuroscience (Lai, Wang et al. 2005) has given us the first glimpse of which cellular motors are involved in transporting  $\text{Ca}^{2+}$  channel complexes to the neuronal surface. They demonstrate in their study that a protein from the dynein motor complex directly interacts with  $\text{Ca}^{2+}$  channels. By performing a yeast two hybrid screen with the C-termini of  $\text{Ca}^{2+}$  channels as baits, the authors identified recently several proteins involved in  $\text{Ca}^{2+}$  channel trafficking and function including endophilin, the protein phosphatase  $2c\alpha$  and the PKC binding protein (enigma homolog) ENH (Chen, Deng et al. 2003; Maeno-Hikichi, Chang et al. 2003; Li, Wang et al. 2005). Several other proteins have previously been found to interact with the  $\text{Ca}^{2+}$  channel C-termini including  $\text{Ca}^{2+}$  channel  $\beta$  subunits, G protein  $\beta\gamma$  subunits,  $\text{Ca}^{2+}$ /Calmodulin and proteins of the tripartite complex MINT and CASK; indicating that the  $\text{Ca}^{2+}$  channel C-terminus is the main locus for the interaction of modulatory, transporting and clustering proteins within the cell. Zhang's group found a new interacting partner at the C-terminus of N- and P/Q-type channels but not L-type channels, Tctex-1 (t complex testis expressed-1), a light chain subunit of the dynein motor protein complex. The interaction was verified using *in vitro* assays (GST fusion protein pull-down assays and co-immunoprecipitation assays) and *in vivo* assays (FRET measurements in Cos-7 cells and Tctex1-  $\text{Ca}^{2+}$  channel complex formation in lysates from adult rat brain). The interaction between the dynein motor and the channel is independent of the  $\text{Ca}^{2+}$  concentration. This implies that the interaction between the  $\text{Ca}^{2+}$  channel complex and the motor protein is not modulated by activity of the channel itself and may suggest that the motor protein is involved in the constitutive transport of the channel to or from the plasma membrane. The interaction site was narrowed down to a 79-140 amino acid stretch within the C-terminus of the N-type channels (aa 2018-2097). The 140 aa binding site was used as a dominant negative construct to investigate the functional consequences of the Tctex-1/  $\text{Ca}^{2+}$  channel interaction in neurons. As expected, the 140 aa binding site acts in a dominant negative fashion by reducing  $\text{Ca}^{2+}$  channel surface expression at the soma and at the neurite extensions. However, the dominant negative effects have to be interpreted carefully since as mentioned above the C-terminus binds various proteins including the  $\text{Ca}^{2+}$  channel  $\beta$  subunits. Therefore the C-terminal derived peptide may interfere with other proteins involved in channel targeting when exogenously expressed.

*What are the possible roles of Tctex-1 in  $\text{Ca}^{2+}$  channel trafficking?*

Microtubule based motor proteins, such as kinesins, dyneins and myosins mediate the transport of intracellular organelles and protein complexes in neurons (for review on dynein see Vallee, Williams et al. 2004). Dynein motors are responsible mainly for the retrograde transport in neurons, i.e. from the axon or dendrites to cell body. Dyneins consist of two heavy chains, two intermediate chains, two light

intermediate chains and a variable number of light chains. Tctex-1 is part of the dynein motor complex and belongs to the family of the light chains. The light chains bind to dynein via the dynein intermediate chain and to several so far identified cargo proteins including small GTPases, lipid associated protein Doc2, Trk receptor, voltage-dependent anion selective channel 1, rhodopsin and now N-type  $\text{Ca}^{2+}$  channels. It is not yet clear where exactly Tctex-1 acts during  $\text{Ca}^{2+}$  channel transport. Several possibilities exist:

1. One option is that Tctex-1 controls the ER/Golgi transport or Golgi exit of the channel complex. Tctex-1 has recently been localized to the Golgi apparatus of fibroblasts (Tai, Chuang et al. 1998). The role of Tctex-1 in ER/Golgi transport would explain the effect of the reduced  $\text{Ca}^{2+}$  channel expression and current in the soma of the hippocampal neurons.

2. Tctex-1 is part of the cytoplasmic dynein complex which is involved in fast retrograde axonal transport. Therefore one has to consider the possibility of a retrograde  $\text{Ca}^{2+}$  channel transport. In fact, Tctex-1 interacts with TrkA receptors and mediates the retrograde signaling between the axonal terminal and the cell soma. The functional consequences of a retrograde  $\text{Ca}^{2+}$  channel transport remains to be elucidated.

3. In addition to retrograde transport cytoplasmic dynein has recently been suggested to drive slow anterograde transport (Vallee, Williams et al. 2004). Blocking anterograde transport of the channels from the soma to the presynapse would explain the effect of the dominant negative mutant on the reduction in  $\text{Ca}^{2+}$  channel staining at terminals.

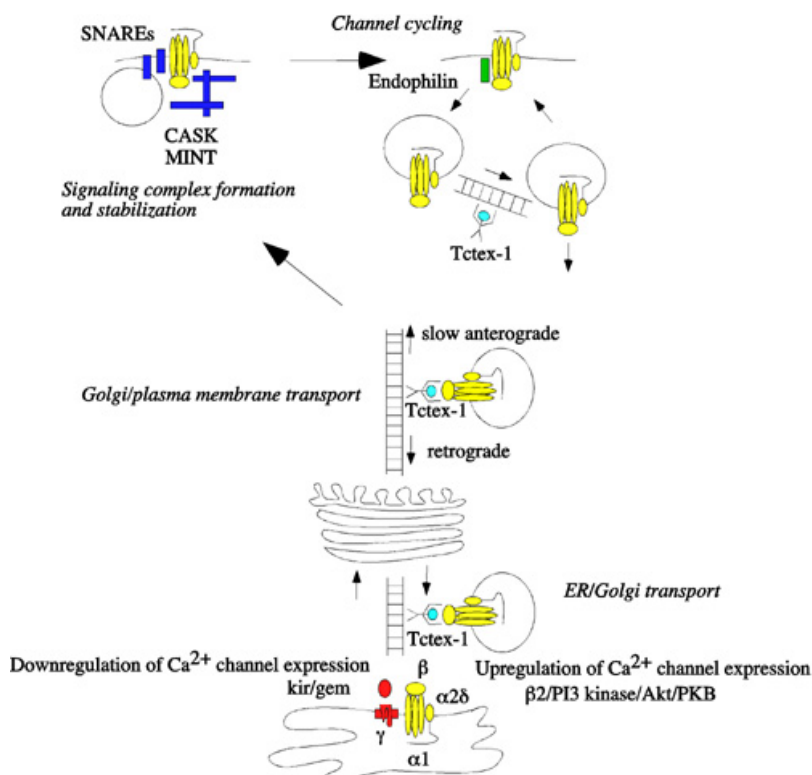
4. A presynaptic function of Tctex-1 should be considered due to the fact that Tctex-1 is found in presynaptic terminals, where the protein is associated primarily with small synaptic vesicle clusters and interacts with Doc2. Doc2, the double C2 domain protein, is localized to the presynaptic terminal and interacts with proteins of the exocytotic machinery such as Munc18 and Munc13-1. Doc2 has also been suggested to be involved in vesicle cycling and vesicle maturation at the transmitter release site (Duncan, Shipston et al. 2000). This may point to a role of Tctex-1 in  $\text{Ca}^{2+}$  channel recycling at the presynaptic terminal and is also suggested by the fact that dynein plays a role in transport between early and late endosomes. The coupling of  $\text{Ca}^{2+}$  channel to endophilin and thus to the endocytotic machineries has recently been shown also by Ji-fang Zhang group (Chen, Deng et al. 2003). This may suggest that clathrin-mediated endocytosis via endophilin/Tctex-1/ $\text{Ca}^{2+}$  channel interaction may lead to recycling of  $\text{Ca}^{2+}$  channels at the presynaptic membrane. Virus mediated expression of Tctex-1 or the dominant negative form in slices and/or organotypic or primary neuronal cultures coupled with electrophysiology and imaging of the dynamic trafficking of presynaptic  $\text{Ca}^{2+}$  channels would be helpful to decipher the presynaptic role of Tctex-1 for  $\text{Ca}^{2+}$  channel transport.

*Future question:*

The identification of new cargos (the N- and P/Q-type channel) of the dynein motor complex opens a new area in  $\text{Ca}^{2+}$  channel trafficking research and immediately raises new questions. Do other motor proteins bind to the channel complex to distinguish between dendritic versus axonal transport? Can the channel be transported alone, or does it have to be embedded into signaling complexes or larger transport packets? Are there fast anterograde mechanisms for transporting the channels and if so, which kinesins are involved? Is  $\text{Ca}^{2+}$  channel trafficking dynamically regulated? The connection between clathrin-mediated endocytosis and early to late endosome transport suggests that  $\text{Ca}^{2+}$  channels may underlie dynamic trafficking within terminals or spines, and may reveal new activity-dependent mechanisms as demonstrated for AMPA/NMDA receptor trafficking.

In summary the paper by Ji-fang Zhang's group finally connects  $\text{Ca}^{2+}$  channel trafficking to cellular motors. It will be interesting to see how many more proteins are able to interact with the  $\text{Ca}^{2+}$  channel C-terminus to clarify the precise subcellular transport and anchoring of a specific  $\text{Ca}^{2+}$  channel complex. In fact as mentioned in the discussion of the Lai et al. paper, a yeast two hybrid screen using the L-type channel C-terminus as bait, identified a different motor protein, a member of the kinesin superfamily for trafficking of L-type channels. We are therefore anxiously waiting for the next report on  $\text{Ca}^{2+}$  channel trafficking from the Zhang laboratory.

**Figure 1:** Transport of  $\text{Ca}^{2+}$  channels to the presynaptic terminal: Tctex-1 may act at multiple trafficking sites



$\text{Ca}^{2+}$  channel trafficking is controlled at various levels in neurons involving multi protein complexes. ER to Golgi and Golgi to plasma membrane transport of the channel complex involves the  $\text{Ca}^{2+}$  channel  $\beta$  and  $\alpha_2\delta$  subunits and may be antagonized by the TARP family members  $\gamma_5$  and  $\gamma_7$  and the small GTPase kir/gem. PI3-kinase can upregulate channel transport via  $\text{Ca}^{2+}$  channel  $\beta_2$  subunits. Arrival of the channel complex at the presynaptic terminal leads to

clustering and colocalization with synaptic vesicles. SNARE proteins as well as the proteins of the

tripartite complex, MINT and CASK, directly interact with the channel and mediate anchoring of the channel at the presynaptic site. Clathrin mediated endocytosis may lead to the recycling of presynaptic channels involving the endophilin/Ca<sup>2+</sup> channel interaction. Tctex-1 may regulate various steps during the channel transport for the following reasons: Tctex-1 is found in the Golgi apparatus and in presynaptic terminals. Tctex-1 is part of the cytosolic dynein, which is involved in slow anterograde, retrograde and early to late endosome transport. Therefore, Tct3x-1 may be involved in regulating the Ca<sup>2+</sup> channel transport between ER to Golgi, Golgi to plasma membrane, soma to presynaptic terminal, recycling within the terminal and retrograde transport to the soma.

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