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## How do T-type calcium channels control low-threshold exocytosis?

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**L**ow-voltage-activated T-type calcium channels act as a major pathway for calcium entry near the resting membrane potential in a wide range of neuronal cell types. Several reports have uncovered an unrecognized feature of T-type channels in the control of vesicular neurotransmitter and hormone release, a process so far thought to be mediated exclusively by high-voltage-activated calcium channels. However, the underlying molecular mechanisms linking T-type calcium channels to vesicular exocytosis have remained enigmatic. In a recent study, we have reported that Ca<sub>v</sub>3.2 T-type channel forms a signaling complex with the neuronal Q-SNARE syntaxin-1A and SNAP-25. This interaction that relies on specific Ca<sub>v</sub>3.2 molecular determinants, not only modulates T-type channel activity, but was also found essential to support low-threshold exocytosis upon Ca<sub>v</sub>3.2 channel expression in MPC 9/3L-AH chromaffin cells. Overall, we have indentified an unrecognized regulation pathway of T-type calcium channels by SNARE proteins, and proposed the first molecular mechanism by which T-type channels could mediate low-threshold exocytosis.

Depolarization-evoked synaptic transmission relies on the calcium (Ca<sup>2+</sup>)-regulated release of quantal packets of neurotransmitters following fusion of synaptic vesicles with the presynaptic plasma membrane.<sup>1</sup> It is well established that neuronal voltage-gated Ca<sup>2+</sup> channels, by converting electrical signals into intracellular Ca<sup>2+</sup> concentration elevations, play a key role in triggering evoked neurotransmitter

release.<sup>2–4</sup> Hence, Ca<sup>2+</sup> entry through high-voltage-activated (HVA) channels (N-, P/Q-, and in some extent and particular cell populations, L- and R-type) into presynaptic nerve terminals in response to action potentials supports a transient Ca<sup>2+</sup> microdomain<sup>5</sup> essential for synaptic exocytosis. However, the observation that some neurons can release functionally significant amounts of neurotransmitter below the threshold of action potentials<sup>6</sup> questioned the possible involvement of another source of Ca<sup>2+</sup> ions, independent of HVA channels activation.

In contrast to HVA channels, low-voltage-activated (LVA) T-type Ca<sup>2+</sup> channels activate in response to subthreshold membrane depolarizations between -65 mV and -50 mV and thus represent an important source of Ca<sup>2+</sup> entry near the resting membrane potential. Hence, besides controlling important physiological processes by regulating neuronal excitability, pacemaker activity and post-inhibitory rebound burst firing, mounting evidences from various neuronal cell types suggest an efficient role of T-type channels in fast and low-threshold exocytosis.<sup>7–10</sup> Until now, however, the mechanism whereby these channels support exocytosis events at the molecular level remained a mystery. In a recent study, we provided compelling evidence of the existence of a Ca<sub>v</sub>3.2/syntaxin-1A molecular complex essential for T-type-dependent exocytosis.<sup>11</sup>

In mammalian synapses, interaction of several members of the vesicle-docking / release machinery (including syntaxin-1A/1B and SNAP-25) onto a *synprint* (*synaptic protein interaction site*) domain

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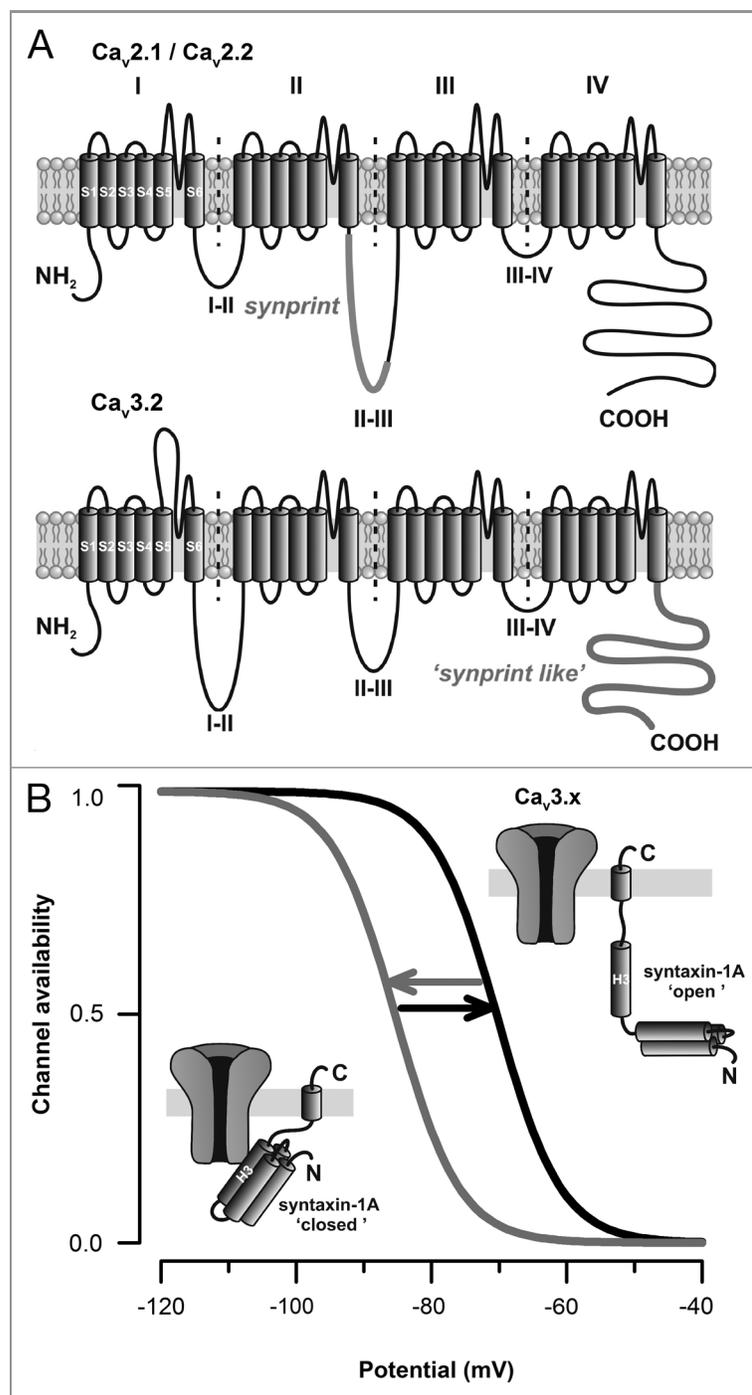
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located within the intracellular loop between domains II and III of the  $Ca_v2.1$ <sup>12</sup> and  $Ca_v2.2$ <sup>13</sup> channels (Fig. 1A, top panel) ensures a close localization of the secretory vesicles near the  $Ca^{2+}$  source. In turn, both syntaxin-1A/1B and SNAP-25 modulate calcium channel activity<sup>14,15</sup> to fine tune  $Ca^{2+}$  entry and synaptic strength. However, in contrast to HVA channels, all of the three T-type channel members (i.e.  $Ca_v3.1$ ,  $Ca_v3.2$  and  $Ca_v3.3$ ) lack the consensus *synprint* site, making the molecular understanding of the involvement of these channels in the exocytosis process quite difficult. Our observation that  $Ca_v3.2$  channels associate with syntaxin-1A in central neurons prompted us to investigate the possible existence of specific  $Ca_v3.2$  channel molecular determinants other than the consensus HVA *synprint* domain. Using biochemical and cellular trafficking approaches, we demonstrated that syntaxin-1A, as well as SNAP-25, interact with the C-terminal domain of  $Ca_v3.2$  channel (Fig. 1A, lower panel). Moreover, using patch-clamp recordings performed on tsA-201 cells expressing  $Ca_v3.2$  channels, we demonstrated that co-expression of a syntaxin-1A in its “closed” conformational state (i.e. the conformation adopted by the syntaxin-1A in isolation or in interaction with Munc18)<sup>16,17</sup> potentially decreases  $Ca_v3.2$  channel availability by shifting the voltage-dependence of inactivation toward more hyperpolarized membrane potentials, similarly to what was previously reported for N- and P/Q-type channels.<sup>14,15,18-20</sup> Interestingly, this regulation was abolished upon co-expression of SNAP-25, and not observed with a constitutively “open” syntaxin-1A (i.e. the conformation adopted upon its association with SNAP-25)<sup>18</sup> (Fig. 1B). Given that syntaxin-1A undergoes a conformational switch from a “closed” to an “open” conformation during the vesicle release cycle,<sup>16,21,22</sup> this suggests that syntaxin-1A may be able to dynamically regulate T-type channel availability during various stages of exocytosis. Interestingly, although T-type channels utilize distinct molecular determinants to interact with SNARE proteins (the C-terminal domain vs. the classical *synprint* of the II-III linker), they are subjected to a similar SNARE

regulation. Does this observation question the molecular mechanism by which binding of syntaxin-1A produces changes in channel gating? Earlier reports have shown

that reorganization of intramolecular interactions among the main intracellular loops of  $Ca_v2$  channels critically influence channel inactivation.<sup>23-30</sup> Mapping the



**Figure 1.** SNARE proteins modulate high- and low-voltage-gated calcium channels via distinct molecular determinants. (A) Membrane topology of voltage-gated calcium channels highlighting the localization of the *synprint* site located within the intracellular linker between domains II and III of  $Ca_v2.1/Ca_v2.2$  channels (top panel), and the “*synprint like*” domain of  $Ca_v3.x$  channels (bottom panel) located within the C-terminal domain of the channel. (B) Voltage-dependence of  $Ca_v3.x$  channel availability during the conformational switch of syntaxin-1A.

intramolecular interactions of T-type channels along with the characterization of the minimal sequence engaged in the interaction with SNARE proteins will provide important structural information on how syntaxin-1A modulates channel gating.

It is well known that direct interaction of SNARE proteins with Ca<sub>v</sub>2.1 and Ca<sub>v</sub>2.2 channels is critical for depolarization-evoked neurotransmitter release. Hence, disruption of the Ca<sup>2+</sup> channel-SNARE proteins coupling by deletion of the *synprint* domain or by peptides derived from the *synprint* sequence, alters synaptic transmission.<sup>31-34</sup> We revealed that similarly to HVA channels, T-type channels-mediated exocytosis relies on a channel-SNARE protein interaction. Indeed, membrane capacitance recordings performed on MPC 9/3L-HA chromaffin cells expressing Ca<sub>v</sub>3.2 channels revealed robust voltage-dependent exocytosis which was totally prevented by co-expression of the Ca<sub>v</sub>3.2 C-terminal domain (i.e the synaptic protein interaction site of Ca<sub>v</sub>3.2). Ablation of Ca<sub>v</sub>3.2-dependent exocytosis most likely results from the specific uncoupling of the channel with SNARE proteins and not from a side

alteration of the exocytosis machinery by itself because no alteration was observed when exocytosis was induced by direct intracellular Ca<sup>2+</sup> elevation. Hence, we showed that similarly to HVA channels, a physical coupling between SNARE proteins and T-type channels is critical for T-type-dependent exocytosis. Considering the relative small conductance of T-type channels<sup>35</sup> and the restricted diffusion of Ca<sup>2+</sup> due to the high Ca<sup>2+</sup> buffering capacity of neuronal cells,<sup>36</sup> it is conceivable that this interaction allows the close localization of the vesicle-docking / release machinery in close proximity to the Ca<sup>2+</sup> source in order to efficiently sense Ca<sup>2+</sup> elevation. However, we cannot exclude the possibility that interaction of T-type channels with SNARE proteins could form a macromolecular complex through which channel conformational changes following membrane depolarization would work as an on/off molecular switch of secretion by controlling the ultimate conformational change of the releasing complex as previously proposed for HVA channels.<sup>37,38</sup> Although this concept still requires further investigation, the use on a non-conducting channel to investigate T-type-dependent secretion would definitively provide

interesting information about the functional importance of T-type channel interaction with SNARE proteins.

Overall, we revealed an unrecognized regulation of low-voltage-activated T-type Ca<sup>2+</sup> channels by SNARE proteins, and provide the first evidence for a molecular mechanism by which these channels could mediate low-threshold exocytosis. We revealed that, although T-type Ca<sup>2+</sup> channels differ from HVA channels by their molecular constituents, they possess the same ability to functionally interact with SNARE proteins, highlighting a key evolutionary mechanism for specialized fast and spatially delimited exocytosis.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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