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Title

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Permalink

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Journal

Nature Communications, 13(1)

ISSN

2041-1723

Authors

Manning, Declan
Santana, L Fernando

Publication Date

2022

DOI

10.1038/s41467-022-35027-5

Peer reviewed

Regulating voltage-gated ion channels with nanobodies

Declan Manning & L. Fernando Santana

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In this work, Morgenstern and colleagues describe an approach involving functionalized nanobodies which decrease the activity of voltage-gated Ca^{2+} channels associated with β_1 subunits and promote their removal from the surface membrane of neurons and muscle.

Voltage-gated Ca^{2+} channels are composed of multiple subunits and are broadly expressed in excitable cells

Voltage-gated Ca^{2+} (Ca_v) channels are broadly expressed in excitable cells throughout the body, where they regulate multiple physiological processes, including cardiac, skeletal, and smooth muscle contraction as well as neuronal excitability, neurotransmitter release, and gene expression. Accordingly, Ca_v channels are a major target for the treatment of cardiovascular and neurological disease. Morgenstern and colleagues¹ describe an approach for controlling the function of Ca_v channels with a specific molecular composition.

Ca_v channels are formed by one of ten pore-forming α_1 subunits, which largely determine their conductive properties. Four of these subunits contribute to L-type Ca^{2+} channels ($\text{Ca}_v1.1-4$), while R-, N-, and P/Q-type channels ($\text{Ca}_v2.1-4$) are composed of their own distinct α_1 subunits.

The Ca_v1 and Ca_v2 subfamilies associate with a range of auxiliary subunits: β , encoded by *CACNB1-4*; $\alpha_2\delta$, encoded by *CACNA2D1-4*; and γ , encoded by *CACNG1-8*^{2,3}. α_1 subunits form a strong interface with β subunits at the intracellular α -interaction domain of the channel^{4,5}. It is believed that Ca_v1 and Ca_v2 channels all assemble with a single β subunit, which serves to promote membrane expression and set channel voltage-dependencies. Expression of β_{1-4} subunits has been detected in the brain. β_1 is also expressed in skeletal muscle, whilst β_2 subunits are expressed in heart, lung, and smooth muscle. Finally, β_3 subunits are expressed highly in smooth muscle.

The β subunit prevents ubiquitination of the α_1/β channel complex, increasing membrane channel density and whole-cell current magnitude⁶. β subunits also confer diverse biophysical properties to the mature Ca_v channels, enabling G protein regulation, setting rates of channel activation and inactivation, and fine-tuning voltage dependency⁷. β subunits also serve as sites for post-translational modification and protein-protein interactions. For example, palmitoylation allows β_{2a} to embed into the plasma membrane, slowing the rate of channel inactivation⁸. More recently, adrenergic stimulation of $\text{Ca}_v1.2$ in the heart was shown to involve an interaction between β_{2a} and the Rad G protein, which becomes phosphorylated by protein kinase A to relieve its constitutive inhibition of $\text{Ca}_v1.2$ ⁹.

Most Ca_v channel agonists and antagonists work by binding to their α_1 subunits. However, because of the broad expression of Ca_v

channels, this pharmacological approach does not afford precise, tissue-specific regulation of Ca^{2+} entry. Gene ablation or siRNA-mediated protein knockdown of β or other subunits approaches could circumvent these limitations, but the interpretation of these experiments is confounded when there are multiple auxiliary subunit isoforms expressed in a cell, some of which have partially overlapping functions.

Nanobodies target ion channels of specific composition with precision

Here, Morgenstern et al.¹ describe an elegant and highly effective strategy to decrease the functional impact of β_1 -associated Ca_v channels using nanobodies.

Nanobodies are recombinant antigen binding fragments, and their small size and folding properties enhance their stability inside of live cells, where they can be utilized as “intrabodies”¹⁰. Morgenstern et al.¹ demonstrate how Ca_v channels may be targeted with functionalized nanobodies, to precisely inhibit channels comprising of specific β subunit isoforms.

In their previous work, Morgenstern et al.¹¹ demonstrated a $\text{Ca}_v\beta$ -targeted nanobody (nb.F3) inhibits $\text{Ca}_v1/2$ channels by initiating their redistribution into endosomes. This nanobody-delivered ubiquitination machinery ($\text{Ca}_v\alpha\beta$ lator) functions as an effective inhibitor of Ca_v channels. In this present work, Morgenstern et al.¹ reveal a refined inhibitor specifically targeted to β_1 -associated Ca_v channels (Chisel-1).

Briefly, the authors identified a nanobody (nb.E8) which selectively binds the $\text{Ca}_v\beta_1$ SH3 domain and inhibits $\text{Ca}_v\beta_1$ -associated voltage-gated Ca_v channels by decreasing open probability and increasing their rate of channel inactivation. Interestingly, nb.E8 also decreases channel activity by reducing channel surface density.

Functionalizing nb.E8 with the Nedd4L HECT domain yielded Chisel-1, which eliminated current through $\text{Ca}_v\beta_1$ -reconstituted $\text{Ca}_v1/\text{Ca}_v2$ and native $\text{Ca}_v1.1$ channels in skeletal muscle. Chisel-1 also decreased depolarization-induced Ca^{2+} entry and excitation-transcription coupling in hippocampal neurons. Notably, Chisel-1 was ineffective against $\text{Ca}_v\beta_2$ -associated $\text{Ca}_v1.2$ channels in cardiomyocytes, underscoring its specificity. In a therapeutic setting, genetically-encoded inhibitors like $\text{Ca}_v\alpha\beta$ lator and Chisel-1 could be selectively expressed within cells of interest, potentially bypassing the off-target effects produced by many traditional Ca_v inhibitors.

Nanobodies could reveal important aspects of ion channel organization and function

The findings by Morgenstern et al.¹ raise multiple intriguing questions. For example, how does binding of the nb.E8 nanobody to $\text{Ca}_v\beta_1$, independent of ubiquitin ligase conjugation, act to reduce the membrane surface density of $\text{Ca}_v2.2$ channels? Does the reduction in channel activity in the presence of nb.E8 prime the channel for

endocytosis? Also, Ca_v channels form clusters in the surface membrane of neurons and muscle^{12,13}. Recent studies indicate that ion channels involved in cooperative signaling cascades co-cluster. This raises the question of whether Chisel-1-bound channels are removed individually within a cluster or if the binding of a subset of channels destines the entire cluster for removal? The latter mechanism would suggest an amplification mechanism which could impact on clustered channels. Future studies should investigate these questions.

Declan Manning¹ & L. Fernando Santana¹✉

¹Department of Physiology and Membrane Biology, University of California, Davis, One Shields Avenue, Davis, CA, USA.

✉ e-mail: lfsantana@ucdavis.edu

Received: 11 November 2022; Accepted: 15 November 2022;

Published online: 09 December 2022

References

1. Morgenstern T. J. et al. Selective posttranslational inhibition of $\text{Ca}_v\beta 1$ -associated voltage-dependent calcium channels with a functionalized nanobody. *Nat. Commun.* <https://doi.org/10.1038/s41467-022-35025-7> (2022).
2. Dong, Y. et al. Closed-state inactivation and pore-blocker modulation mechanisms of human $\text{Ca}_v2.2$. *Cell Rep.* **37**, 109931 (2021).
3. Wu, J. et al. Structure of the voltage-gated calcium channel $\text{Cav}1.1$ complex. *Science* **350**, aad2395 (2015).
4. Davies, A. et al. The $\alpha 2\delta$ subunits of voltage-gated calcium channels form GPI-anchored proteins, a posttranslational modification essential for function. *Proc. Natl Acad. Sci. USA* **107**, 1654–1659 (2010).
5. Pragnell, M. et al. Calcium channel beta-subunit binds to a conserved motif in the I-II cytoplasmic linker of the alpha 1-subunit. *Nature* **368**, 67–70 (1994).
6. Altier, C. et al. The $\text{Cav}\beta$ subunit prevents RFP2-mediated ubiquitination and proteasomal degradation of L-type channels. *Nat. Neurosci.* **14**, 173–180 (2011).
7. Canti, C., Bogdanov, Y. & Dolphin, A. C. Interaction between G proteins and accessory subunits in the regulation of 1B calcium channels in *Xenopus* oocytes. *J. Physiol.* **527**, 419–432 (2000).
8. Chien, A. J., Carr, K. M., Shirokov, R. E., Rios, E. & Hosey, M. M. Identification of palmitoylation sites within the L-type calcium channel $\beta 2a$ subunit and effects on channel function. *J. Biol. Chem.* **271**, 26465–26468 (1996).

9. Liu, G. et al. Mechanism of adrenergic $\text{Ca}_v1.2$ stimulation revealed by proximity proteomics. *Nature* **577**, 695–700 (2020).
10. Dong J. X. et al. A toolbox of nanobodies developed and validated for use as intrabodies and nanoscale immunolabels in mammalian brain neurons. *Elife* **8**, (2019).
11. Morgenstern T. J., Park J., Fan Q. R., Colecraft H. M. A potent voltage-gated calcium channel inhibitor engineered from a nanobody targeted to auxiliary $\text{Ca}_v\beta$ subunits. *Elife* **8**, (2019).
12. Dixon, R. E., Navedo, M. F., Binder, M. D. & Santana, L. F. Mechanisms and physiological implications of cooperative gating of clustered ion channels. *Physiol. Rev.* **102**, 1159–1210 (2022).
13. Sato, D. et al. A stochastic model of ion channel cluster formation in the plasma membrane. *J. Gen. Physiol.* **151**, 1116–1134 (2019).

Acknowledgements

This work was supported by grants from the NIH (HL152681, NS114210, and 1OT2OD026580).

Author contributions

D.M. and L.F.S. wrote the commentary.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to L. Fernando Santana.

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