Functional Modulation of the Cav3.1 Calcium Channel by Alternative Splicing at the C-terminus

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Abstract

The *CACNA1G* gene encodes Cav3.1, the alpha-1G subunit of a low-voltage-activated T-type calcium channel, which plays crucial roles in cardiac and smooth muscle cells and neurons by influencing the transmembrane potentials and regulating intracellular Ca^{2+} signaling. Different splice variants of Cav3.1 have been described, and alternative splicing of Cav3.1 can alter channel kinetics, localization, and cytosolic Ca^{2+} trafficking. Here, we analyze the function of two exons, termed E34 and E35, in the intracellular C-terminus. E34 and E35 are preferentially included in nerve tissue postnatally but mostly skipped in embryonic tissues. To examine the physiological properties of these splice variants, we recorded channel activity by two-electrode voltage clamp. We discovered that including either or both E34 and E35 in Cav3.1 may facilitate channel trafficking. To investigate the mechanism of E34 and E35 splicing regulation, we generated minigene reporters and tested them against specific splicing factors that may modulate their inclusion and/or skipping. We also investigated the effects of the calcium binding protein calmodulin on the channel activity of the different splice variants. Our data indicate that alternative splicing at E34 and E35 of Cav3.1 may regulate neuron excitability and modulate the intrinsic firing pattern by controlling Ca^{2+} influx through the channel.

Introduction

Cav3.1, comprised of at least 38 exons, is an ion channel that mediates cell signaling by conducting ions across the cell membrane. It is alternatively spliced at several sites. It forms four transmembrane domains (DI, DII, DIII and DIV); each domains consists of six membrane-spanning segments, including a pore loop, cytoplasmic loops, and extracellular connecting loops (Fig. 1). Cav3.1 is widely expressed in neuronal, cardiac, kidney and smooth muscle cells, where it serves calcium homeostasis and cell excitability by triggering low-threshold spikes, which in turn stimulate a burst of action potentials by other voltage-gated ion channels[1]. We focused on alternative splicing of exon 34 and 35, which results in both structural changes at the C-terminus and electrophysiological activity changes controlling calcium entry. Here, four splicing variants and one mutation (Fig. 2) were generated to express mouse Cav3.1 channels in *Xenopus* oocytes following cRNA injection. Two-electrode voltage clamp (TEVC) technique was used to study the electrophysiology of mCav3.1 ion channel *in vitro*.

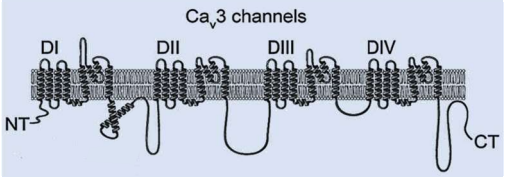

Figure 1. Structural layout of Cav3 channels, comprising four-domains (DI to DIV). x 6 transmembrane helices structure are also found in Cav3.1[2].

[1] Perez-Reyes, Edward. "Molecular physiology of low-voltage-activated t-type calcium channels." Physiological reviews 83.1 (2003): 117-161.

[2] Chemin, Jean, et al. "Calmodulin regulates Cav3 T-type channels at their gating brake." Journal of Biological Chemistry (2017): jbc-M117.

Results

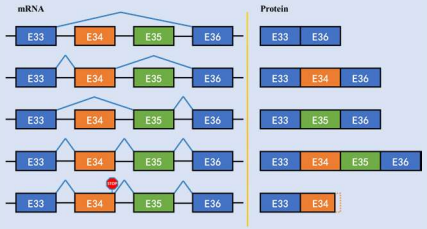

Figure 2. Cloning of alternative splicing variants of mouse Cav3.1 channel. mCav3.1-E33-36 has the exon sequence 33-36 and skips exon 34 and 35. Three more splice variants, including either or both exon 34 or 35, were cloned by RT-PCR, and confirmed by DNA sequencing. One mutant clone contains a stop codon in exon 34, generating a channel truncated at the C-terminus.

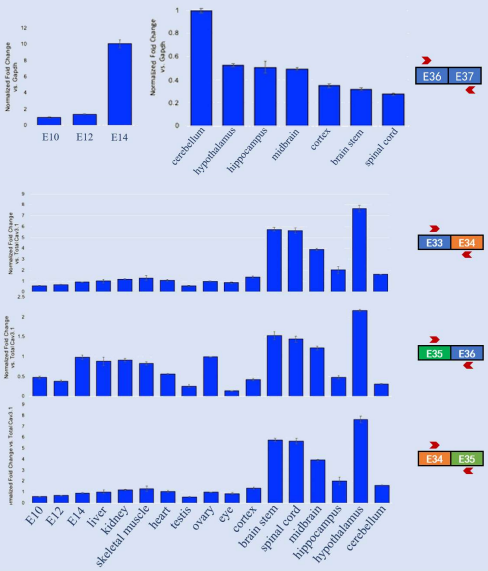

Figure 3. RT-qPCR analysis of mCav3.1 expression in whole embryos at E10, E12, and E14, and in neuronal tissue samples from adult mice. Data from embryo are normalized to E10, while data from neuronal tissue are normalized to cerebellum, the sample that shows the highest level of expression of total mCav3.1 when normalized to Gapdh. qPCR primers are shown as red makers, and their location on the transcript is shown.

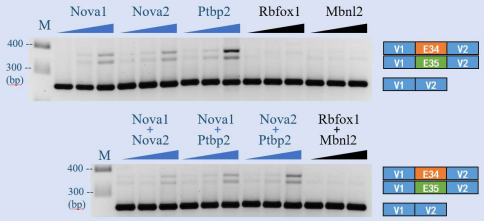

Figure 4. A minigene splicing assay recapitulates Cacnalg alternative splicing *in vitro*. Exons 34 and 35 of Cacnalg were cloned into pSPL3 vector. Constant amounts of minigene were co-transfected into HEK293T cells with increasing amounts of constructs expressing various splicing factors (Noval , Nova2, Ptbp2, Rbfox1 , and MbnI2). Total RNA was collected 48 hours post-transfection and analyzed by semi-quantitative RT-PCR. The location of RT-PCR primers (red markers) on the minigene construct is shown.

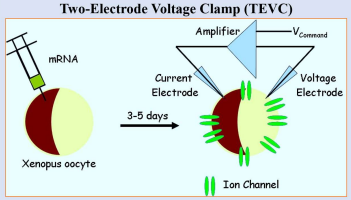

Figure 5. TEVC method. -80mV to 80mV voltage pulses were generated in oocytes injected with mCav3.1 splice variant cRNAs. The current electrode was used to record ion trafficking of the four mCav3.1 splicing isoforms and mutation described in Fig. 2.

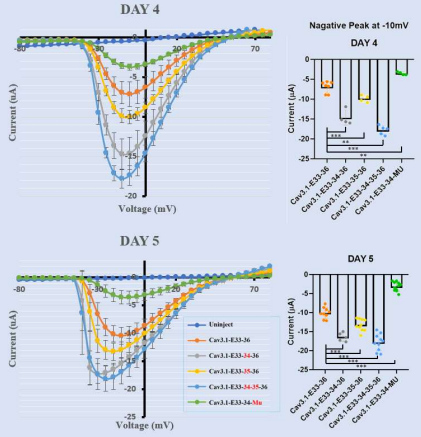

Figure 6. Current-Voltage (I-V) relation of mCaV3.1 channels at Day 4 and 5 after injection with the splice variants and mutation cRNAs indicated in Fig. 2. mCav3.1 channel activity increases when E34 and E35 are included.

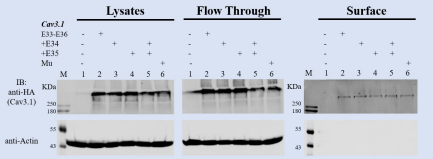

Figure 7. Western blot analysis of cell surface mCav3.1 expression in injected oocytes at day 5. An HA-tag was attached at the N-terminal end of mCav3.1. Equal surface protein expression of mCav3.1 was shown in all four splice variants and mutation.

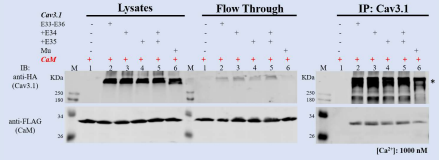

Figure 8. Western blot analysis of co-immunoprecipitation (CO-IP) of mCav3.1 and CaM interaction. All four mCav3.1 splicing isoforms and mutation interact with CaM.

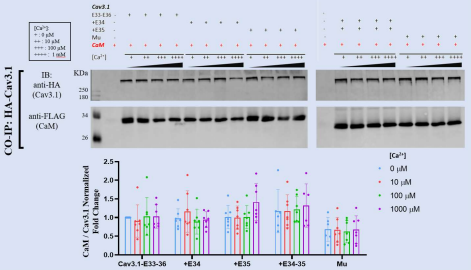

Figure 9. Western blot analysis of impact of Ca^{2+} concentration on the interaction between mCav3.1 and CaM. Different Ca^{2+} concentrations do not appear to significantly affect the interaction between mCav3.1 and CaM. The C-terminus of the ion channel mediates interaction between mCav3.1 and CaM.

Conclusions

1. Expression of mCav3.1 and its splice variants is developmentally regulated. E34 and E35 inclusion is differentially regulated in different neuronal tissues.

2. Nova1, Nova2, and Ptbp2 promote inclusion of E34 and E35 of mCav3.1 *in vitro*, while Rbfoxl and MbnI2 do not appear to regulate mCas,3.1 splicing by minigene assay.

3. Inclusion of alternatively spliced E34 and/or E35 affects the activity of mCav3.1 ion channel via its C-terminus, which regulates voltage-dependent influx of calcium. Truncation of the C-terminal part of the channel blocks gating properties of mCav3.1.

4. C-terminus of Cav3.1 mediates interaction between CaM and mCav3.1, but alternative splicing at C-terminus dose not appear to modulate this interaction.

Acknowledgments

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