Genotyping of CRISPR-Edited Mice and Axonal Localization Patterns of Calmodulin 1 3'UTR Isoforms

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Abstract

Calmodulin (CaM) is a key calcium-signal transducer in multiple cell types ad is known to influence neuron differentiation, development, and maturation. Calmodulin 1 (Calm1) is one of three genes that encode for the CaM protein. Because this gene is subject to alternative polyadenylation (APA), a common mechanism for increasing transcript diversity, it produces transcripts with either a long 3'UTR (Calm1-L) or a short 3'UTR (Calm1-S). While it is known that the long Calm1-L is expressed in neural tissues, the function of *Calm1*-L is still unknown. To study the function of Calm1-L, CRISPR/Cas9 was utilized to generate a mouse model lacking expression of the long isoform. Through genotyping PCR, we confirmed the biogenesis of Calm1-L was impaired. Interbreeding of CRISPR/Cas9 knockout mice produced three genotypes: *Calm1* WT △3'UTR, homozygous △3'UTR, and heterozygous △3'UTR. The homozygous deletion mice exhibit impaired DRG axon development. We performed RNAscope fluorescent *in situ* hybridization (FISH) with probes designed to detect Calm1-L or all Calm1 isoforms. Through the application of FISH on mouse primary hippocampal cells, we discovered transcript localization patterns of Calm1-L versus Calm1-S in axons and cell bodies.

Background

Calmodulin (CaM)

- Ion sensor and transducer of Ca^{+} signals(1)
- CaM important in guiding axon projection(1)
- Mammals encode 3 CaM genes (*Calm1*, *Calm2*, *Calm3*) (1)
- Knockdown of *Calm1* found to cause defects in the creation of hindbrain neurons(1,2,3)

Alternative Polyadenylation

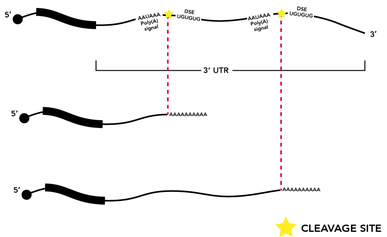


Figure 1 Alternative polyadenylation commonly leads to the expression of two alternative length 3'UTR isoforms

CRIPSR-Cas9 Genome Editing System

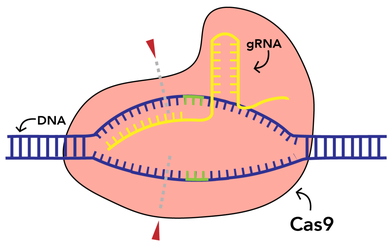


Figure 2 CRISPR-Cas9 genome editing is a useful approach to generate mice that lack *Calm1-L* expression

Cortex Northern Blot

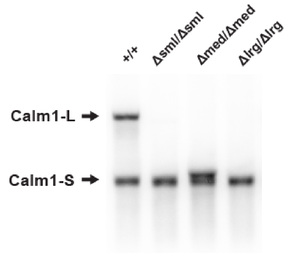


Figure 3 CRISPR-Cas9 approach successfully abolished *Calm1-L* without impairing *Calm1-S* expression

Methods

Primary Hippocampal Neuron Culture: Hippocampal cells from postnatal day 0-1 mouse pups dissected and dissociated. Cells plated on cover glass for imaging experiments. At DIV 1 AraC added to reduce non-neuronal populations. The cultures were maintained until DIV7 at 37°C with 5% CO_2.

PCR Genotyping: Genomic DNA (gDNA) isolated from tail-snips of mice by overnight incubation in lysis buffer. gDNA then precipitated and resuspended in water for PCR. m.g.7 and m.g.8 (shown in figure 8) were used. PCR performed using Taq Polymerase with Standard Taq Buffer (NEB) with 2 µL of gDNA preps.

Fluorescent *in situ* Hybridization: RNAscope multiplex fluorescent v2 assay according to manufacturer's instruction probe solution using Calm1 probes (seen in figure 4). A Leica SP8 TCS confocal microscope was used for imaging.

Results

Analysis of Localization of Patterns of *Calm1* Isoforms

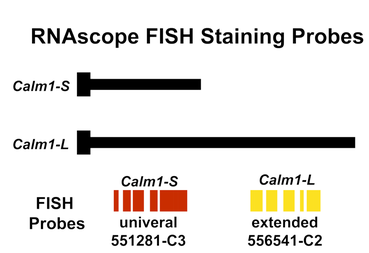


Figure 4 Schematic of probes used in RNAscope FISH staining

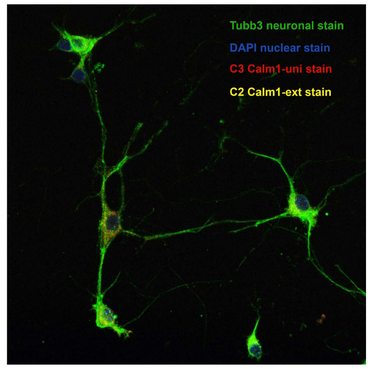
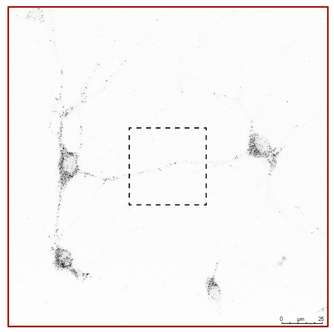


Figure 5 FISH image of DIV7 Hippocampal neural cells from WT mouse



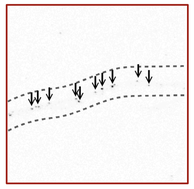
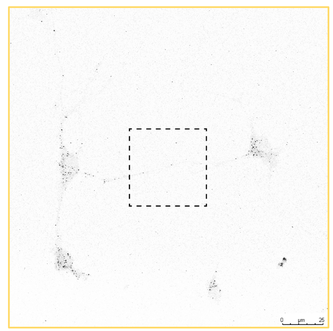


Figure 6 Image of *Calm1-S* isoform transcript localization found in a DIV7 hippocampal neuron cell



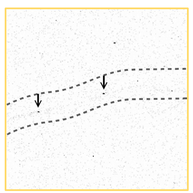


Figure 7 Image of *Calm1-L* isoform transcript localization found in a DIV7 hippocampal neuron cell

*Signals enhanced for visualization purposes

Results

Genotyping of CRISPR edited mice

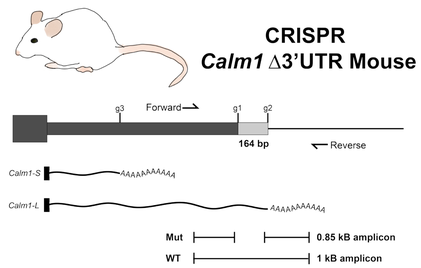


Figure 8 Schematic of *Calm1* △3'UTR mouse genotyping PCR primers

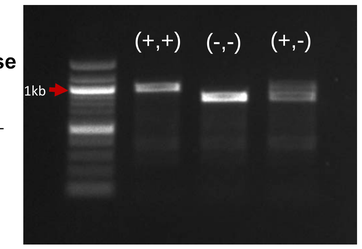


Figure 9 Agarose gel showing three genotypes of CRISPR edited mice assessed through genotyping PCR. Deletion of Calm1-L is signified through the cut of 164 bp

Conclusion

- Successful deletion of 3'UTR extended region impaired biogenesis of Calm1-L
  - Identification of interbred knockout mice through genotyping PCR
- Both long and short isoforms of Calm 1 3'UTR display transcript localization in hippocampal neuron cells as shown through FISH staining of DIV7 hippocampal neuron cells

Future Directions

- Evaluation of transcript localization through distal measurements of localization in neurite from soma
- Optimization of cell culturing to improve growth of neurites
- Produce full images of HIPP cell neurites by stitching together imaging from FISH staining

References

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4. Tushev et al. (2018) Neuron. 98(3):495-511

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