Characterization of the sodium calcium exchanger NCX-6 in *C. elegans*

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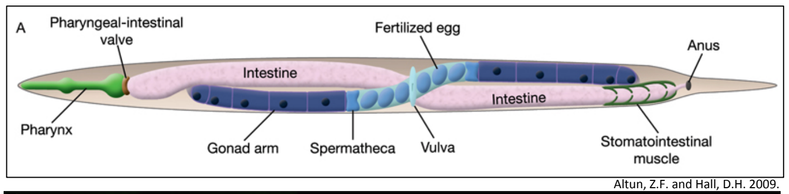
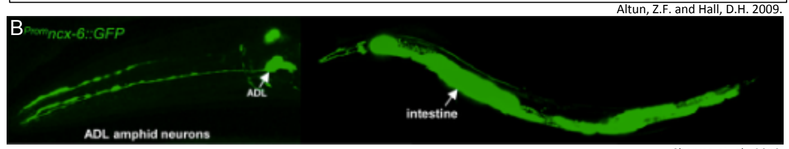
Abstract

The exchanger protein NCX-6 belongs to the sodium calcium exchanger (NCX) family, a group of transporter proteins that regulate Ca^{2+} homeostasis and electrical capacitance by rapidly pumping calcium ions out of the cell. Expression of sodium calcium exchangers in *C. elegans* has been reported in diverse tissues and cell types, such as sensory neurons, interneurons, motor neurons, muscles cells and intestinal tissue, with even more diverse expression in mammals. Abnormal function of these calcium exchangers has been implicated in Parkinson's disease, heart disease, hypertension, and diabetes, thus understanding the exact function of Na^{+}/Ca^{2+} exchangers in physiological pathways is crucial in developing effective treatments and biological markers. Although the NCX family has been broadly studied, much is still unknown with regard to the specific function of NCX genes and proteins. Thus, the characterization of NCX-6 provides novel insight into the calcium dynamics controlled by Na^{+}/Ca^{2+} exchangers and their affect on a stereotyped intrinsic rhythm of the intestine.

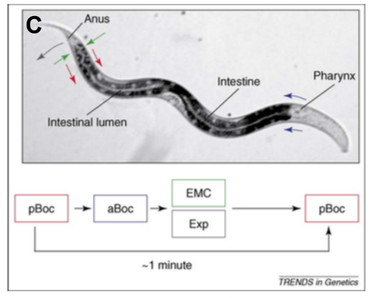
Introduction



Altun, Z.F. and Hall, D.H. 2009.



Sharma et. al., 2013.



Briancy and Hekimi, 2006.

Figure 1. (A) *C. elegans* alimentary system in a hermaphroditic adult. **(B)** Expression pattern of *ncx-6* in *C. elegans*. The *ncx-6* gene is exclusively expressed in the intestine as well as a pair of head neurons, termed the ADL. **(C)** Defecation motor program (DMP) cycle in *C. elegans*, characterized by three distinct steps executed in a rhythmic pattern: pBoc, aBoc, and Exp.

Methods

Genetics

- Wildtype (N2) males containing the GCaMP transgene were crossed with hermaphrodite *ncx-6(gk182649)* mutants. Cross progeny were genotyped visually for the GCaMP transgene and sequenced for the *ncx-6* mutation.
- N2 animals positive for GFP under the *ncx-6* promoter were selected and used for analysis of the temporal expression pattern of NCX-6.

DMP Assay

- Day 1 adult GCaMP positive animals (N2 and *ncx-6* mutants) were observed for six consecutive expulsions, starting with the Exp of the first cycle and continuing for the following five DMP cycles. The presence or absence of an Exp step was recorded as well the time between incidents of expulsion.
- A percentage of successful Exp events was calculated and a Z-test used to determine the significance of the collected data.

Fluorescent Imaging

- Wildtype (N2) animals containing a GFP transgene under the *ncx*-6 promoter were imaged using an Zeiss Axiocam MRm camera on a Zeiss Axio Imager M2 microscope under 5x, 10x, and 20x magnifications and visualized on an adjacent monitor using AxioVision SE64 Rel. 4.0 software.
- All stages of the *C. elegans* life cycle were imaged, including egg, larval stage 1 (L1), larval stage 2 (L2), larval stage 3 (L3), larval stage 4 (L4), and day 1 adult, as well as an adult male.

Results

Temporal Expression Patten of *ncx-6*

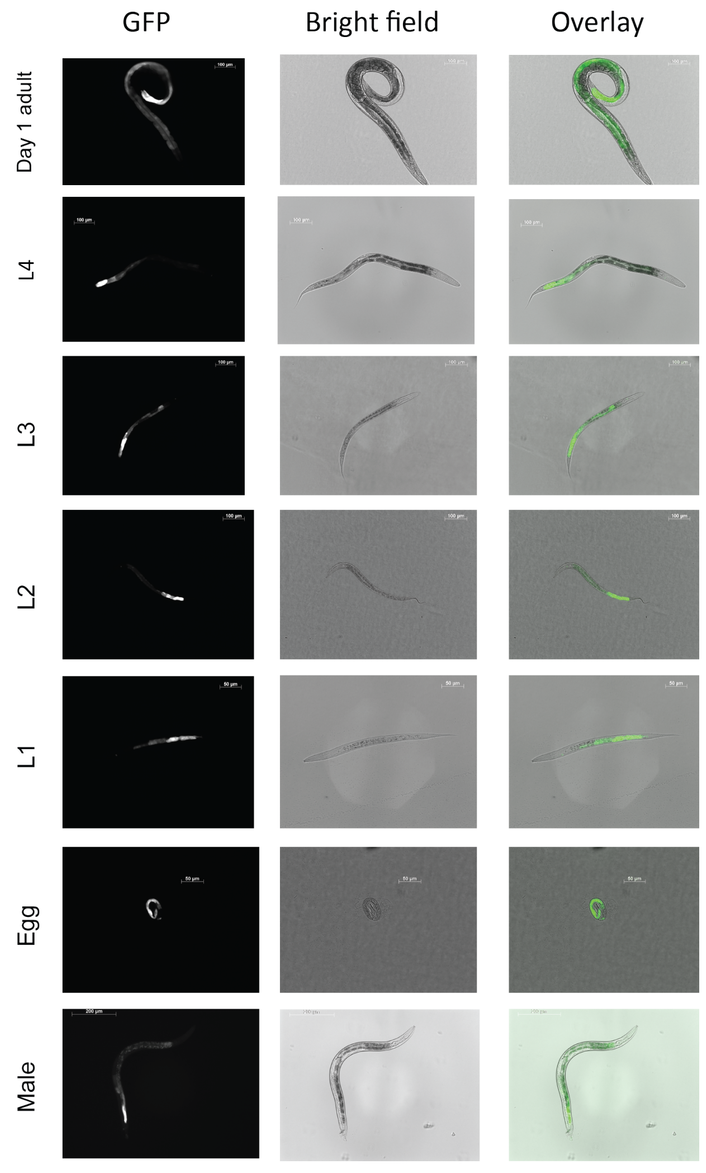


Figure 2. *ncx-6* is expressed in *C. elegans* throughout development and in both sexes. In all life stages imaged, *ncx-6* is expressed predominately in the intestine.

DMP assay

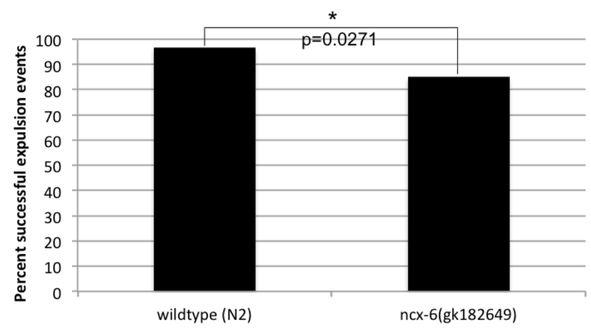


Figure 3. *ncx-6(gk182649)* mutants were found to have a significantly lower percentage of successful expulsion events of the DMP cycle when compared to wildtype (N2) animals. (p=0.0271)

Conclusion

An analysis of the temporal expression pattern of *ncx-6*, visualized through GFP expressed under the *ncx-6* promoter, revealed that *ncx-6* is expressed in *C. elegans* throughout development and in both sexes [Figure 2]. In all life stages imaged, *ncx-6* was expressed in the intestine. This suggests that NCX-6 plays a role in the DMP cycle of C. elegans, as it is the major process that occurs in the intestine over the lifespan of the animal. Furthermore, the results of the DMP assays reveal that *ncx-6* mutants have a significantly lower percentage of successful expulsion steps when compared to wildtype (N2) animals [Figure 3]. Thus, we can speculate that NCX-6 plays a role in the proper execution of the expulsion step of the DMP cycle.

Future Research

Future research will seek to further characterize the *ncx-6* mutant and understand the role of NCX proteins in the calcium dynamics of the intestine. Studies will examine the function of NCX-6 in intestinal calcium oscillations linked to the defecation motor program (DMP) using a genetically encoded calcium imager called GCaMP to visualize calcium dynamics *in vivo.* Additionally, future research will seek to identify the subcellular localization of the NCX-6 exchanger protein using GFP tagged *ncx-6*.

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