Unlike Drosophila elav, the *C. elegans* elav orthologue *exc-7* is not panneuronally expressed

Kenneth Pham$^1$ and Oliver Hobert$^{1\S}$

$^1$Columbia University, Department of Biological Sciences, HHMI, New York, NY

$\S$To whom correspondence should be addressed: or38@columbia.edu

**Figure 1:** Expression pattern of *exc-7::gfp* in the OH16020 strain. *exc-7* was endogenously tagged at its genomic locus with a *gfp::3xflag* tag (A). Embryonic expression (B) was observed in the excretory canal cell (arrow) and other unidentified cells (*). Broad expression was observed in L1 (C) and young adult head (D'), mid body (D''), and tail (D'''), including the excretory canal cell at both stages (arrow). In young adult, individual neurons expressing *exc-7* were identified in the head (E') and in the ventral nerve cord (E''). All *exc-7* expressing neurons are listed in panel (F) (grey box). Scale bar, 10 µm.

**Description**

We are interested in identifying genes that are expressed in a panneuronal manner throughout the nervous system (Stefanakis et al., 2015). The *Drosophila elav* locus is a panneuronally expressed RNA binding protein (Campos et al., 1987; Robinow and White, 1988). Elav protein staining is routinely used in *Drosophila* to identify neurons and cis-regulatory control regions from the *elav* locus are routinely used as panneuronal Gal4 drivers (Berger et al., 2007; Luo et al., 1994; O’Neill et al., 1994; Osterwalder et al., 2001; Robinow and White, 1991). Based on sequence homology, the *C. elegans* *exc-7* locus is the sole *C. elegans* orthologue of *elav* (Fujita et al., 2003; Fujita et al., 1999; Loria et al., 2003; Samson, 2008). Previous expression pattern analyses have shown that *exc-7* is expressed only in a subset of neurons of the nervous system (the expressing neurons were mostly unidentified) (Fujita et al., 1999; Loria et al., 2003). However, reporter gene constructs previously used to infer *exc-7* expression did not contain all intergenic region of the large *exc-7* locus. Therefore, the possibility remained that through the use of more distal cis-regulatory elements, *C. elegans* *exc-7* could also be panneuronally expressed, like its fly orthologue. To address this possibility, we tagged endogenous *exc-7*
with gfp::3xflag at its C-terminus using CRISPR/Cas9 genome engineering (Dokshin et al., 2018) and examined its expression. We cloned gfp from the che-1(ot856/che-1::gfp) allele (Leyva-Díaz and Hobert, 2019), inserted it into the pMiniT 2.0 vector (NEB), and used that resulting plasmid for subsequent cloning of the gfp tag.

Embryonic expression of exc-7 was first observed at the bean stage. By reverse lineaging with use of SIMI-Biocell software (Schnabel et al., 1997), we confirm the identity of one of the expressing cells at this stage as the excretory canal cell (Fig. 1B, arrow). In L1 animals, broad expression in the head, ventral nerve cord (VNC), and tail was observed (Fig. 1C). In young adults, expression is notably observed in vulva cells (Fig. 1D’”). In the nervous system specifically, expression is observed in many neurons throughout the body (Fig. 1D’-D’’’), but unlike Drosophila Elav, exc-7::gfp is not panneuronally expressed. We used the NeuroPAL transgene (https://www.biorxiv.org/content/10.1101/676312v1) to individually identify each neuron in which exc-7 is expressed in the young adult worm. Sites of expression are listed in Fig. 1F and some examples of neuronal expression are shown in Fig. 1E’. Expression in all neurons is at least several fold more intense than UPN::NLS::TagRFP-T signal from NeuroPAL. We confirmed previously reported expression in cholinergic VNC MNs, but absence of GABAergic VNC MNs (Fig. 1E’’), consistent with previous reports (Fujita et al., 1999; Loria et al., 2003) and consistent with exc-7 functioning in cholinergic, but not GABAergic neurons to control alternative splicing (Norris et al., 2014). exc-7::gfp is also expressed in some non-neuronal cell types, including muscle and hypodermis, but not in the gut (Fig. 1D’-D’’’’). A previous report showed that exc-7 is only transiently and weakly expressed in the excretory cell, which, based on exc-7’s excretory mutant phenotype, has puzzled researchers (Fujita et al., 2003). We find that the gfp tagged exc-7 locus is strongly and continuously expressed in the excretory canal cell (Fig. 1B-D’, arrow). We conclude that unlike its fly orthologue elav, exc-7 is not a panneuronally expressed gene.

Reagents

OH16020: exc-7(ot970[exc-7::gfp::3xflag])

The strain is available through the CGC.

Acknowledgments: We thank Chi Chen for expert DNA injection, Eduardo Leyva-Díaz for advice with CRISPR genome editing, and Neda Masoudi for help with embryonic lineaging.

References


Funding: This work was supported by the HHMI.

Author Contributions: Kenneth Pham: Investigation, Methodology, Visualization, Writing - review and editing. Oliver Hobert: Funding acquisition, Supervision, Writing - original draft.

Reviewed By: Matthew Buechner

History: Received October 19, 2019 Accepted October 30, 2019 Published October 30, 2019

Copyright: © 2019 by the authors. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Citation: Pham, K; Hobert, O (2019). Unlike Drosophila elav, the C. elegans elav orthologue exc-7 is not panneuronally expressed. microPublication Biology. https://doi.org/10.17912/micropub.biology.000189