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

Kenneth Pham¹ and Oliver Hobert¹§

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

§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.

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References


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