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

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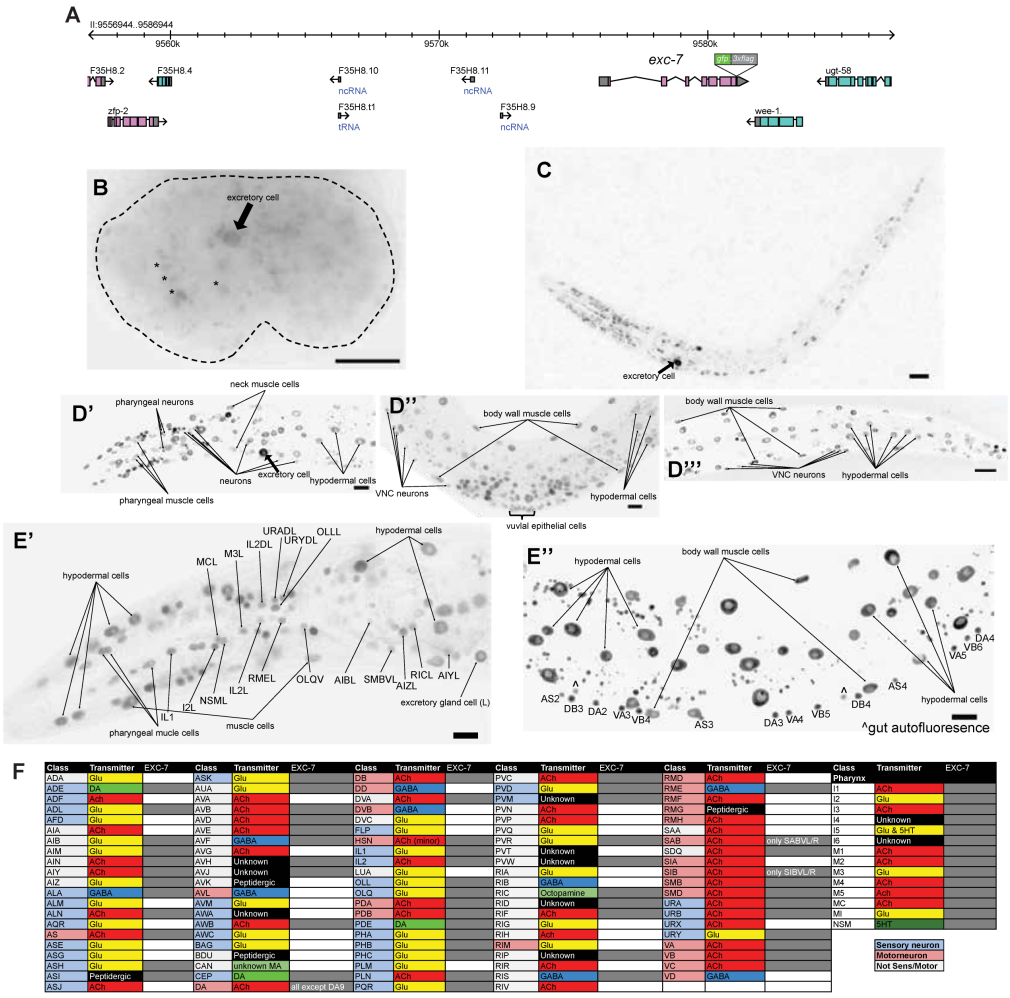

Figure 1: Expression pattern of *exc-7::gfp* in the OH16020 strain. *exc-7* was endogenously tagged at its genomic locus with a *gfp::3xflag* tag (A). Embryonic expression (B) was observed in the excretory canal cell (arrow) and other unidentified cells (*). Broad expression was observed in L1 (C) and 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-Diaz 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* it 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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