Analysis of \textit{unc-62} expression pattern in \textit{C. elegans} embryonic AWC neurons

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Abstract

The \textit{Caenorhabditis elegans} UNC-62 homothorax/Meis/TALE homeodomain protein functions sequentially to regulate general identity of the AWC olfactory neuron pair and the stochastic choice of asymmetric AWC subtypes during embryogenesis. Here we analyze the expression pattern of \textit{unc-62} during AWC development using an integrated \textit{unc-62::GFP} fosmid rescuing transgene. UNC-62::GFP was not detected in AWC neurons in early or late embryos. These results are consistent with previous single-cell RNA sequencing data and also suggest an undetectable level of \textit{unc-62} expression and/or low stability of UNC-62 protein in AWC neurons during embryogenesis.

Figure 1. Expression patterns of an integrated \textit{unc-62::GFP} fosmid transgene in embryonic AWC neurons: (A) Genomic structure and position of the \textit{unc-62} locus and gene loci near \textit{unc-62} in chromosome V. The genomic region of the
unc-62::GFP fosmid clone is shown at the bottom. All UNC-62 protein isoforms are tagged with GFP at the C-terminus from the unc-62::GFP fosmid transgene (Van Nostrand et al., 2013). (B–D) Representative images of UNC-62::GFP expression from an integrated unc-62::GFP fosmid transgene in a gastrula (B), a 1.5-fold stage embryo (C), and a 3-fold stage embryo (D). hlh-16::H1-wCherry and odr-1p::TagRFP expressed from integrated transgenes were used as early and late AWC markers, respectively. Insets in panels B–D are magnified by 2-fold. Scale bar, 10 μm. Anterior to the left in B and C.

Description
The UNC-62 homeodomain protein regulates AWC general identity and subsequently plays a cell autonomous role, determined by mosaic analysis, in AWC asymmetry during embryogenesis (Hsieh et al., 2021). An integrated unc-62::GFP fosmid transgene, in which all UNC-62 protein isoforms are tagged with GFP at the C-terminus (Van Nostrand et al., 2013) (Figure 1A), rescued unc-62(lf) mutant phenotypes of AWC general identity, determined by odr-1p::DsRed expression, and AWC asymmetry, determined by str-2p::GFP expression (Hsieh et al., 2021). These results suggest that UNC-62::GFP fusion protein expressed from the unc-62::GFP fosmid transgene is functional for AWC development. It has been shown that this integrated unc-62::GFP fosmid transgene is expressed in sensory neurons, touch neurons, interneurons, ventral nerve cord motor neurons, and head motor neurons, but it is not expressed in AWC neurons in late-stage larvae or young-stage adult worms using the multicolor transgene NeuroPAL (Reilly et al., 2020).

The AWC neurons are born near the end of gastrulation; AWC asymmetry is established around the 1.5-fold and 3-fold embryonic stage (Sulston et al., 1983; Chuang and Bargmann, 2005). To determine whether unc-62 is expressed in AWC neurons at the embryonic stages of AWC development, the expression pattern of the integrated unc-62::GFP fosmid transgene (Van Nostrand et al., 2013) (Figure 1A), was analyzed with integrated hlh-16::H1-wCherry or odr-1p::TagRFP transgene, early or late AWC marker, respectively. UNC-62::GFP was not detected in AWC neurons at the end of gastrulation, 1.5-fold, or 3-fold embryos (Figure 1B–D). Consistent with our results, single-cell RNA sequencing data revealed a very low expression level of unc-62 in AWC during early embryogenesis as well as an undetectable level of unc-62 in AWC in the later embryonic stage and second-larval stage (Cao et al., 2017; Packer et al., 2019). Together, these results suggest that unc-62 may be expressed at an undetectable level and/or UNC-62 protein may have a very short half-life in embryonic AWC neurons.

Reagents

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
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<tbody>
<tr>
<td>SD1871</td>
<td>wgIs600 [unc-62::GFP fosmid (derived from unc-62 fosmid clone WRM061dC01); unc-119(+)]</td>
<td>Van Nostrand et al., 2013</td>
</tr>
<tr>
<td>RW10588</td>
<td>unc-119(ed3); zuIs178 [his-72(1kb 5′ UTR)::his-72::SRPVAT::GFP::his-72 (1KB 3′ UTR) + 5.7 kb Xbal – HindIII unc-119(+)]; stIs10544 [hlh-16::H1-wCherry::let-858 3′ UTR]</td>
<td>Murray et al., 2012</td>
</tr>
<tr>
<td>IX5658</td>
<td>wgIs600; stIs10544</td>
<td>This study</td>
</tr>
<tr>
<td>IX3577</td>
<td>wgIs600; vyls56[odr-1p::TagRFP] III (Cochella et al., 2014)</td>
<td>This study</td>
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References


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