A missense mutation separates distinct functions of the Zic-family transcription factor REF-2

Michael P. Hart¹ and Oliver Hobert²§

¹Department of Genetics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, USA
²Columbia University, Howard Hughes Medical Institute

§To whom correspondence should be addressed: or38@columbia.edu

Figure 1: ref-2(ot762) affects neuron development. (A) Fluorescent micrographs of unc-53::gfp (DA and AS) and unc-47::mCherry (DD and VD) in L4 wild-type control and ot762 mutant animals, with ventral nerve cord motor neurons indicated by asterisks (scale bar 50µm in all panels). (B) Quantification of the number of VNC neurons expressing unc-53::gfp and unc-47::mCherry in controls and ot762 mutants at L1 and L4 stages (mean ± standard deviation). (C) Confocal micrographs of rab-3::tagRFP (all VNC neurons) in L4 control and ot762 mutant. The average number of rab-3::tagRFP(+) cells in the central region of the VNC decreases from 42.3 to 21. (D) Amino acid sequence of C. elegans of the fourth and fifth Zn finger of REF-2 aligned with orthologs from other species and location of amino acid substitutions caused in ot327 (Let = lethal phenotype) and ot762 (Unc = uncoordinated phenotype) alleles indicated below sequence. Hs = Homo sapiens, Ci = Ciona intestinalis, Dm = Drosophila melanogaster, Hv = Hydra vulgaris (Hv) , Ce = C. elegans. Blue indicates zinc coordinating cysteine and histidine residues, grey indicates complete conservation in species shown.
(E) Fluorescent micrographs of ttx-3::gfp (AIY interneuron) in L1 control and ot762 mutant. (F) Quantification of the percent of worms with ttx-3::gfp expression in AIY in controls and ot762 mutants at the L1 and L4 stages (mean ± standard deviation). In all images, anterior is to the left.

Description

To better understand motor neuron specification, we have conducted an EMS-induced mutant screen in which we sought to identify mutants with defects in the expression of an unc-53::gfp marker (hdIs1 transgene), which is normally expressed in the cholinergic DA and AS motor neurons (Wacker et al., 2003). As previously reported, this screen identified the zinc finger transcription factor bnc-1 (Kerk et al., 2017). Another allele identified from this screen is ot762. Unlike bnc-1 mutant alleles, ot762 mutant animals are Unc and Egl. ot762 mutants show a decrease in the number of unc-53::gfp expressing neurons in the L4, but not L1 stage, indicating a loss of unc-53::gfp expression in the postembryonically generated AS neurons, but not the embryonically born DA neurons (Fig.1A,B). The lineal sister of the cholinergic AS neurons are the GABAergic VD motor neurons (Sulston, 1976). We find that an unc-47::mCherry marker (otIs348; Gendrel et al., 2016)) also fails to be expressed in postembryonically generated VD motor neurons of ot762 mutants; in addition, there is also a reduction in the number of embryonically generated DD neurons (Fig.1A,B). Analysis of a panneuronal marker, rab-3, indicates a reduced number of neurons in the ventral nerve cord (Fig.1C), supporting the possibility that AS, DD and/or VD neurons may not be generated.

Using a combined polymorphic mapping and whole genome sequencing pipeline (Minevich et al., 2012) we found that ot762 animals harbor a missense mutation in the 5th zinc finger domain of the highly conserved zinc finger transcription factor REF-2, called Zic1/2/3 in vertebrates and Opa in flies (Alper and Kenyon, 2002; Aruga, 2004) (Fig.1D). The mutation results in the substitution of a highly conserved serine to a leucine within the last Zn finger domain of REF-2. This specific amino acid position is predicted to contact DNA (Benos et al., 2002). Previous analysis in C. elegans has shown that ref-2 null mutants display an L1 larval lethal phenotype, possibly due to a disruption of the excretory system (Bertrand and Hobert, 2009; Bordet and Bertrand, 2018). However, while ot762 animals are Unc (consistent with a function in motor neuron development), they do not die at L1. Therefore, ot762 separates distinct functions of ref-2 in different cell types. ref-2 has also previously been shown to act upstream of the terminal selector ttx-3 to control AIY interneuron specification (Bertrand and Hobert, 2009). We find that ot762 animals display strong defects in ttx-3::gfp expression (Fig.1D,E), albeit at a somewhat lesser penetrance than the ot327 null allele (Bertrand and Hobert, 2009).

ref-2 is expressed in a number of neuroblasts, but not in the mature, adult nervous system (Bertrand and Hobert, 2009). Among the neuroblasts that express ref-2 are the P neuroblasts (Alper and Kenyon, 2002; Bertrand and Hobert, 2009), which give rise to a number of motor neurons, including the AS and VD sister neurons (Sulston, 1976). Together with the ref-2(ot762) mutant phenotype that we describe here, as well as with P cell developmental defects described in ref-2 mutants (Alper and Kenyon, 2002), this suggests that ref-2 acts at some point in the P lineage to affect the differentiation of the AS and VD motor neurons. The phenotype that we observe in the AIY neurons of ot762 is a reflection of the previously described function of ref-2 in the neuroblast that generates the AIY neuron (Bertrand and Hobert, 2009). DD neuron differentiation defects observed in ot762 mutants may be a reflection of ref-2 function in embryonic neuroblasts akin to the function of ref-2 in the AIY-generating neuroblast.

Reagents

OH12225 ref-2(ot762); hdIs1[unc-53::gfp]; otIs348[unc-47::mCherry]

Acknowledgments: We thank Kelsey Roberts for assistance and John Kerk and Paschalis Kratsios for discussions.

References


**Funding:** Howard Hughes Medical Institute

**Author Contributions:** Michael P. Hart: Formal analysis, Methodology, Writing - review and editing, Conceptualization, Investigation. Oliver Hobert: Funding acquisition, Conceptualization, Writing - original draft.

**Reviewed By:** Roger Pocock

**History:** Received March 6, 2020 Accepted March 13, 2020 Published March 16, 2020

**Copyright:** © 2020 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.