A single amino acid change in the EGL-46 transcription factor causes defects in BAG neuron specification

Rasoul Godini$^1$, Kasper Langebeck-Jensen$^2$ and Roger Pocock$^1$

$^1$Development and Stem Cells Program, Monash Biomedicine Discovery Institute and Department of Anatomy and Developmental Biology, Monash University, Melbourne, Victoria 3800, Australia

$^2$Biotech Research and Innovation Centre, University of Copenhagen, Ole Maaløes Vej 5, Copenhagen, Denmark

§To whom correspondence should be addressed: roger.pocock@monash.edu

$^*$These authors contributed equally.

**Figure 1:** (A) Schematic of the *egl-46* genomic locus showing the *rp4* genetic lesion (TGC>TAC). (B) Schematic of the EGL-46 protein showing the amino acid change (C185Y) caused by *rp4*. (C) Quantification of *Pgcy-33::gfp* expression defects in *egl-46(rp4)* animals. Transgenic expression of a fosmid (WRM0636bB06) containing the entire *egl-46* genomic locus rescues the loss of *Pgcy-33::gfp* expression in the BAG neurons observed in *egl-46(rp4)* mutant animals. Circles indicate *gfp* expression level in the pair of left and right BAG neurons.

**Description**

The BAG neurons control multiple aspects of *Caenorhabditis elegans* behavior, such as sensing environmental gases (oxygen and carbon dioxide), regulation of systemic fat levels and egg laying (Brandt et al. 2012; Guillermin et al. 2011; Juozaityte et al. 2017; Zimmer et al. 2009). To identify factors that control BAG specification, we performed a forward genetic mutagenesis screen using the *Pgcy-33::gfp* reporter, which is exclusively expressed in the BAG neurons. We
isolated a new allele (rp4) that exhibits a loss of Pgcy-33::gfp expression. Using the one-step whole-genome sequencing and SNP mapping strategy (Doitsidou et al. 2010) we mapped the genetic lesion to the egl-46 gene, encoding a zinc finger transcription factor homologous to mammalian INSM1/2, which we had previously shown to be important for BAG specification (Rojo Romanos et al. 2015). The new lesion we identified egl-46(rp4) (TGC>TAC) causes a single amino acid change in a highly conserved cysteine residue (C185Y) that lies in the first zinc finger domain of EGL-46, which would be predicted to affect DNA binding. Analysis of Pgcy-33::gfp expression in the rp4 allele reveals that it exhibits the same phenotype as the previously published rp13 deletion allele, which is an out-of-frame deletion that removes the zinc finger domains (Rojo Romanos et al. 2015). Therefore, rp4 acts as a strong loss-of-function/null allele and may be of use to those researchers interested in elucidating additional functions of EGL-46.

Methods
In the forward genetic screen, the BAG reporter strain Pgcy-33::gfp; Pdop-3::rfp was mutagenized using ethyl methanesulfonate. Mutants with decreased GFP expression in the BAG neurons were isolated using the automated COPAS biosorter platform. The one-step whole-genome sequencing and SNP mapping strategy (Doitsidou et al. 2010) was used to identify the genetic lesion of the isolated rp4 allele. Phenotypic analysis of Pgcy-33::gfp BAG expression was performed as described previously (Rojo Romanos et al. 2015).

Reagents
RJP22 rplIs3(Pgcy-33::gfp); vslIs3(Pdop-3::rfp)
RJP56 egl-46(rp4); rplIs3(Pgcy-33::gfp); vslIs3(Pdop-3::rfp)
RJP4585 egl-46(rp4); rplIs3(Pgcy-33::gfp); vslIs3(Pdop-3::rfp); rpEx2046 (WRM0636bB06) 1ng/µl + Punc-122::gfp 30ng/µl Line 1
RJP4586 egl-46(rp4); rplIs3(Pgcy-33::gfp); vslIs3(Pdop-3::rfp); rpEx2047 (WRM0636bB06) 1ng/µl + Punc-122::gfp 30ng/µl Line 2

Strains will be available at the CGC.

References

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