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

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**A**

\textbf{egl-46 (K11G9.4)}

\textbf{rp4}  
TGC>TAC

**B**

\textbf{EGL-46}  
\textbf{Zinc fingers}  
286 aa

\textbf{rp4}  
C185Y

**C**

\begin{tabular}{|l|l|l|l|l|}
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 &  &  &  &  \\
Wild-type &  & \begin{tikzpicture}[scale=0.5]
\draw[fill=green] (0,0) circle (0.2cm);
\draw[fill=white] (0.5,0) circle (0.2cm);
\draw[fill=white] (1,0) circle (0.2cm);
\draw[fill=white] (1.5,0) circle (0.2cm);
\draw[fill=white] (2,0) circle (0.2cm);
\end{tikzpicture} &  & \begin{tikzpicture}[scale=0.5]
\draw[fill=green] (0,0) circle (0.2cm);
\draw[fill=white] (0.5,0) circle (0.2cm);
\draw[fill=white] (1,0) circle (0.2cm);
\draw[fill=white] (1.5,0) circle (0.2cm);
\draw[fill=white] (2,0) circle (0.2cm);
\end{tikzpicture} &  & 50 \\
\textit{egl-46(rp4)} &  & \begin{tikzpicture}[scale=0.5]
\draw[fill=green] (0,0) circle (0.2cm);
\draw[fill=white] (0.5,0) circle (0.2cm);
\draw[fill=white] (1,0) circle (0.2cm);
\draw[fill=white] (1.5,0) circle (0.2cm);
\draw[fill=white] (2,0) circle (0.2cm);
\end{tikzpicture} &  & \begin{tikzpicture}[scale=0.5]
\draw[fill=green] (0,0) circle (0.2cm);
\draw[fill=white] (0.5,0) circle (0.2cm);
\draw[fill=white] (1,0) circle (0.2cm);
\draw[fill=white] (1.5,0) circle (0.2cm);
\draw[fill=white] (2,0) circle (0.2cm);
\end{tikzpicture} &  & 61 \\
\textit{egl-46(rp4)};  
\textit{fosmid rescue line 1} &  & \begin{tikzpicture}[scale=0.5]
\draw[fill=green] (0,0) circle (0.2cm);
\draw[fill=white] (0.5,0) circle (0.2cm);
\draw[fill=white] (1,0) circle (0.2cm);
\draw[fill=white] (1.5,0) circle (0.2cm);
\draw[fill=white] (2,0) circle (0.2cm);
\end{tikzpicture} &  & \begin{tikzpicture}[scale=0.5]
\draw[fill=green] (0,0) circle (0.2cm);
\draw[fill=white] (0.5,0) circle (0.2cm);
\draw[fill=white] (1,0) circle (0.2cm);
\draw[fill=white] (1.5,0) circle (0.2cm);
\draw[fill=white] (2,0) circle (0.2cm);
\end{tikzpicture} &  & 43 \\
\textit{egl-46(rp4)};  
\textit{fosmid rescue line 2} &  & \begin{tikzpicture}[scale=0.5]
\draw[fill=green] (0,0) circle (0.2cm);
\draw[fill=white] (0.5,0) circle (0.2cm);
\draw[fill=white] (1,0) circle (0.2cm);
\draw[fill=white] (1.5,0) circle (0.2cm);
\draw[fill=white] (2,0) circle (0.2cm);
\end{tikzpicture} &  & \begin{tikzpicture}[scale=0.5]
\draw[fill=green] (0,0) circle (0.2cm);
\draw[fill=white] (0.5,0) circle (0.2cm);
\draw[fill=white] (1,0) circle (0.2cm);
\draw[fill=white] (1.5,0) circle (0.2cm);
\draw[fill=white] (2,0) circle (0.2cm);
\end{tikzpicture} &  & 57 \\
\hline
\end{tabular}

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

\textbf{Description}

The BAG neurons control multiple aspects of \textit{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 \textit{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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