Identification of Fibroblast Growth Factor Signaling Components

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Communication between cells is necessary for correct cell development, and therefore correct organism development. One crucial way that cells communicate is through receptor tyrosine kinases (RTKs). RTKs are a class of cell surface receptors that can be used to interpret signals from other cells and transduce those signals into specific cellular responses. FGFRs are a type of RTK that phosphorylate precise tyrosine residues. The phosphorylation of these residues causes an interaction between proteins that eventually activate genes through the RAS/MAPK cascade. In humans, there are four FGFRs which are involved in a multitude of complex processes such as cell proliferation, cell migration, and cell survival (Szebenyi and Fallon, 1999). Mutation in FGFRs can cause ruinous developmental disorders involving improper fusing of bones, including dwarfism, craniosynostosis, and thanatophoric dysplasia (Hibberd C. E., et al., 2016).

In order to closely look at the mechanism and structure of these pathways, we use the model organism, *C. elegans*. *C. elegans,* a roundworm, is particularly well suited for our studies for numerous reasons. For example, unlike mammals that contain numerous FGFRs, *C. elegans* only contain one FGFR, the EGL-15 receptor which makes for a much simpler system. Similar to the human FGFR, EGL-15 is involved in numerous processes. EGL-15 is imperative for sex myoblast migration and fluid homeostasis in *C. elegans* (Szewczyk and Jacobson, 2003; Devore, et al., 1995; Huang and Stern, 2004; Borland, et al., 2001; Branda and Stern, 2000; Branda and Stern, 1999). Defects in these processes result in easily observable, striking phenotypes that can be used to discover components of the EGL-15 signaling pathway. EGL-15 activation is tightly balanced in the organism, and hyperactivation of this receptor results in increased fluid uptake. Normally, a protein named CLR-1 inhibits EGL-15 (Kokel et al., 1998). When CLR-1 is absent, EGL-15 is no longer inhibited, which results in increased EGL-15 activity which results in the accumulation of an excessive amount of clear fluid within the body of the worm. This phenotype is called the "Clr" phenotype. In order to suppress the effects of a defective CLR-1 protein, another component of the EGL-15 signaling pathway must be altered so that EGL-15 activity can be restored to normal. Genes that, when mutated, can suppress the Clr phenotype associated with the hyperactivation of EGL-15 are referred to as *soc (suppression of Clr)* genes. The isolation of suppressor of Clr (*soc*) mutants has led to the identification of many of the core components of EGL-15 signaling. A previous *soc* screen identified the SEM-5 adaptor protein that links EGL-15 activity to other pathways, such as the RAS/MAPK pathway.

Interestingly, a mutation in *egl-15, n1457,* that truncates the EGL-15 protein and eliminates the known known SEM-5 binding sites (amino acids Y1009 and Y1087) on EGL-15 (Lo, et al., 2010) does not confer a Soc phenotype indicating that a key component that links activated EGL-15 to SEM-5 has yet to be identified. To identify these missing components, we conducted a modified, "enhancer" *soc* screen in an *egl-15(n1457)* background. To date, we have identified 10 mutations that result in a Soc phenotype in an *egl-15(n1457)* background that are

not mutations in the *egl-15* gene itself. Using CRISPR/Cas9, we are introducing mutations in the known SEM-5 binding sites (Y1009 and Y1087) to determine if the Soc phenotype in these newly identified genes is solely dependent on these binding sites. We have also begun to use whole-genome sequencing to determine the identity of these 10 *soc* mutations and have potentially identified the identity of one novel *soc* gene as *cca-1*. We are currently performing RNAi experiments to verify that *cca-1* is a *bona fide soc* gene. Preliminary results indicate that *cca-1* may act as a *soc* gene. *cca-1* can partially suppress the Clr phenotype (Figure 1). Additional genetic analyses and whole-genome sequencing is underway to determine the molecular identities of the remaining suppressors.

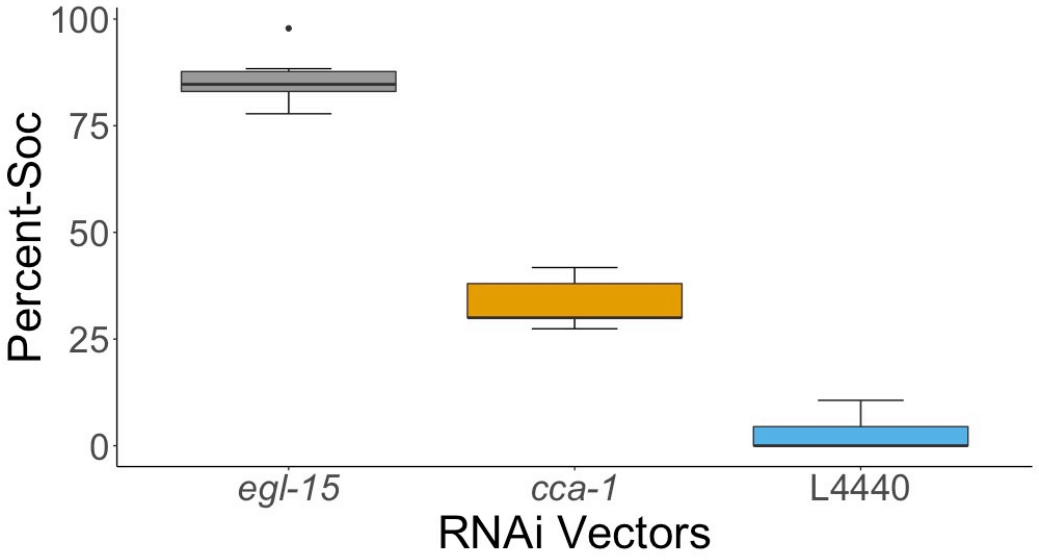

Figure 1. Box and whisker plots of preliminary RNAi verification of *cca-1* as a possible *soc* gene. *egl-15* as a positive control, and L4440 as a negative control. All box plots are significantly different from each other $p < .05$.

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