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Whalen Abstract

Identification of *lin-35* suppressors in *C. elegans*

Proper development of an organism requires the precise regulation of many genetic factors. Mis-regulation of gene expression will often result in diseases, such as cancer. This can be caused by mutations in key classes of genes required for cellular development. One class of genes known to play a role in cancer prevention are tumor suppressor genes. In humans, retinoblastoma (*Rb*) is a tumor suppressor gene that, when inactivated, promotes cancer. *Rb* is highly conserved between humans and other organisms, meaning it has remained essentially unchanged throughout evolution. This makes it possible to use model organisms, such as the nematode *C. elegans*, to further our understanding of *Rb*. *C. elegans* is ideally suited for these studies because they have a short generation time, are cheap to maintain, have well established genetic tools, and a single hermaphrodite can have 300 offspring at a time. The *C. elegans* equivalent to the human *Rb* gene is a gene called *lin-35*. Due to the conserved nature between human *Rb* and *C. elegans lin-35*, we can use the *C. elegans lin-35* gene to further understand the human *Rb* gene and its role in cancer progression. We have been performing RNAi screens to identify potential genes interacting with *lin-35* in the *C. elegans lin-35* pathway.

RNAi is a well-established genetic tool used to silence gene expression. Developed in the early 2000s, it uses an organism's natural ability to use double-stranded RNA molecules to target and degrade specific messenger RNA (mRNA) molecules. When mRNA is broken down, the organism can no longer produce its corresponding protein product. When no protein is made from a gene, the gene is effectively silenced. In *C. elegans*, RNAi can be administered via feeding. In other words, an RNAi effect can be elicited simply by replacing standard bacterial food with RNAi bacteria. Due to the simplicity of feeding, it is possible to screen a large number of candidate genes at a time.

In our study, which is based on a previous study by Polley and Fay (2012), we screened 371 genes via RNAi for their ability to suppress *lin-35* associated larval lethality. Suppression of *lin-35* lethality is defined by the presence of a viable *lin-35* mutant, which was identified by using a green fluorescent marker. Our list of potential suppressors were identified in the following manner: The initial screen was conducted by students in the Spring 2015 Genetics (BIOL 22700) course. They identified 155 out of 371 genes as potential suppressors. In the Lo lab, through additional experiments, we first narrowed the list of potential suppressors to 45, and then using more stringent conditions, have identified the five strongest *lin-35* suppressors. For each experiment, we included known suppressors of *lin-35* as positive controls to ensure all reagents and techniques functioned properly. RNAi bacteria that did not target a gene was included in each experiment as a negative control.

On-going experiments include the further characterization of the five strongest suppressors. A better understanding of how these genes are working to inhibit *lin-35* function in *C. elegans* will potentially further our understanding of the Rb gene in humans and its role in cancer development.

References:

Polley, SRG, and Fay DS. 2012. A network of genes antagonistic to the LIN-35 retinoblastoma protein of *Caenorhabditis elegans*. Genetics 191:1367-1380.