Mechanisms of Gene Silencing: Genetic Analysis by a First-Year Scientific Research Learning Community

Rhese Thompson, Tika Zbornik, and Dr. Catherine Reinke

Department of Biology, Linfield College: McMinnville, Oregon

Abstract

Our broad goal is to use *Drosophila melanogaster* to identify new genes required for a form of gene regulation known as gene silencing. As participants in iFOCUS, an interdisciplinary freshman orientation camp for undergraduate sciences that introduces students to scientific research, we learned how classical genetics is used to identify novel genes essential for a particular biological process such as gene silencing. Specifically, we learned how to determine which chromosomes are present in a fruit fly by observing that fly's physical characteristics, or phenotypes. We further learned how mutations in genes are mapped to a particular location on a chromosome using *Drosophila*. Previous work in our lab indicated that the Regena/NOT2 gene is essential for microRNA-mediated gene silencing. We set out to locate the nucleotide change that is responsible for the Regena/NOT2 mutant phenotype. To do this, we started a genomics learning community and taught 8 additional first-year Linfield students how to analyze DNA sequence data. Through our collective efforts, we determined that the existing DNA sequence data only covered a portion of the Regena gene, and additional DNA needed to be sequenced. We performed fly crosses, selected relevant progeny, and prepared and genomic DNA from these flies to amplify the Regena gene by polymerase chain reaction (PCR). Amplified DNA has been sent for DNA sequencing, and sequence analysis is ongoing.

What is the nucleotide change in the Regena/NOT2 gene that results in the mutant phenotype?

Current Models of miRNA-mediated Gene Silencing

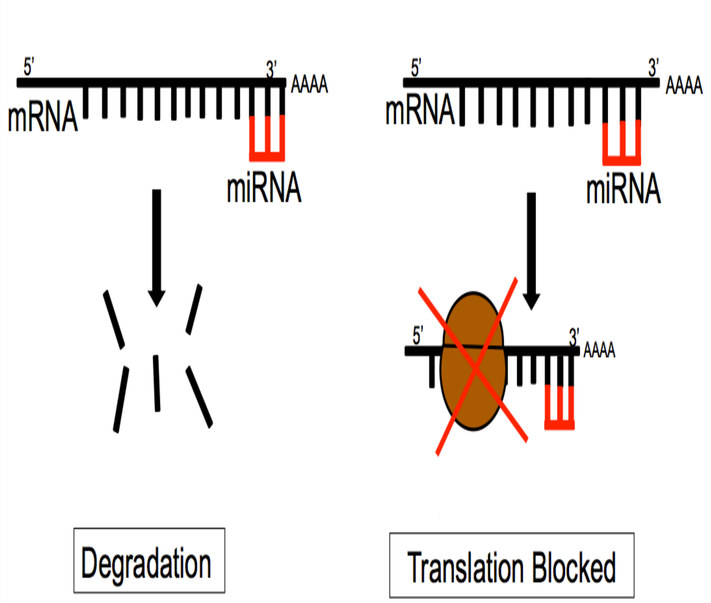

Figure 1: Mechanisms of microRNA-mediated gene silencing
Messenger RNAs (mRNAs) are generally translated into proteins, but in some cases, microRNA (miRNA) mediated gene silencing results in either A: mRNA degradation, the breakdown of the mRNA into individual nucleotides, or B: translation inhibition, where the miRNA prevents the translation of the mRNA and generation of protein by the ribosome.

Wild type and Mutant Gene Silencing Phenotypes

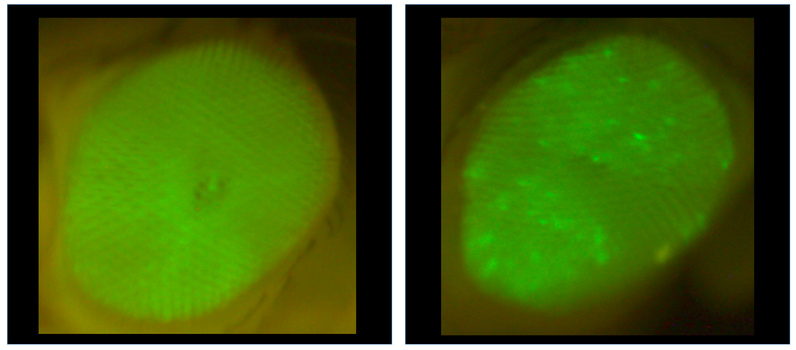

Figure 2: Visualization of decreased gene silencing; comparison with a wild type eye. A transgene encoding green fluorescent protein (GFP) and sensitive to miRNA-mediated gene silencing is expressed in the eye. A: The silenced (wild type) phenotype, demonstrating low-level GFP expression. B: The disruption of silencing (mutant) phenotype, demonstrating higher GFP expression in clones of cells.

Generation of a Fly Line for Genomic DNA Extraction

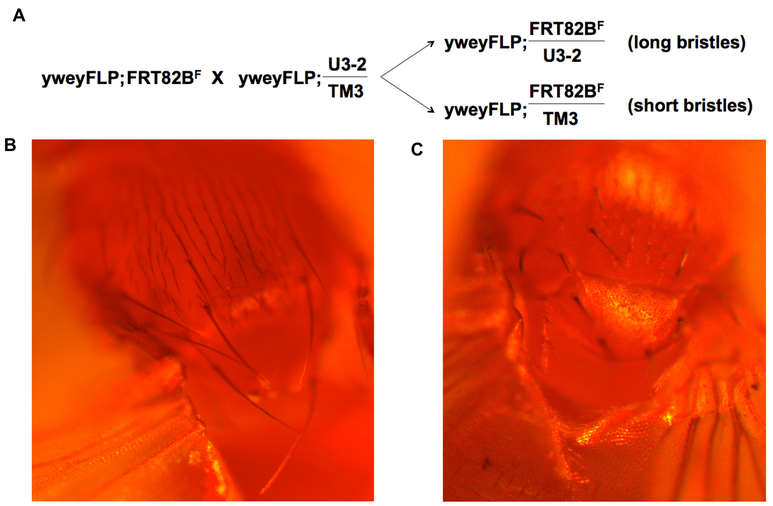

Figure 2: Generation of flies for the extraction of genomic DNA . A: Flies containing the mutation in Regena were crossed to flies containing the original mutagenized chromosome (prior to mutation) to generate heterozygous flies for genomic DNA extraction and additional DNA sequencing. Progeny of interest were selected on the basis of particular *Drosophila* phenotypes. B: Wild type flies have long bristles. C: Flies with TM3 have short bristles.

Amplification of the Regena Gene

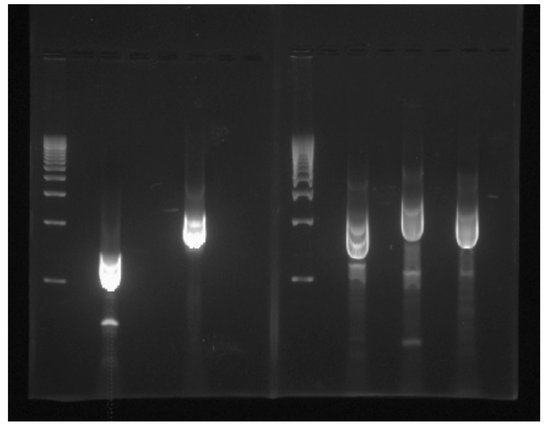

Figure 4: Amplification of the Regena gene for subsequent sequence analysis. Five sets of primers were used to amplify a total of four kilobases (kb) of DNA, including the coding region of the Regena gene, by polymerase chain reaction (PCR). PCR products were run on an agarose gel to verify the sizes of the amplified gene fragments.

Summary of DNA Sequence Data

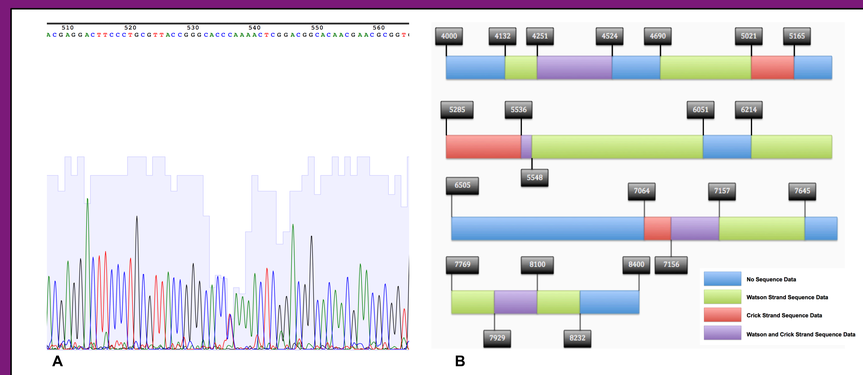

Figure 5: Regena DNA sequence analyzed to identify the induced mutation and a summary of the current analyzed sequence data. A: DNA sequence data that was analyzed by members of the genomics learning community. Analysis involved deciding which portions of the sequencing reads were reliable and which portions of the gene require resequencing. B: Nine forward and eight reverse primers were used to sequence the coding region of the Regena gene. We aimed to analyze sequence data from the Watson and Crick strands. Blue= no sequence data, Green= Watson strand sequence data, Red= Crick strand sequence data, and Purple=sequence data from both strands.

Future Experiments

Future experiments will consist of analyzing newly-generated DNA sequence data in order to obtain coverage of both the Watson and Crick strands of Regena. As a control, wild type DNA will also be sequenced and analyzed. These experiments will involve amplification of the DNA by PCR, sending the amplified DNA for sequencing, and analysis of the sequenced DNA.

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