Identifying a new gene required for microRNA-mediated gene silencing in *Drosophila melanogaster*

Michael Morin, Sean Bowden, and Catherine Reinke
Department of Biology, Linfield College, McMinnville, Oregon

Linfield College

Linfield College

BACKGROUND

Mature microRNAs (miRNA) are ~22 nucleotide long single-stranded ribonucleic acids essential for gene silencing. Silencing occurs when miRNAs are processed via endonucleolytic cleavage and subsequently associate with the miRNA-induced silencing complex (miRISC). miRISC binds via complementary base pairing to target mRNAs, and target mRNAs are silenced by either mRNA degradation, translational block, or both. Knowledge of all genes required for silencing is incomplete.

We aim to determine the molecular mechanism of silencing by identifying and characterizing genes required for silencing. A forward genetic screen was performed using EMS mutagenesis of *Drosophila melanogaster* to generate mutant lines with disrupted gene silencing as visualized by a GFP-based fluorescent reporter of silencing. Locations of EMS-induced mutations are being mapped by determination of recombination frequencies between these mutations and molecularly defined P-element insertions. Preliminary recombination mapping reveals that our mutation of interest (I1-5) is found within a discrete region of the genome that lies on chromosome 3R. A new fly line has also been generated to assist with this preliminary recombination mapping. Future deficiency mapping and complementation tests combining the mutation and alleles of candidate genes will reveal the location of our mutation, and lead us to identify a gene required for microRNA mediated gene silencing.

microRNA biogenesis and RISC function

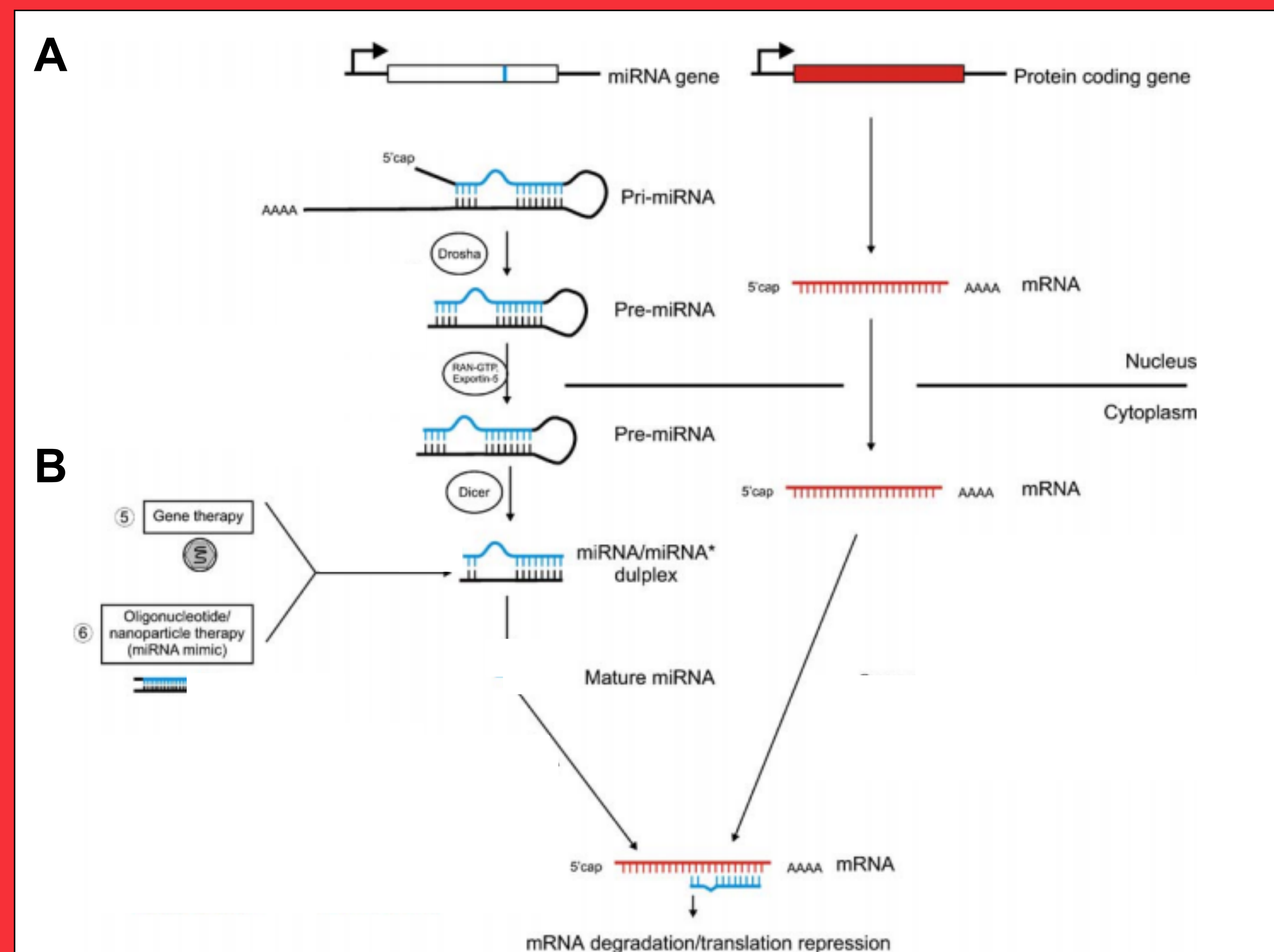

Figure 1: Biogenesis of microRNA and mRNA.
(A) MiRNA genes are transcribed and processed to yield short double-stranded RNAs. After further processing, a single miRNA strand is incorporated into the miRNA-induced Silencing Complex (miRISC), which binds to complementary target mRNAs and silences gene expression by means of mRNA degradation, translation block, or both.
(B) Several miRNA-based therapeutics designed to regulate target gene expression are currently in clinical trials.

Modified from Li, Feng, Coukos, and Zhang. 2009. Therapeutic MicroRNA Strategies in Human Cancer.

EXPERIMENTAL APPROACH AND RESULTS

A forward genetics screen to identify new genes required for miRNA-mediated gene silencing

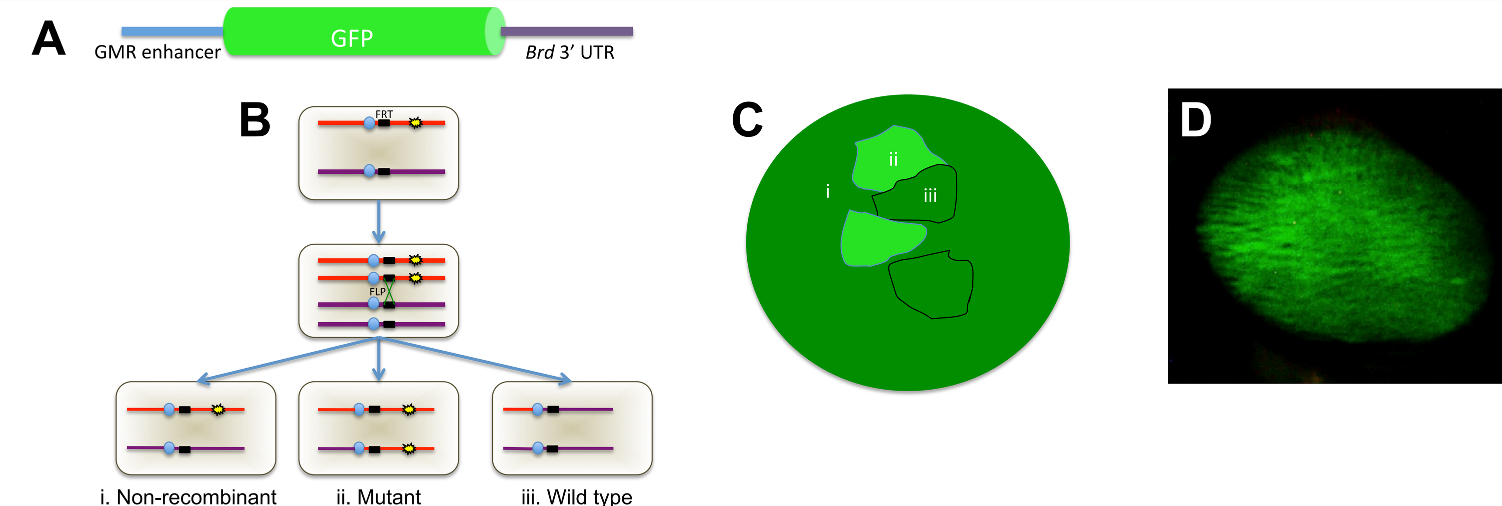

Figure 2: Isolation of mutants and detection of miRNA activity. The eye-specific GFP reporter is regulated by miRNA binding to the target 3' untranslated region (3'UTR) (A). EMS mutagenesis was performed and mitotic recombination was used to generate clones of cells homozygous for induced mutations (B). Predicted GFP expression levels in the adult eye if a gene required for silencing is disrupted; corresponding chromosomes shown in B (C).The I1-5 mutant demonstrates a defect in silencing (D).

Recombination mapping with molecularly defined P-element insertions

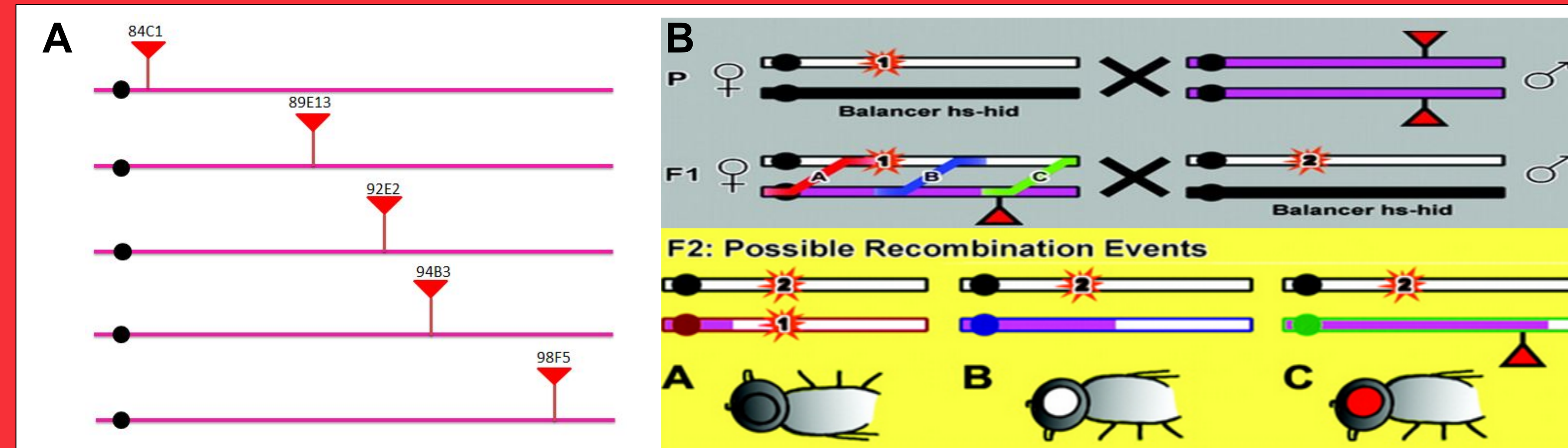

Figure 3: Molecularly defined P-element insertions and observations of recombination. Locations of P-element insertions used for recombination mapping (A). Parental cross between a fly line with a specific P-element insertion and flies carrying the mutation of interest (P), F1 cross between flies heterozygous for both the mutation and a specific P-element and flies carrying the mutation of interest (F1), and flies observed for evidence of a recombination event (F2). (Zhai '04)

Frequency of recombination between our mutation and a cytologically-defined P-element insertion

Table1: Phenotypes of flies assayed for recombination between the mutation and each P-element insertion. Numbers of flies of each phenotype observed were used to determine the predicted distance between the mutation and each P-element insertion.

P-Element Insertions	Red Eyes & Short Bristles	Red Eyes & Long Bristles	White Eyes & Short Bristles	White Eyes & Long Bristles	Total Flies Observed	Recombination Distances (cM)
84C1	1033	963	872	32	2900	0.01103448276
89E13	439	389	383	4	1215	0.00329218107
92E2	1082	1064	1001	10	3157	0.003167564143
94B3	837	203	708	7	2255	0.00310421286
98F5	368	253	376	101	1098	0.09198542805

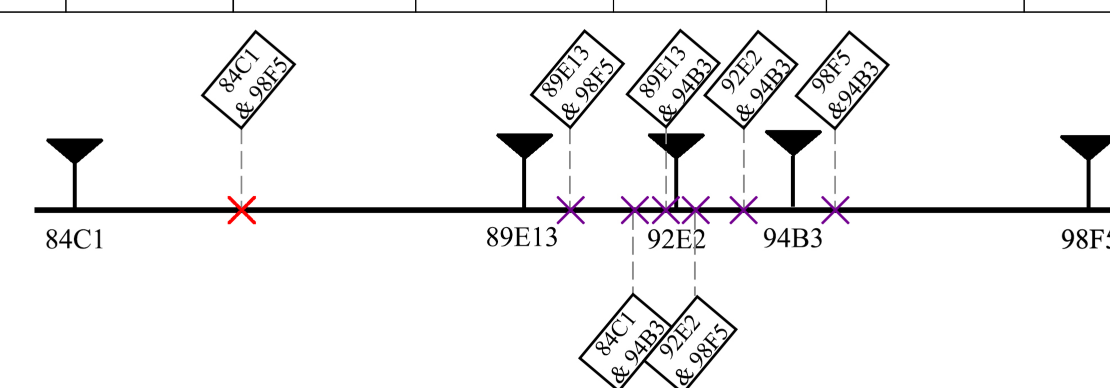

Figure 4: Projected position of the I1-5 mutation. The Xs indicate possible locations of the mutation based on recombination frequencies from Table 1.

Figure 4 by: Alleta Maier

A newly-engineered fly to simplify recombination mapping

Figure 5: A new fly line for streamlined data collection for recombination mapping. Further recombination mapping will be aided by generation of a fly line that allows for removal of uninformative progeny though the use of a heat-shock hid transgene. Brief heat shock kills all uninformative flies that contain a balancer chromosome unrelated to the mapping protocol.

Heat shock in practice with newly-engineered fly

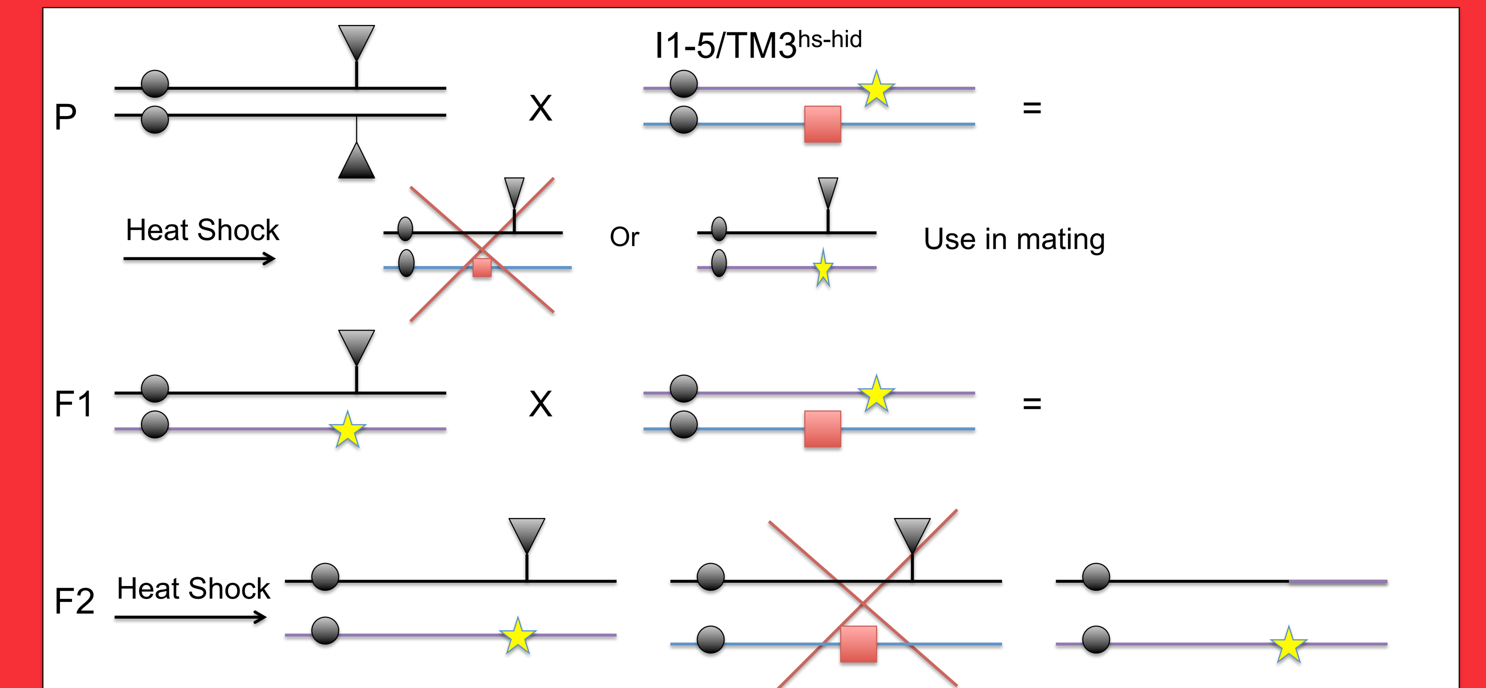

Figure 6: Heat shock protocol and resulting progeny. Four days after a mating has been set, larva are heat shocked at 37°C for one hour to kill unwanted flies containing the heat shock-hid transgene. The overall number of flies to be sorted is reduced, simplifying scoring of informative recombinant flies in the F2 generation.

FUTURE DIRECTIONS

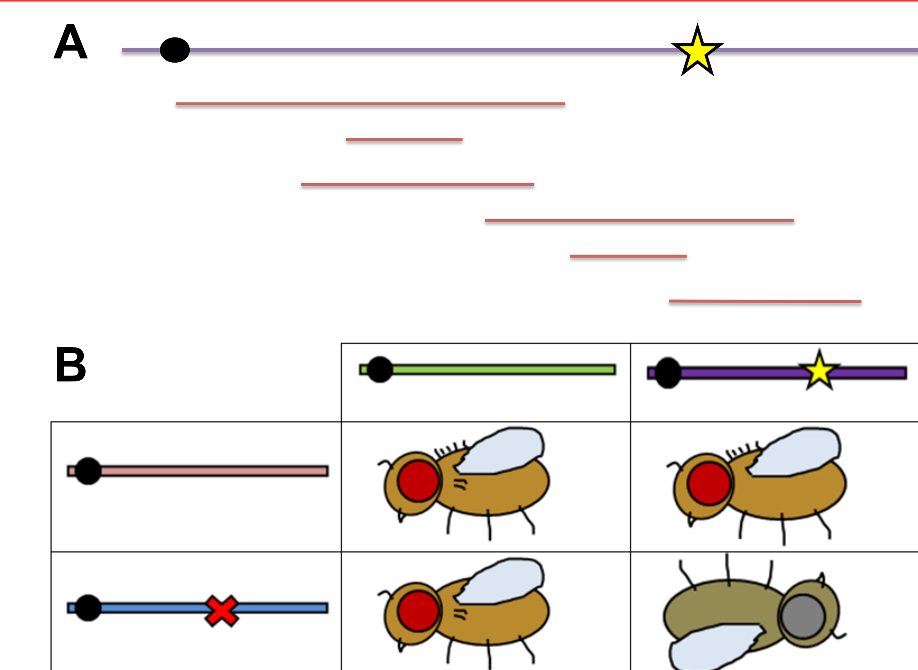

Figure 7: Deficiency Mapping and Complementation Tests. Flies carrying the mutation of interest will be crossed with flies carrying a chromosome with a small deletion or deficiency. Viable offspring are produced if the mutation lies outside of the deficiency region (A). Hypomorphic alleles of candidate genes will be tested for their ability to complement the mutation of interest. Failure to complement will reveal the gene disrupted in our mutant line. Thanks to collaborators K. Bates and D. Cannon.