ABSTRACT

microRNAs (miRNAs) are one class of small non-coding ribonucleic acid (RNA) molecule essential to development and homeostasis in plants and animals. miRNAs silence gene expression through complementary base pairing with target gene messenger RNAs and association with the miRNA-induced Silencing Complex (miRISC). The identification and characterization of cellular factors required for miRNA-mediated gene silencing is incomplete. A forward genetic screen was carried out in Drosophila melanogaster to generate flies defective for gene silencing. Silencing was assayed by expression of a Green Fluorescent Protein (GFP) reporter fused to the exon 3' untranslated region (3'UTR) regulated by miRNAs. Genetic analysis revealed that the CCR4-NOT deadenylase-complex subunit Regena is required for miRNA-mediated silencing of the reporter. In addition, perturbation of the Regena gene altered Drosophila eye development and resulting adult eye morphology.

miRNAs are thought to silence target gene expression through a combination of translational repression and target mRNA degradation, though the detailed mechanism of this process is a matter of controversy. Novel genetic reagents to explore miRNA function in vivo have been generated and characterized. Ongoing efforts aim to explore whether Regena is required to silence other miRNA targets in vivo, and whether Regena is required for miRNA-mediated silencing of the reporter. In addition, perturbation of the Regena gene altered Drosophila eye development and resulting adult eye morphology.

FUTURE PLANS

The role of Regena in cell viability and normal eye development

We assayed the role of Regena in eye development by generating whole eyes homozygous for the mutation in Regena, using mitotic recombination and a nonfunctional allele of the cell lethal gene hid. The results of this perturbation are seen as malformation of some ommatidia, or eye facets (arrow). Loss of Regena function has a mild effect on Drosophila eye morphology.

Exploration of a putative role for Regena in gene silencing during early development

We analyzed one mutant from the screen (U3-2) and performed deficiency mapping and complementation testing in order to determine the identity of the gene that is essential for silencing the GFP reporter (Figure 3). Deficiency mapping narrowed the genomic region of interest to a ~16.4kb region containing 5 genes. We tested the ability of characterized alleles of these genes to complement the genetic lesion in our mutant. Noncomplementation was observed when our mutant flies were crossed with flies containing a nonfunctional allele of the Regena gene. This suggests that Regena is required for gene silencing by miRNAs.

We have identified Regena(NOT2) as an essential gene required for miRNA activity in vivo. Regena has been previously characterized as a subunit of the CCR4-NOT deadenylase complex, a highly-conserved, multi-subunit complex implicated in gene silencing via target mRNA transcript degradation and translational repression. Controversy exists as to whether translational repression necessarily precedes transcript degradation. Further analysis of our mutant is likely to inform this debate.

The microRNA pathway

Figure 1: Gene silencing requires the generation of a functional miRNA-induced Silencing Complex (miRISC). miRNA genes are transcribed from genomic DNA and processed to yield short ~22 nucleotide double-stranded RNAs. A single miRNA strand is incorporated into the miRNA-induced Silencing Complex (miRISC), which binds to complementary target miRNAs and silences gene expression by means of miRNA degradation or translation block.

EXPERIMENTAL APPROACH

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

A forward genetics screen was conducted to isolate mutants with altered gene silencing. A GFP reporter was used to assay miRNA activity. The GFP reporter is sensitive to miRNA activity because it is fused to a 3' untranslated region (3'UTR) regulated by miRNAs in the Drosophila eye. An increase in GFP fluorescence indicates a loss of gene silencing (Figure 2C). An alternate lacZ reporter is also being used to evaluate gene silencing (Figure 2D). An increase in lacZ expression indicates a loss of gene silencing. Given that a mutation disrupting miRNA function is likely to be lethal, mitotic recombination was used to generate clones of cells in the eye that are homozygous for the mutation, and can be used to assay GFP fluorescence in adult eyes, or lacZ expression in larval eye discs.

Discovery of the Regena gene

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The CCR4-NOT deadenylase complex is essential for gene silencing

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