Problem set 8 answers

1. Feeding *C. elegans E. coli* that express double stranded RNA from the *unc-54* gene, which encodes the major form of muscle specific heavy chain myosin, causes the animals to be paralyzed because they lack this myosin. You mutagenize wild-type worms with EMS and screen the F2 progeny for worms that are no longer paralyzed when fed these bacteria. The mutations that cause this resistance are recessive and are also resistant to RNAi for other genes. From what you know about RNAi, what could be the normal functions of the genes that are mutated?

Since they are involved in implementing RNAi, they could be components involved in dicer or RISC complex function.

2. RNAi in *C. elegans* can effectively silence gene function though a posttranscriptional process. RNAi can be induced globally in worms simply by feeding the animals bacteria that express double-stranded RNA representing the gene being silenced. While how RNAi induces mRNA degradation is understood, it is less clear how the effects of RNAi spread from cell to cell; for example, from the gut to the remaining cells of the body. To identify genes involved in the spreading process, investigators screened for mutants defective in RNAi spreading. They mutagenized animals that expressed three transgenes. One transgene expresses GFP in the pharynx, the feeding organ, and the second expressed GFP in the body wall muscles, which are used for locomotion. The third transgene expressed double-stranded RNA to GFP in the pharynx. In the absence of this third transgene, GFP is expressed in both the pharynx and the body wall muscles; in its presence, all GFP expression is lost. The investigators then screened for recessive mutations in genes required for RNAi.

Describe how the screen was done. Include the generation that was screened, whether the animals were crossed or selfed, and the phenotype the investigators were looking for. Describe the difference between the phenotypes of mutants generally defective in RNAi, (for example, in the genes encoding the Risc components) and of mutants defective in spreading of the RNAi effect.

The screen was conducted by mutagenizing the strain that carries the three transgenes and by looking in the F2 (for zygotic genes) or the F3 (for maternal effect genes) for animals that express GFP. If GFP is expressed in the pharynx but not in the muscles the mutants would be defective in spreading. Mutants generally defective in RNAi would express GFP in both tissues.

3. *C. elegans* neurons are particularly insensitive to the effects of RNA interference. For example, if you have a strain that expresses Green Fluorescence Protein (GFP) in neurons, feeding bacteria that express double stranded GFP RNA doesn't have any effect on the levels of GFP. By contrast, feeding these bacteria to animals that normally express GFP in muscles eliminates this GFP fluorescence. You find this irritating because you want to carry out an RNAi screen for genes involved in the formation of chemical synapses between neurons. You decide that one approach to this problem would be to

screen for mutants with neurons that are sensitive to RNAi.

a. Using EMS and the transgenic strain that expresses GFP in neurons, how would you screen for these mutants?

You would screen for mutants that no longer express GFP in the neurons when fed bacteria that express GFP dsRNA.

b. You isolate a mutant that has the expected phenotype, and it defines a single gene on chromosome II. You predict that this mutant now allows the canonical RNAi pathway to be effective in neurons. Describe a simple genetic test of this prediction.

Make the double mutant that contains the new mutation on II and a mutation that disrupts RNAi. When fed bacteria that express GFP dsRNA, neuronal GFP expression should still be present.

4. You are interested in identifying genes involved in the production of wing veins. In general terms, describe three approaches that you can use that are necessary wing veins. What tools would you need for each screen.

A direct screen for mutants with wing vein defects. A mutagen to induce the mutations.

A mosaic screen for mutants for wing vein defects. A mutagen, a transgene where a wing enhancer drives FLP expression, FRT sites on the mutagenized chromosome and its homolog, a cell authonomous marker (e.g., a transgene that expresses GFP in wing cells) that is on the homolog of the mutagenized chromosome.

An RNAi screen for defects in wing veins. A strain carrying a wing enhancer driving Gal4. Stains that have UAS sequences upstream of inverted repeats of the genes that will be screened.