RNAi in *C. elegans*

A cure for the circuitous worm.

The *rol-6* gene encodes a collagen protein that is similar to human collagen α1. In *C. elegans*, *rol-6* is involved in cuticle formation. The *rol-6* gene is not essential; worms that are homozygous for loss of function alleles of *rol-6* have no obvious phenotype. However, dominant mutations in *rol-6* cause the worms to roll in a circle rather than travel in the normal sinusoidal pattern.

For this experiment, you will be given worms with a dominant mutation in the *rol-6* gene. The proper name for this mutant is *rol-6(su1006)* where "su1006" represents that exact mutant allele that causes this phenotype. You will also be given some wild type worms so that you can observe the normal movement pattern.

You will then use RNAi (also known as RNA interference) to knockdown expression of the mutant *rol-6* allele. What do you expect will happen to the worms after the RNAi treatment?

What is RNAi?

RNA interference is the phenomenon of turning off expression of a specific gene by introducing double stranded RNA (dsRNA) molecules that match the nucleotide sequence of that gene. Gene expression is silenced by degradation of the mRNA. The dsRNA enters the cytoplasm and there it is incorporated into a multi-protein complex, the RISC complex. At this point, mRNA from the cognate gene will be taken into the RISC complex containing the dsRNA and then the mRNA will be cleaved by an RNase enzyme that is part of the complex.

RNAi was a serendipitous discovery by scientists working to solve a totally different question. The result was unanticipated and it took the work of many scientists to eventually figure out how RNAi works. Two of those scientists, Craig Mello and Andy Fire, received the Nobel Prize in 2006 for their contributions to this effort.

It is very easy to perform RNAi in *C. elegans*. dsRNA is delivered to the worm cells via the intestine. Worms eat bacteria that express dsRNA for a specific gene. The dsRNA then can move out of the intestine into other tissues.

RNAi is a fabulous tool for geneticists. With RNAi, one can investigate the function of any gene for which the nucleotide sequence is known. In addition, RNAi has promising therapeutic potential. What diseases do you think might be able to be treated with RNAi?

For more information about the genetic and molecular details of *rol-6* visit:
http://www.wormbase.org/db/gene/gene?name=WBGene00004397;class=Gene
For more information about RNAi visit:
http://www.pbs.org/wgbh/nova/sciencenow/3210/02.html
http://www.rnaiweb.com/

PROCEDURES

LAB 1: Begin RNAi treatment

You will need the following supplies for the RNAi treatment.

-solution of 0.1M IPTG (isothiopropylgalactopyranoside)
-solution of 100 mg/ml ampicillin
-liquid culture of L4440:*rol-6* (*rol-6* RNAi)
-liquid culture of L4440 (negative control)
-three 60 mm petri dishes with nematode agar
- *rol-6(su1006)* worms and wild type worms (young larvae)

Prepare worm plates for RNAi:

-place 120 µl of 0.1 M IPTG onto the agar surface of each of the petri dishes
-place 12 µl of 100 mg/ml ampicillin onto the agar surface of each of the petri dishes

Wait until the IPTG and ampicillin soak into the plates before proceeding

-place 200 µl of L4440:rol-6 onto the agar surface of two of the petri dishes.
-place 200 µl of L4440 onto the agar surface of the other petri dish.
-label plates appropriately and place plates in the safety hood for one hour

Transfer one tube of thawed *rol-6* larvae onto a plate seeded with each bacterial strain (*rol-6* RNAi and control). Transfer one tube of wild type worms onto a plate seeded with the *rol-6* RNAi bacteria.

Incubate at 20º C for 5 days.

LAB 2: Observations

Remove worm plates from 20º incubator.

Compare the movement of worms on the three different petri dishes.

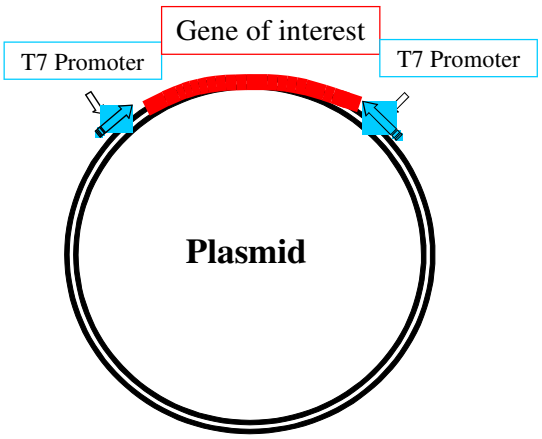
Fill out the following table:

Worm genotype	RNAi treatment	# worms with normal movement	# worms with rolling movement	# worms not moving

Possible Discussion Points for Lab instructor:

What if *rol-6* was an essential gene? How would that change the results of this experiment?

How can we get bacteria to make dsRNA?



How might RNAi be used as a therapy? What might be the technical hurdles for this type of therapy?

Explain why IPTG is added to the plates.

Lab Prep
Prep for Worm RNAi lab

Months or years prior to lab:

Grow worms on large seeded plates (as many as you can handle). Let them starve and become mostly L1s. Freeze using standard freezing buffer. (wash worms off plates with sterile water, spin worms and reduce down to about 0.5 ml per plate, add equal volume of freezing solution: see www.wormbook.org Maintenance of C. elegans for recipe). Aliquot 150 ul of worms + solution into 600 ul tubes. Put tubes in freezer boxes, then put those boxes into a styrofoam box and place in -80 freezer. Remove from styrofoam box and place in freezer racks 2 days later.

Several weeks prior to lab:

Check frozen worm supplies (-80 C freezer). Need 2 tubes of rol-6 and one tube of wild type worms for each group.

Order worm plates from Lab Scientific (#NEM0339). Need 3 plates per lab group.

One day prior to lab:

-liquid culture of pL4440:rol-6 bacteria in 50 ml TSB + Amp (in a 250 ml flask)
-liquid culture of pL4440 bacteria in 50 ml TSB + Amp (in a 250 ml flask)
-shake at 30ºC O/N
-take out worm plates, open package and place plates at room temperature. This will allow the plates to be sufficiently dry for the experiment.

Supplies Lab day #1:

Liquid cultures
5 ml of 0.1 M IPTG
1 ml of 100 mg/ml Ampicillin
P1000, P200, P20 micropipettors

Sterile tips (blue and yellow)

Each Group
-dissecting scopes
-3 unseeded plates (60 mm)
-paintbrushes with green fishing line(=worm picks)

Lab day #2:

dissecting scopes
Autoclave waste repository