BIO 251

Lab Handout Week 11

Inhibition of *unc-22* function using double stranded RNA mediated interference (dsRNAi) by feeding

By Kirsten Crossgrove at University of Wisconsin-Whitewater

Purpose: This lab will introduce you to one of the techniques used to perform 'reverse genetics.'

Background: As you learned in lecture, double stranded RNA mediated interference (dsRNAi) is a technique that allows the specific inactivation of a gene by exposing on organism to double stranded RNA with the same sequence as the gene. This allows researchers to see the phenotype of a loss of function of that gene, which can provide insight into the normal function of the gene product. We discussed the mechanism of dsRNAi in lecture (for a review, see the animation at http://www.nature.com/nrg/journal/v2/n2/animation/nrg0201_110a_swf_MEDIA1.html).

- How does the RISC complex know which mRNA to degrade?

Today you will be using dsRNAi to disrupt the expression of the *unc-22* gene. First, let's find out some information about *unc-22*. As you learned in lecture, the *C. elegans* research community uses a database called Wormbase to store information on many aspects of *C. elegans* biology. You will use Wormbase to find out some information about *unc-22*. Then, you will use the BLAST tool at the National Center for Biotechnology Information to see what human protein is most closely related to the protein coded by the *unc-22* gene. Finally, you will search the Online Mendelian Inheritance in Man (OMIM) database to find out the function of the human protein.

1. Go to Wormbase (http://www.wormbase.org) and enter 'unc-22' in the Find (any gene) box and click on 'Search'.
2. This should bring up a Gene Report for *unc-22*.
3. You will need to answer the following questions on the lab quiz using information from the Gene Report.
    a. What is the name of the protein encoded by *unc-22*?
    b. What is the role of this protein in muscle contraction?
    c. What is the length (number of amino acids) of the longest protein predicted to be encoded by *unc-22*?
    d. What is the phenotype of *unc-22* mutants?
4. In the 'Gene Models' table, click on '6839aa' in the last cell of the row starting with 'ZK617.1a.1'. You should get the amino acid sequence of one isoform of the protein coded by *unc-22*.
5. Select the entire sequence and copy it.
6. Open the BLAST search engine (http://www.ncbi.nlm.nih.gov/BLAST).
7. Select 'Protein-protein BLAST (blastp)' from the top of the 'Protein' list of links.

8. Paste the sequence into the 'Search' window. Do not change any other settings. Click on the 'Blast!' button.
9. You will get a new screen that includes a 'Format!' button. Click on this. Your results may take a few minutes to load.
10. When your results come up, scroll down to the list of proteins that show sequence similarity to your protein. The first things you should see are the different isoforms of *unc-22*. The number shown to the right is the probability that the sequences shared by these proteins are due to random chance. Scroll down the list until you see the first human protein. You can click on the number to the left to get a full description for any entry. Remember the name of the protein, you'll need it for the quiz!
11. When you have found the name of the human protein. Go to the Online Mendelian Inheritance in Man (OMIM) site (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM). Enter the name of the protein in the search box and click on 'Go.' You will most likely get multiple entries, Select the first one that actually has the name of the protein in its title.
12. Make a note of the function of this protein in humans.

Once you have completed this exercise, read the rest of this handout and then take the quiz on D2L. When you have finished taking the quiz, go to 'Surveys' in D2L and take the survey 'Worm survey 1.'

Experiment Overview

Now that you have learned about the *unc-22* gene, you are ready to do some reverse genetics.

- Based on your understanding of dsRNAi and what you have learned about *unc-22*, formulate an hypothesis to explain what will happen if you inactivate *unc-22* using dsRNAi.

You will be using the feeding method of dsRNAi. Specifically, you will be provided with agar plates seeded with a culture of bacteria cells that contain a plasmid with a portion of the *unc-22* gene cloned in between two T7 RNA polymerase promoters. The bacteria cells contain the T7 RNA polymerase gene under the control of lac operon regulatory elements. This allows expression of T7 polymerase to be controlled by IPTG (recall that IPTG is used as a stable replacement for lactose to remove repressor from the operator). When IPTG is added to a culture containing these cells, the cells will begin to synthesize RNA from both T7 RNA polymerase sites. This will produce double stranded RNA. Using a protocol developed by Kamath and colleagues (2000), cultures were grown for eight hours on the day prior to lab. One hundred microliters of culture was then added to NGM agar plates containing 100 ug/ul carbenicillin (a more stable form of ampicillin) and 1mM IPTG.

- What is the purpose of having a stable form of ampicillin in the plates?

These plates were kept at room temperature overnight. During this time, double stranded RNA began to be synthesized. Today, the bacteria cells should be full of double stranded RNA. You will transfer 2-4 L4 larvae from provided N2 (wild type) plates to each seeded plate.

- Why should you use L4 larvae?

Remind yourself of the methods for transferring worms and recognizing L4 larvae. Try to transfer as little bacteria as possible from the original plate to the RNAi plate. When you have

successfully transferred 2-4 L4 worms (you must show me your plate), it will be kept in a 16°C incubator.

Preparation of plates for dsRNAi feeding:
You will be working in groups four. Each group will receive 4 NGM agar plates with 100 ug/ml carbenicillin, 1mM IPTG and 100 µl of bacteria cells containing either the dsRNAi feeding vector (L4440) or the feeding vector with the unc-22 gene in it (L4440/unc-22).

- What is the purpose of using cells with only the vector in them?

Follow the protocol below to prepare your plates.

dsRNAi protocol:

1.) Check your plates to make sure you have two that say 'L4440' and two that say 'L4440/unc-22'.	**If you do not, notify the instructor.**
2.) Label all four of your plates with the following information: 1. your group number 2. AM or PM	**Label on the bottom of the plate and try to write near the edge of the plate so you don't obscure view of the worms.**
3.) Using the provided N2 plate, transfer 2-4 L4 larvae to each plate.	**Each person in the group should start one plate.**
4.) Have the instructor look at your plate to make sure you have transferred L4's.	
5.) Store your plate at 16°C.	**Bring your plate to the front of the room and the instructor will take it to the incubator.**

Predictions:

Next week you will look at the phenotypes of the worms. You will be given plates with N2 (wild type) worms and *unc-22* mutant worms to use as a reference when scoring phenotypes.

- What do you predict you will see in the plates with cells containing L4440 plasmid?
- What do you predict you will see in the plates with cells containing L4440/unc-22 plasmid?

Conclusions:

- How efficient is the dsRNAi technique in mimicking the *unc-22* mutant phenotype?

Sources:
Kamath, R.S., Martinez-Campos, M., Zipperlen, P., Fraser, A.G., and Ahringer, J.A. 2000. Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in *Caenorhabditis elegans*. Genome Biology, 2: 1-10.