Inducing RNAi of dpy11 and bli-1 genes in C. *elegans*

(in progress)

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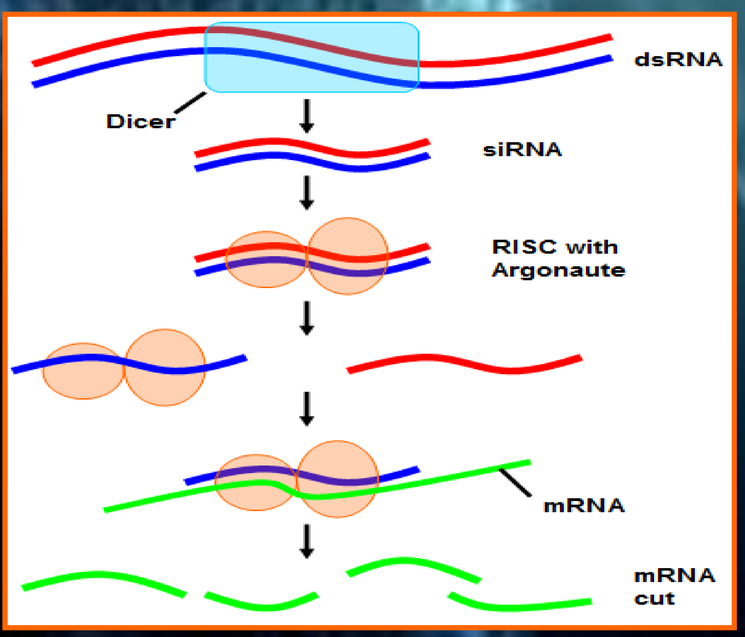
Background

C. elegans (*Caenorhabditis elegans*) is a small roundworm that lives in temperate soil. Due to its small size, short life span, transparent body, and effective reproduction rate, it is used as an model for studying RNA interference(RNAi). RNAi is a cellular mechanism used to protect cells from RNA viruses, it acts by destroys dsRNA which results in silencing gene function. RNAi can also be used as an important tool to study gene functions.

Mechanism of RNA interference (RNAi)

In C. *elegans*, dsRNA is recognized by a protein called Dicer which cleaves dsRNA into small interfering RNAs (siRNA). The siRNA binds to RISC (RNA – inducing silencing complex) with an endonuclease called Argonaute (Ago). After one strand of the siRNA is degraded, the other strand is used to bind to the complementary mRNA. The mRNA is then cleaved by Argonaute and thereby the gene function is blocked.



Abstract

RNA interference (RNAi) is a biological process in which RNA molecules block gene expression, typically by causing the destruction of specific mRNA molecules. C. elegans is an excellent model system to study because many single gene mutations have been identified and the worms' transparency assist morphological observations. We are studying RNAi techniques by feeding E coli to interrupt the dqy-11 and bli-1 genes in C. *elegans*, resulting in morphological changes. RNAi constructs were first transformed in E. *coli* and C. *elegans* are fed E. *coli* bacteria containing relevant dsRNA for genes to be silenced. Phenotypic changes in offspring will be observed and documented to verify RNAi had been achieved. Further studies will examine the feasibility of fluorescent dye to enhance offspring traits. These studies have implications for novel medical treatments and gene therapy.

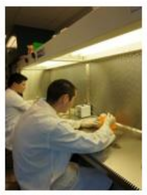
Inducing RNAi by Feeding

Protocol

1. Wild-type C.*elegans*, grown on NGM-*lite* media (0.6g/300ml NaCl, 1.2g/300ml Tryptone, 0.9g/300ml KH2PO4, 0.15g/300ml K2HPO4, and 6g/300ml Agar) were assessed for growth rate and life stage.
2. E. *coli* feeding strain with bli-1 and dpy-11 are grown under 37° C overnight in incubator.
3. The E. coli cultures were seeded onto NGM-*lite* plates to incubate for one day at room temperature.
4. Wild-type C.*elegans* at life stage L1 and L2 are transferred via sterile toothpicks under 35X microscope to the NGM-*lite* amp/IPTG plates that were seeded with E. *coli*.
5. After 55 hours, observations are made for phenotypic changes.

Make NGM-*lite* Medium

Pour Plates



Grow E. *coli* Overnight Cultures

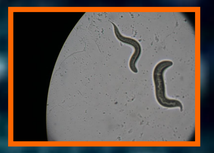
Seed NGM-*lite* plates with E. *coli*

Transfer C.*elegans* to OP50-seeded NGM-*lite* plates

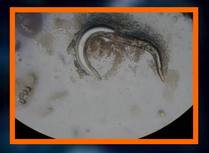
Transfer C.*elegans* to NGM-*lite* amp/IPTG plates

Seed NGM-*lite* amp/IPTG plates with E. *coli*

Wild type



Dpy-11



bli-1

Results to date 4/2/2015

C.*elegans*	**Dumpy-11**		
Trial	I	II	III
dpy-11 (nos. of worms)	19	105	98
Total number of worms	378	1532	1013
Concentration	5.02%	6.85%	9.67%
C.*elegans*	**Blister-1**		
Trial	I	II	III
bli-1 (nos. of worms)	62	94	219
Total number of worms	285	493	1598
Concentration	21.8%	19.07%	13.7%

Conclusion:

RNA interference of C. *elegans* requires diligent and consistent attention to growth media, worm life-stage and feeding stock conditions. Our low induction rates were most likely a result of inconsistent worm staging. Future experiments will pay closer attention to staging, concentration and incubation conditions. In addition, we will be investigating the use of fluorescent dyes for phenotypic identification and analysis.

Reference

1. Grishok, A. (2005) RNAi mechanisms in *Caenorhabditis elegans*. *FEBS Letters*, 579 (26), 5932-5939.
2. Micklos. D., Nash, B., Hilgert, U.(2013). *Genome Science*. Cold Spring Harbor Laboratory Press. 511-673, 647-648.