

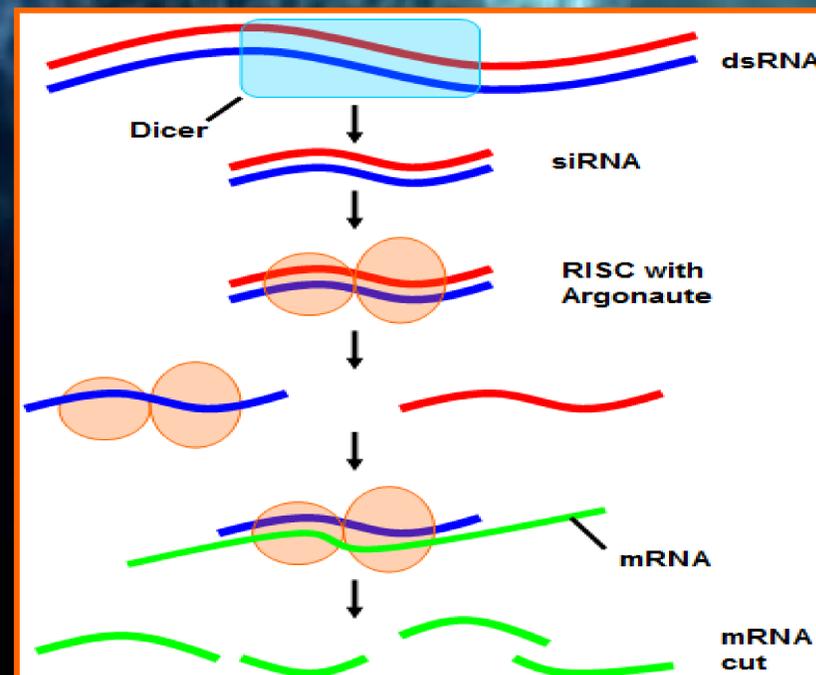


Background

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 a model for studying RNA interference (RNAi). RNAi is a cellular mechanism used to protect cells from RNA viruses, it acts by destroying 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 *dpy-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 dyes to enhance the visualization of phenotypes and more experiments will be set up to optimize experimental conditions to improve the transformation rate. These studies have implications for novel medical treatments and gene therapy.

Inducing RNAi by Feeding



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

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.



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

Table 1: Experiment of Dumpy-11 Construct

| Experiment | I | II | III |
|--|-------|-------|-------|
| <i>dpy-11</i> (nos. of worms) observed | 19 | 105 | 98 |
| Total number of worms examined | 378 | 1532 | 1013 |
| Transformation rate | 5.02% | 6.85% | 9.67% |

Table 2: Experiment of Blister-1 Construct

| Experiment | I | II | III |
|---------------------------------------|-------|--------|-------|
| <i>bli-1</i> (nos. of worms) observed | 62 | 94 | 219 |
| Total number of worms examined | 285 | 493 | 1598 |
| Transformation rate | 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.
3. Inducing RNAi by Feeding. (n.d.) *Carolina Biological Supply Company*