Introducing RNAi in *C. elegans*

**Introduction**

RNA interference, or RNAi, is a common way used to reduce gene expression. A double-stranded RNA would be delivered into the cell and processed into short interfering RNA (siRNA) by enzymes Dicer and Drosha (NCBL). Then, the siRNA would bind to the RNA-induced silencing complex RISC, together pairing with the mRNA of a gene and degrading it so that no translation would happen (NCBL). In this lab, RNAi was introduced in *C. elegans* by feeding them with bacteria HT115 (DE3) which contain a plasmid with dsRNA gene. IPTG would be used to express T7 polymerase in bacteria to transcribe the dsRNA. Once the worms take up the dsRNA, RNAi can happen throughout the body because the worms’ cells contain pores formed by protein SID-1 to transport the dsRNA (LM 110). The target of RNAi would be the unc22 gene, which code for proteins that regulate normal muscle function, so it is expected that RNAi treated worms would show uncoordinated movement (WormBase). This lab examined the effectiveness of RNAi by observing phenotypes and performing a RT-qPCR for the unc22 mRNA, and I hypothesized that RNAi would be highly efficient in degrading unc22 mRNA and causing uncoordinated movement in *C. elegans*.

**Methods**

* **Setting up RNAi (pg. 115, Protocol 23)**

Each group was assigned with either a “Control” or a “RNAi” plate containing *C. elegans* fed with HT115 (DE3). The Control bacteria had a wildtype plasmid without dsRNA gene, and the RNAi bacteria had the plasmid with it. IPTG mixed with M9 buffer was added to express T7 RNA polymerase to transcribe the dsRNA, if the bacteria had the gene, for the worms to take up.

* **Measure phenotypes (pg. 116, Protocol 24)**

After incubation, 20 large and 20 small worms were counted for both Control and RNAi plates, and their movement was compared to the movement before incubation. Their phenotype was recorded as either twitching or not twitching.

* **Isolation of Total RNA from *C. elegans* (pg.116-118, Protocol 25)**

The worms were centrifuged and sonicated to break open cells, allowing lysis buffer to enter. Ethanol, Wash Buffer I and II were added to wash. RNase-free water was used to elute the RNA.

* **RT-qPCR (pg. 112-114, “Reverse Transcriptase and Quantitative PCR (RT-qPCR)”)**

RT-qPCR uses reverse transcriptase to create cDNA based on the RNA template and uses Taq DNA polymerase to create the dsDNA product based on the cDNA template. It could show levels of the amplified product as it progresses because SYBR green was added as a dye, which would only fluoresce and be detected when it intercalates between the two strands of DNA, allowing the amount of DNA to be visualized by looking at the fluorescence intensity. In this experiment, 100 ng of extracted RNA, primers specifically designed for unc22, SYBR green, reverse transcriptase, and Taq DNA polymerase were used. In addition, cdc42 was also amplified as a standard. The cycles taken for the fluorescence intensity to plateau were recorded for all the samples and represented by CT.

* **Analyzing qPCR and Phenotype Data (pg. 120, Protocol 26)**

The averages of duplicate CT measurements for both unc22 and cdc42 were calculated, and each group’s ΔCT was calculated using (unc22 aveCT) – (cdc42 aveCT) so that the unc22 CT were normalized. Then, the aveΔCT for Control groups was calculated to be used as a standard. The ΔΔCT for RNAi groups were calculated using (ΔCT for RNAi) – (aveΔCT for Control) to determine how many more cycles did RNAi unc22 take compared to Control unc22. Since each more cycle indicates a doubled DNA amount, the fold change for each RNAi group was calculated using 2-∆∆CT. The aveΔCT for RNAi and the standard deviations of ΔCT for Control and RNAi groups were also calculated. A bar graph was drawn to compare the aveΔCT for Control and RNAi groups. A t-test was performed between ΔCT for Control and RNAi groups to see the significance of mean difference.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Control Large | Control Small | RNAi Large | RNAi Small |
| Twitching | 1 | 0 | 15 | 13 |
| No Twitching | 19 | 20 | 5 | 7 |
| % Twitching | 5% | 0% | 75% | 65% |

**Results**

Table 1. Phenotype and % twitching for Control/RNAi, large/small worms. This table was generated by counting worms and observing phenotype under a light microscope.

Figure 1. Average ΔCT and standard deviation for Control and RNAi worms. This figure was generated by calculating the average ΔCT and standard deviations of Control and RNAi groups in Excel and graphing them. A t-test was performed, with a p-value of 6.27611E-05.

Ave: 2.197407

Stdev: 0.631171

Ave: -0.24764

Stdev: 0.2715

|  |  |  |  |
| --- | --- | --- | --- |
|  | Fold change | High/low fold change | % Twitching |
| Group 2 | 0.084609 | High | Large 70%, small 70% |
| Group 4 | 0.164898 | High | Large 40%, small 25% |
| Group 6 | 0.296162 | Low | Large 70%, small 70% |
| Group 8 | 0.171609 | High | Large 90%, small 95% |
| Group 10 | 0.229840 | Low | Large 20%, small 30% |
| Group 12 | 0.235334 | Low | Large 70%, small 55% |

Table 2. Fold change and corresponding phenotype. This table was generated by matching each RNAi group’s fold change with corresponding % twitching to show potential relationships.

I observed that, before RNAi, all the worms moved normally without twitching. After RNAi, almost all Control worms still moved normally with few of them twitching, whereas most RNAi worms were uncoordinated and twitched a lot. We see in Table 1 that the % twitching for Control worms were both close to zero and much smaller than those for RNAi worms. In Figure 1, we see that the Control average ΔCT was close to zero, and the RNAi average ΔCT was approximately 2. In Table 2, some groups such as Groups 2, 4, and 8 had high fold changes, and the others had low. Some groups such as Groups 2, 6, 8, and 12 had large % twitching, and some had small numbers.

**Discussion**

Based on the qPCR data in Figure 1 and Supplemental Figure 3, it is observed that RNAi unc22 mRNA spent more cycles completing the qPCR, meaning there were less starting mRNA templates. It confirmed that RNAi decreased unc22 mRNA level. According to the phenotype data in Table 1 and Supplementary Figure 2, RNAi treated worms showed an increased % twitching compared to Control worms, confirming my prediction that RNAi would result in uncoordinated movement. These conclusions can be related because muscle function in *C. elegans* is affected by unc22 protein. In *C. elegans*, unc22 is a gene expressing twitchin in muscle cells throughout the life cycle to regulate normal contraction and relaxation (Moerman et al., 1988). When unc22 mRNA is degraded, twitchin is not produced, and the muscle would lose normal function and result in twitches, as shown by the worms in RNAi groups. As a result, the twitching phenotype I got for RNAi worms was caused by the decreased unc22 mRNA level, and my hypothesis was confirmed.

My conclusion is supported by phenotype caused by other unc22 mutations. In the paper “Additional sequence complexity in the muscle gene, unc-22, and its encoded protein, twitchin, of *Caenorhabditis elegans*” by Benian et al, the authors mentioned a null mutation in unc22 could result in “pronounced body surface twitch,” “impaired movement,” and “disrupted muscle structure” (Bernian et al., 1993). In addition, they inserted a transposon into the gene to create a shift in reading frame and also discovered a “weak phenotype” (Benian et al., 1993). They analyzed this weak phenotype as a result of mRNA splicing that created partially translated unc22 protein. Although the phenotype was weak, it still indicated that incomplete expression of unc22 could result in uncoordinated movement. Therefore, it is further confirmed that both a degradation of unc22 mRNA and a repressed/reduced transcription of unc22 can result in muscle dysfunction.

In human, there is also a homologue of unc22 named TTN, which codes for titin (WormBase). Titin is a protein providing stiffness to cardiac muscles, so mutations in the TNN gene would result in dilated cardiomyopathy and heart failure (LeWinter, 2013). Compare to unc22, both have a similar function of maintaining normal muscle organization.

According to the fold changes in Table 2, it was shown that RNAi can decrease at least two third of the mRNA level. Combining this with the % twitching and the outside resources discussed, my hypothesis was correct such that RNAi was highly effective in degrading unc22 mRNA and causing uncoordinated movement in *C. elegans*.

A close up of a piece of paper

Description automatically generated**Supplemental Results**

Supplementary Figure 1. Screen shot showing class unedited qPCR data.

A screenshot of a cell phone

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Supplementary Figure 2. Screen shot showing class phenotype data.

**A close up of text on a white background

Description automatically generated**Supplementary Figure 3: Excel sheet screenshot showing calculations for Avg CT, ΔCT, ΔΔCT, and Fold Change 2-ΔΔCT.

**References**

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