Ziyue Chen A14524683 Section D01

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 C_T.

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

The averages of duplicate C_T measurements for both unc22 and cdc42 were calculated, and each group's ΔC_T was calculated using (unc22 aveC_T) – (cdc42 aveC_T) so that the unc22 C_T were normalized. Then, the aveΔC_T for Control groups was calculated to be used as a standard. The $\Delta\Delta C_T$ for RNAi groups were calculated using (ΔC_T for RNAi) – (aveΔC_T 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 $2^{-\Delta\Delta CT}$. The aveΔC_T for RNAi and the standard deviations of ΔC_T for Control and RNAi groups were also calculated. A bar graph was drawn to compare the aveΔC_T for Control and RNAi groups. A t-test was performed between ΔC_T for Control and RNAi groups to see the significance of mean difference.

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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.

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

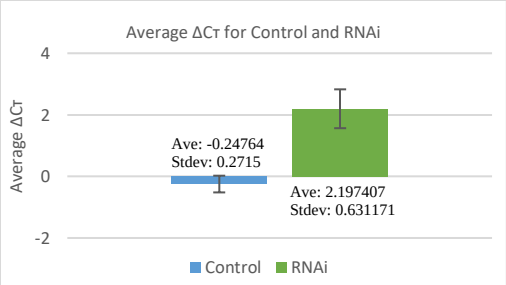

Figure 1. Average ΔC_T and standard deviation for Control and RNAi worms. This figure was generated by calculating the average ΔC_T and standard deviations of Control and RNAi

	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 ΔC_T was close to zero, and the RNAi average ΔC_T 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*.

Supplemental Results

Well	Group Name	Target Nam	Cr
A1	Group 1	cdc42	20.32538
A2	Group 1	cdc42	20.26558
A3	Group 2	cdc42	20.09044
A4	Group 2	cdc42	20.56345
A5	Group 3	cdc42	25.65091
A6	Group 3	cdc42	26.00033
A7	Group 4	cdc42	22.8531
A8	Group 4	cdc42	23.08328
A9	Group 5	cdc42	19.65854
A10	Group 5	cdc42	19.73877
A11	Group 6	cdc42	19.57649
A12	Group 6	cdc42	19.7619
B1	Group 7	cdc42	19.58208
B2	Group 7	cdc42	20.12663
B3	Group 8	cdc42	19.52774
B4	Group 8	cdc42	20.13961
B5	Group 9	cdc42	19.15357
B6	Group 9	cdc42	19.28294
B7	Group 10	cdc42	19.34201
B8	Group 10	cdc42	19.85022
B9	Group 11	cdc42	19.85415
B10	Group 11	cdc42	19.64485
B11	Group 12	cdc42	21.12057
B12	Group 12	cdc42	21.05497
E1	Group 1	unc22	19.70835
E2	Group 1	unc22	19.55637
E3	Group 2	unc22	23.78145
E4	Group 2	unc22	23.50327
E5	Group 3	unc22	25.96166
E6	Group 3	unc22	25.96116
E7	Group 4	unc22	25.18659
E8	Group 4	unc22	25.45522
E9	Group 5	unc22	19.03693
E10	Group 5	unc22	19.63416
E11	Group 6	unc22	21.17973
E12	Group 6	unc22	21.17446
F1	Group 7	unc22	19.20013
F2	Group 7	unc22	19.86824
F3	Group 8	unc22	22.53867
F4	Group 8	unc22	21.71901
F5	Group 9	unc22	19.11219
F6	Group 9	unc22	19.15962
F7	Group 10	unc22	21.25226
F8	Group 10	unc22	21.68729
F9	Group 11	unc22	19.50776
F10	Group 11	unc22	19.60554
F11	Group 12	unc22	22.95501
F12	Group 12	unc22	22.8997

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

			small worms		large worms	
Group#	RNAi (R) or Control (WT)	Phenotype Notes	n	% twitch	n	% twitch
D01 1	WT	twitching	20	25	20	10
D01 2	R	twitching	20	70	20	75
D01 3	WT	no twitching	20	0	20	0
D01 4	R	twitching	20	25	20	40
D01 5	WT	no twitching	20	0	20	5
D01 6	R	twitching	20	70	20	70
D01 7	WT	no twitching	20	0	20	0
D01 8	R	twitching	20	95	20	90
D01 10	R	twitching	20	30	20	20
D01 12	R	twitching	20	55	20	70

Supplementary Figure 2. Screen shot showing class phenotype data.

	Group Nam	Target Nam	Cт	Ave Cт		Group Nam	Target Nam	Cт	Ave Cт
Control	Group 1	cdc42	20.32538	20.29548	RNAi	Group 2	cdc42	20.09044	20.32695
		cdc42	20.26558				cdc42	20.56345	
	Group 3	cdc42	25.65091	25.82562		Group 4	cdc42	22.8531	22.96819
		cdc42	26.00033				cdc42	23.08328	
	Group 5	cdc42	19.65854	19.69866		Group 6	cdc42	19.57649	19.66919
		cdc42	19.73877				cdc42	19.7619	
	Group 7	cdc42	19.58208	19.85436		Group 8	cdc42	19.52774	19.83367
		cdc42	20.12663				cdc42	20.13961	
	Group 9	cdc42	19.15357	19.21826		Group 10	cdc42	19.34201	19.59612
		cdc42	19.28294				cdc42	19.85022	
	Group 11	cdc42	19.85415	19.7495		Group 12	cdc42	21.12057	21.08777
		cdc42	19.64485				cdc42	21.05497	
	Group 1	unc22	19.70835	19.63236		Group 2	unc22	23.78145	23.64236
		unc22	19.55637				unc22	23.50327	
	Group 3	unc22	25.96166	25.96141		Group 4	unc22	25.18659	25.32091
		unc22	25.96116				unc22	25.45522	
	Group 5	unc22	19.03693	19.33554		Group 6	unc22	21.17973	21.1771
		unc22	19.63416				unc22	21.17446	
	Group 7	unc22	19.20013	19.53419		Group 8	unc22	22.53867	22.12884
		unc22	19.86824				unc22	21.71901	
	Group 9	unc22	19.11219	19.13591		Group 10	unc22	21.25226	21.46978
		unc22	19.15962				unc22	21.68729	
	Group 11	unc22	19.50776	19.55665		Group 12	unc22	22.95501	22.92736
		unc22	19.60554				unc22	22.8997	

	Group Nam	ΔCт	Ave ΔCт	SD	ΔΔCT	$2^{-\Delta\Delta CT}$	t-test
Control	Group 1	-0.66312	-0.24764	0.2715	/	/	6.27611E-05
	Group 3	0.135791					
	Group 5	-0.36311					
	Group 7	-0.32017					
	Group 9	-0.08235					
	Group 11	-0.19285					
RNAi	Group 2	3.315414	2.197407	0.631171	3.563052	0.084609	
	Group 4	2.352718			2.600356	0.164898	
	Group 6	1.507902			1.755539	0.296162	
	Group 8	2.295166			2.542803	0.171609	
	Group 10	1.873659			2.121296	0.22984	
	Group 12	1.839583			2.087221	0.235334	

Supplementary Figure 3: Excel sheet screenshot showing calculations for Avg C_T, ΔC_T, $\Delta\Delta C_T$, and Fold Change $2^{-\Delta\Delta CT}$.

References

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