Suppression of Argonaute 2 Transcript Levels in Du182A Cells

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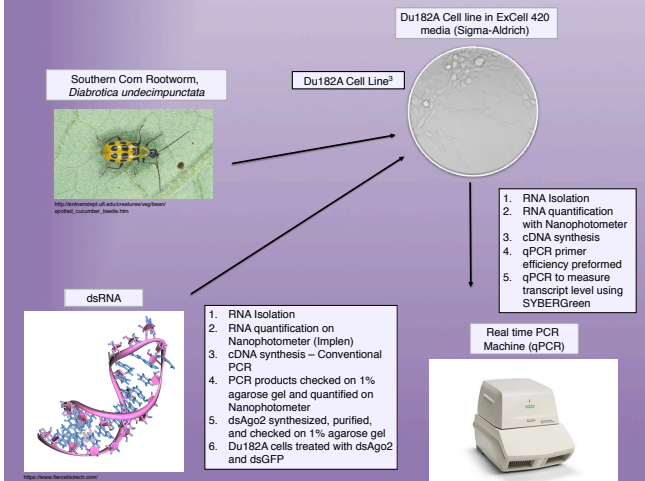
Abstract

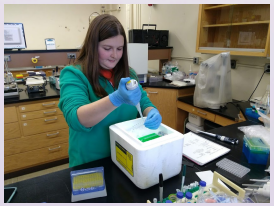
RNA interference (RNAi) uses double-stranded RNA (dsRNA) molecules to degrade and suppress the transcript level of a complementary mRNA target[1]. The RNAi pathway is complex and includes many different proteins, like argonautes, in the core machinery. Argonautes are dsRNA binding proteins which help recognize and cleave target mRNA molecules. In our experiments, we attempted to suppress the transcript level of argonaute 2 (Ago2) in a *Diabrotica undecimpunctata* cell line (Du182A) using dsRNA, with the idea of disrupting the RNAi pathway using an RNAi of RNAi technique. Ago2 transcript levels were suppressed following treatment with dsRNA. Future experiments can now use this technique, with some modification to better understand the RNAi pathway.

Purpose

To determine if treatment with dsRNA can suppress the transcript level of Ago2 in Du128A cells.
Hypothesis: Treatment of Du182A cells with dsAgo2 will suppress Ago2 mRNA.
Prediction: Suppression of the Ago2 transcript levels will occur in cells treated with dsAgo2.

Study System & Materials and Methods



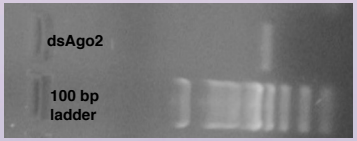


Kyah preparing for qPCR

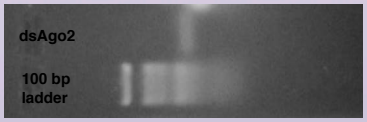
Karen pipetting into 96-well plate for qPCR

Results

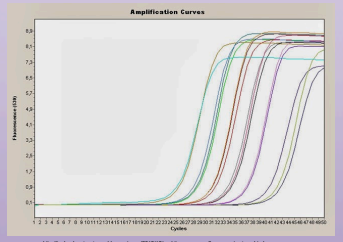
dsRNA Template on 1% Agarose gel



dsRNA Synthesis on 1% Agarose gel



Example qPCR Fluorescence Results



This is an example of qPCR results. During thermal cycling, templates go through the repeated steps of denaturing, annealing, and extension. SYBRGreen then binds double-stranded DNA and fluoresces. Fluorescence increases as more copies of the PCR product are synthesized. The cycle at which the fluroescence crosses a threshold is called the Ct value, which is used for analysis of transcript level.

Table 1. qPCR results

Control - dsGFP											
Group	Ct RpS	Ct RpS	Avg RpS	Avg All RpS	Ct Ago	Ct Ago	Avg Ago	Avg All Ago	ddCt	Avg ddCt	Fold
G1	23.94	22.13	23.03	22.66	25.13	24.85	24.99	23.78	1.13	0.83	0.5623
G1	23.65	22.83	23.24		24.07	23.86	23.97			-0.4	1.3182
G2	22.04	21.35	21.7		22.31	22.47	22.39			-0.43	1.3492
										Avg Fold Δ	1.0765
Experimental – dsAgo 2											
Group	Ct RpS	Ct RpS	Avg RpS	Avg All RpS	Ct Ago a	Ct Ago b	Avg Ago	Avg All Ago	ddCt	Avg ddCt	Fold
Ago 1	23.64	22.66	23.15	23.15	24.89	24.52	24.7	24.6	1.45	0.43	0.7441
Ago 2	25.37	23.29	24.33		25.69	25.54	25.62			0.16	0.8939
Ago 3	23.28	22.05	22.66		23.47	23.24	23.36			-0.43	1.3508
Ago 4	23.13	21.83	22.48		24.89	24.43	24.66			1.05	0.4825
										Avg Fold Δ	0.8678

Ct values analyzed using the ΔΔCt method[4]. Results indicate there is a 13% reduction in transcription level on Du182A cells treated with dsAgo2.

Conclusions

- The Du182A cell line is sensitive to treatment with dsRNA
- These experiments lay the foundation for further experimentation with this cell line to identify potential new targets for insect control through RNAi.
- The mechanism of RNAi suppression can be further explored because of the evidence of knockdown

Future Directions

With only 13% knockdown, we would like to adjust our procedures to obtain a more physiologically relevant silencing of transcript level.

We can explore the role of Ago2 in the RNAi pathway and the effects suppression of Ago2 transcript levels on RNAi responses. These experiments can also be extended to include other components of the RNAi pathway, including dsRNA uptake and export mechanisms.

In addition to the RNAi pathway, we can observe the effects of suppression of Ago2 transcripts on anti-viral immunity, a parallel pathway to that of RNAi.

Injection or feeding of *D. undecimpunctata* larvae or eggs with dsAgo2 can be done to observe any physiological or phenotypic consequences.

References

1. Fire, *et al.* 1998. *Science* **391**, 806-811.
2. Velez, *et al.* 2016. *PLoS ONE* **11**, e0157520.
3. Lynn and Stoppleworth. 1984. *In Vitro* **20**, 365-368.
4. Livak and Schmittgen. 2001. *Methods* **25**, 402-408.

Table 2: Primers used for dsRNA synthesis and qPCR

dsRNA	Sequence	Size	Ref.
Ago2	TAATACGACTCACTATAGGGATCTCTTGGATTCAATGGGA TAATACGACTCACTATAGGGCCTGATTCGCAACATATACC	366 bp	2
qPCR			
Ago2	AGCCCTGATTCGCAACATAT TCTCCTGTCTGGGTGGTT	109	2
RpS3	GGGCTTGCTATGGTGTCTTG GAGTGGATCATAAGACCATCTAC	200	N/A

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