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Investigating RNAi in Manduca sexta using BAPCs

Kierra Holloman

Investigating RNA interference in *Manduca sexta* using Branched Amphiphilic Protein Capsules (BAPCs)

Kierra Holloman, Neal Dittmer, Michael Kanost
Kansas State University Department of Biochemistry and Molecular Biophysics
Developing Scholars, Office of Undergraduate Research and Creative Inquiry

Abstract

The goal of this research is to find an improved method for interfering with gene expression in insects, which can be important agricultural pests. Genes contain the information that tell cells/organisms how to function. When a gene is expressed, a copy of the gene is made of RNA; this copy is known as messenger RNA (mRNA). One method to study a gene's importance would be to interfere with its expression and observe the effect it has on the organism. This can be accomplished by a technique called RNA interference (RNAi). When double stranded RNA (dsRNA) is introduced into a cell, it is recognized as being non-natural to the cell and a possible indication of viral infection. If the cell finds an mRNA that is a match to the dsRNA, it is targeted for degradation. In RNAi, if we introduce dsRNA that matches a gene we are interested in, the corresponding mRNA will be degraded. This method has proven to be very useful for studying gene function. However, this technique has not been very successful in butterflies and moths, possibly because of enzymes that break down the dsRNA before it can get into cells. A method to protect the dsRNA could make this method more effective. Branched Amphiphilic Peptide Capsules (BAPCs) are small protein nanocapsules that have been used successfully to deliver dsRNA in insects. We want to test if BAPCs will be helpful to deliver dsRNA in the caterpillar of the moth *Manduca sexta*. To test this technique, we targeted the two genes that encode for insecticyanin, a protein that is responsible for the green color of the caterpillars. If successful, the color of the caterpillars should be lighter green or white. Caterpillars were fed a solution that contained dsRNA for insecticyanin complexed with BAPCs. The caterpillars were monitored for a week to observe their color pattern. We also monitored the color of their hemolymph (blood) which is also green because of the presence of insecticyanin. In preliminary experiments, caterpillars fed the dsRNA/BAPC complexes looked similar to caterpillars fed a sugar solution. This suggests that either RNAi didn't work or was too minimal to affect insecticyanin protein levels.

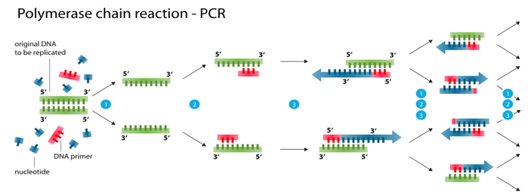
Hypothesis

BAPCs will be an efficient method for delivering dsRNA to cells by protecting it from degradation.

How we will test the hypothesis

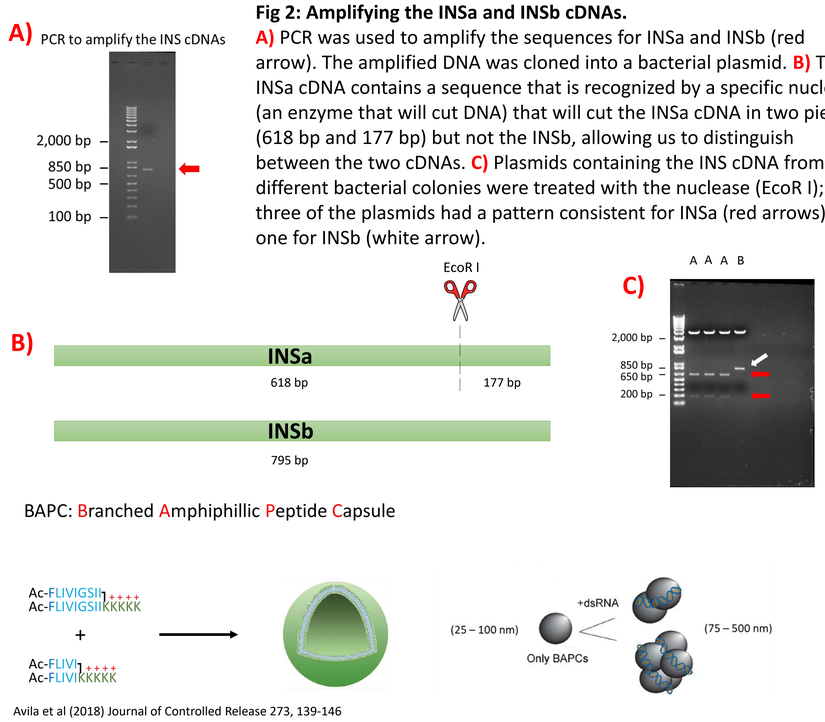
- We chose to knock down the two genes for Insecticyanin (INS).
  - The INS protein binds a pigment that makes the caterpillars green; absence of this pigment should affect the color of the caterpillars.
  - These genes are expressed in tissues that previously have been difficult to get RNAi to work.
- First, we needed to clone the INS-a and INS-b cDNAs.
- Next, we used the INS-a cDNAs as template to make the dsRNA.
- dsRNA was then complexed with the BAPCs.
- The complexes were fed to the caterpillars.
- The caterpillars were observed everyday for a week, and the amount of INS in the hemolymph (blood) was monitored by measuring its absorbance at different light wavelengths.

Methods



https://en.wikipedia.org/wiki/File:Polymerase_chain_reaction.svg

Fig 1: How PCR Works.
Step 1, heat is used to separate the DNA. Step 2, a small piece of DNA (primer) pairs with the appropriate strand of DNA. Step 3, the enzyme DNA polymerase synthesizes new DNA. Each cycle of PCR doubles the amount of DNA. After 30 cycles of PCR 1 billion copies of DNA will be made.



Avila et al (2018) Journal of Controlled Release 273, 139-146

Fig 2: Amplifying the INSa and INSb cDNAs.
A) PCR was used to amplify the sequences for INSa and INSb (red arrow). The amplified DNA was cloned into a bacterial plasmid. **B)** The INSa cDNA contains a sequence that is recognized by a specific nuclease (an enzyme that will cut DNA) that will cut the INSa cDNA in two pieces (618 bp and 177 bp) but not the INSb, allowing us to distinguish between the two cDNAs. **C)** Plasmids containing the INS cDNA from four different bacterial colonies were treated with the nuclease (EcoR I); three of the plasmids had a pattern consistent for INSa (red arrows) and one for INSb (white arrow).

Fig 3: Formation of BAPC/dsRNA complexes.
Two branched peptides are mixed together to form the capsules; the water soluble portions (green) are on the inside and outside of the capsule, while the water insoluble portions (blue) are in the middle. When the dsRNA is mixed with the capsules, it wraps around the outside. This technique has been used to deliver dsRNA to insects (Avila et al., 2018).

The caterpillars were fed a sucrose solution that contained:
1. BAPC/dsRNA complexes
2. BAPC only
3. dsRNA only
4. Sucrose only

Fig 4: Feeding the caterpillars the BAPC/dsRNA complexes.
Newly hatched caterpillars were fed a sugar solution containing the BAPC/dsRNA complexes (a newly hatched caterpillar is only 5 millimeters, or 1/5 of an inch, long!). We then observed the caterpillars for 7 days to see what color they were. The image on the right shows caterpillars that are newly hatched, one day old, and 7 days old.

Results

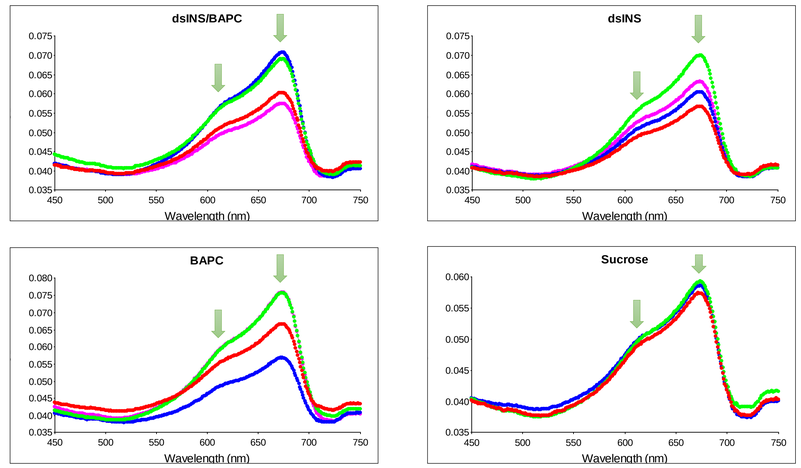


Fig 5: The absorbance spectrum of the caterpillars hemolymph (blood).
Insecticyanin also turns the caterpillars hemolymph green. We measured the absorbance of light at different wavelengths for the hemolymph as a method to determine the relative abundance of insecticyanin, which absorbs light at 630 nm and 680 nm (green arrows). The graphs above show the absorbance spectrum for each of the four groups of caterpillars (four caterpillars per group).

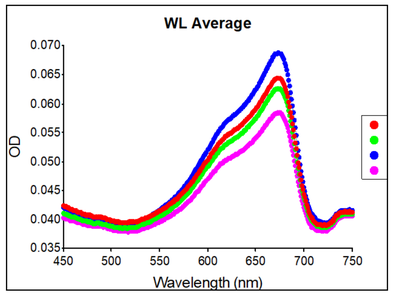


Fig 6: Average absorbance spectrum for each of the four groups.
For each of the groups above, the individual spectrums were averaged together and plotted on the same graph. The average for the sucrose treatment, which we expected to be the highest, was actually the lowest, indicating that it had the least amount of insecticyanin.

Conclusion

- No differences were observed between the different treatments or wavelength scans.
- It is possible that INS transcript levels were reduced, however, the knockdown was not great enough to affect protein levels.
- It is possible that we knocked down one of the genes but not the other, therefore, the protein levels were still high.
- The next step will be to determine transcript levels by quantitative RT-PCR.
- If RNAi was not successful, it is possible that the dsRNA/BAPC complexes were trapped in the gut.
- It is also possible that we needed to treat the caterpillars for a longer period time rather than feeding them the dsRNA just once.

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