Genetics of a Transgenic Beetle (*Tribolium castaneum*) Carrying Green Fluorescent Protein

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Abstract

We found that a transgenic line of *Tribolium castaneum*, previously characterized for the expression of green fluorescent protein (GFP) in the oenocyte (OE strain), shows unusual genetics based on the GFP expression patterns. The larval individuals of OE line showed GFP expressions are not homogeneous among individuals; the expressions are in their oenocyte or hindgut, or in both organs. We provide two alternative hypotheses for this phenomenon: H1) variable expressions of a GFP gene in different stages or H2) more than one GFP transgenes. We tested this hypotheses by examining the GFP expression patterns in larval stages over the period of one week. In addition, we crossed the OE line to wildtype to understand the segregation pattern of the GFP phenotypes. We found that the GFP expression pattern in the OE changes within a molting instar leading to an conclusion accepting the H2) variable expressions of a GFP transgene in OE line, while the genetic crossing is in progress to test the H1) multiple GFP transgene insertions.

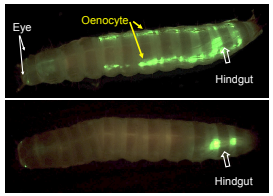

Fig.1. Variations in expression of GFP in OE transgenic line of *T. castaneum*. Upper panel shows expressions in eyes, oenocytes, and hindgut. Lower panel shows expression lacking in oenocytes.

Purpose

To understand the source of variations in expression of GFP transgene in the OE line of *T. castaneum*.

Questions, Hypotheses, and Predictions

Question: What causes the variation of GFP expressions in the OE transgenic line? Two alternative hypotheses below are constructed.

Hypothesis 1: A GFP transgene varies its expression depending on age of the molting stage.

Hypothesis 2: Multiple GFP transgenes segregate in the OE line.

Study System

Daily observations of GFP expression patterns were made for multiple individuals in 5 consecutive days. In additton, we have set up single pair mating between OE line to a wildtype to examine the individuals of F2 of this crossing (data not available yet).

Methods and Experimental Design

- For observation of GFP expression patterns over time (hypothesis 1), we collected ~5th larval instar and maintained individually in 1oz solo cups with ~1 gram flour. Each individuals were daily observed by using a fluorescent microscope for GFP expression patterns for 5 consecutive days.
- For genetic study (hypothesis 2), we collected males of wild type pupae and females of OE line. Single pair mating was made for establishing three families. F1 was self crossed for obtaining F2, but is in progress without data obtained yet.

Results

We found that the OE line of *T. castaneum* showed a difference in expression of GFP throughout its life stages (Fig. 1). In the larvae stage, you can see that the posterior expression of GFP is there and at relatively the same strength throughout this whole life stage. The lateral side (oenocytes) of the specimen in the larvae stage is different. After each molt the larvae shows a strong GFP and as it ages the GFP becomes weaker and weaker until the next molt. As shown in the pictures below Specimen D was observed from the dates of 11/03/2017-11/07/2017 and the lateral expression of GFP becomes weaker and weaker as the days go on. Specimen G was observed from the dates of 11/06/2017-11/09/2107 and in the pupae stage the only visible GFP would be in the specimens eyes and the posterior end with weak signal in between abdominal segments. The expression of GFP in the pupae stage stays fairly constant as it ages in this stage.

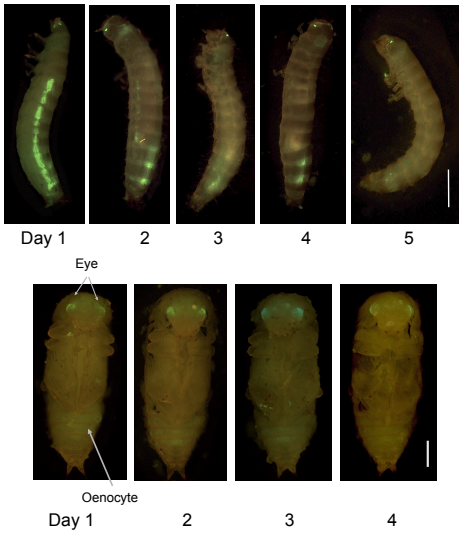

Fig.2. Changes in expression of GFP in OE transgenic line over time. Upper panel shows expressions in 6th larval instar after molting. Lower panel shows in the pupal stage. Scale bar is for 1 mm.

Conclusions

We found that the variations in expression of GFP in OE transgenic line is caused by stage specific expressions. GFP expression in the oenocyte is high after molting, but decreased over time until the next molting. Pupae stage shows constitutive, but low levels of GFP expressions in the oenocytes.

Future Directions

Although we concluded that the oenocyte GFP expression is dependent on the developmental stages, we can not rule out possibility of two different GFP transgenea in this strain (hypothesis 2), each for expression in oenocytes and in hindgut. We currently established single-pair crossings to test this hypothesis. Segregation pattern in the F2 of this crossings will provide us the conclusion.

Decreased GFP in the oenocyte is an interesting phenomenon because GFP molecule is highly stable in the tissue in general. Therefore, the decreased GFP is likely caused by degeneration of oenocytes over time in the molting instar. This hypothesis needs to be tested in further experiments.

Beetle oenocyte is the tissue responsible for production of the most important insect development hormone ecdysone. The OR strain will be an important resource for the study of insect hormone metabolism.

References

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