Relative expression of basic metabolic genes in the anterior midgut of *Manduca sexta*

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Abstract

The surface area to volume ratio decreases as animals grow resulting in a decrease in relative surface area for gas exchange and nutrient uptake. Larger animals may compensate by increasing surface area with in-folding, increasing the density of transport proteins or decreasing mass-specific metabolic rate. *Manduca sexta* grow 10,000-fold over an 18 day period, molting through five stages, or instars. Animals grow bigger by increasing cell size during intermolt, while cell number increases by cell division during molting. We investigated whether development has an effect on the expression of basic metabolic genes. RNA was extracted from anterior midgut tissue from 4th and 5th instar larvae. qRT-PCR was used to measure the relative expression of select genes involved in glycolysis, the pentose phosphate pathway (PPP), and the cytoskeleton. Target gene expression was normalized to mRNA levels of the ribosomal housekeeping gene 18s. Actin expression increased 3.7 fold from 4th to 5th instar. The PPP gene Glucosamine-6-phosphate isomerase decreased by 70 fold from 4th to 5th instar while the other genes tested remained unchanged. Actin and glycolysis gene Glucose-6-phosphate isomerase were the only genes that showed any change in expression over 5th instar. Both decreased by about 4 fold from day 1 to day 3 of 5th instar. The expression of these genes suggests a complicated picture of gene expression over larval development.

Introduction

As animals grow larger their metabolism slows down. We are interested in understanding the mechanisms that contribute to this decrease in mass-specific metabolic rate. Tobacco Hornworm larvae *Manduca sexta* are good model organisms because they increase 10,000-fold in weight with limited changes in body plan (Yeoh, et al., 2011).

M. sexta grow through five larval instars, separated by periods of molting. The total number of cells increases during molting and cell size increases during intermolt. One explanation for decreased mass-specific metabolic rate is a decrease in cell-specific metabolic rate as diffusion becomes a limiting factor in larger cells. We hypothesize that the expression of select metabolic genes might be upregulated in compensation.

A complicating factor in the analysis of qRT-PCR data is the need for a housekeeping gene (HKG) with constant expression to normalize technical variation between samples. Previous work has suggested that common HKGs are unsuitable for the normalization of qRT-PCR data because their expression is variable throughout development (McCurley and Callard, 2008). We investigated a number of metabolic genes that have been previously used as HKGs.

Results

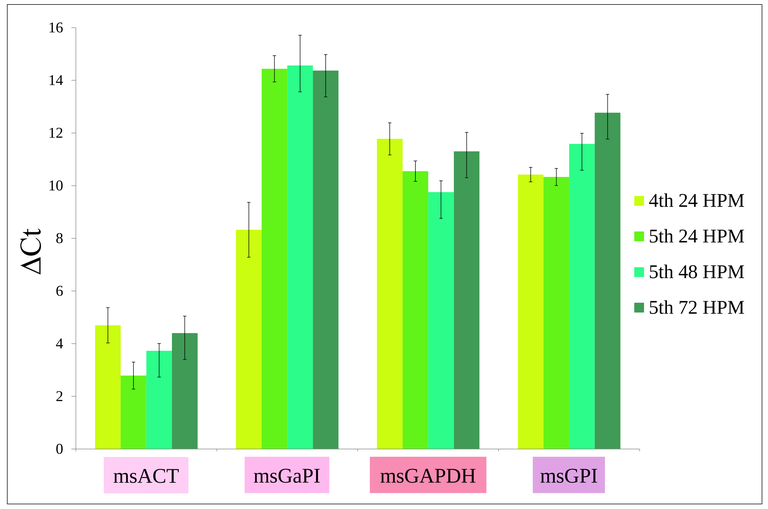

Figure 2. Comparison of ΔCt values of metabolic genes for 4th and 5th instar anterior midgut tissue (average ± 1 STD, n= 5-6 per age).

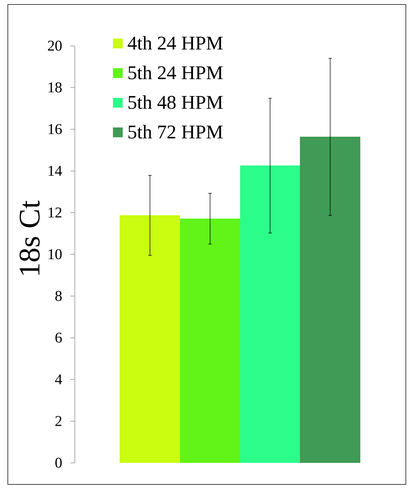

Figure 3. Comparison of 18s Ct values over 4th and 5th instar (average ± 1 STD, n= 20-24).

Materials and Methods

Gene	Protein	Function
msACT	Actin	Important cytoskeleton protein
msGaPI	Glucosamine-6- phopshate isomerase (deaminase)	Converts D-glucosamine 6-phosphate into D-fructose 6-phosphate in the PPP.
msGAPDH	Glucose-6-phosphate dehydrogenase	Converts glucose-6-phosphate to 6-phosphogluconolactone while reducing NADP+ to NADPH in the pentose phosphate pathway (PPP).
msGPI	Glucose-6-phosphate isomerase	Second enzyme in glycolysis that reversibly converts glucose-6-phosphate to fructose-6-phosphate.

Table 1. Target genes under study (Berg et al., 2010).

Manduca sexta were raised on an artificial wheat germ diet (Carolina Biological) and kept at 27 °C on a 16L: 8D photoperiod. Anterior midgut tissue was collected from 4th instar and 5th instar animals 24, 48, & 72 hours post molt (HPM). Tissue was stored at - 80 °C. Total RNA was extracted using TRIzol reagent (Invitrogen) and treated for DNA contamination using a Turbo DNA-Free kit (Ambion). A TAQman Reverse Transcription Kit (Applied Biosystems) was used to reverse transcribe RNA (100 ng/µL) to cDNA.

Primers were designed using Primer Express (Applied Biosystems), synthesized (Operon), and optimized. 18s was used to normalize the data. Quantitative real-time PCR using SYBR green was run in triplicate in 96-well optical plates (Applied Biosystems). Each age studied had 5-6 biological replicates. Mixed-effects models using the statistical program R were used to analyze the data.

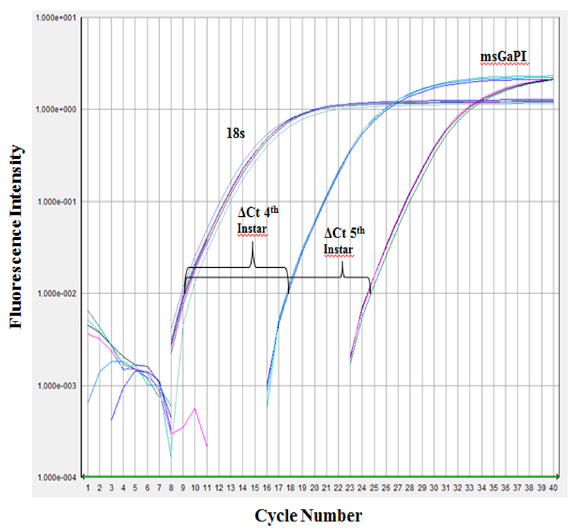

Figure 1. Graphical representation of ΔCt values for msGaPI.

Results

- There was a decrease in 18s expression (decreased Ct value) over 5th instar for all genes tested, (Mixed-Effects Model, t = 2.8859-3.41428, p = 0.0098- 0.0029).
- Actin expression was upregulated from 4th to 5th instar (Mixed-Effects Model, t = 2.54, P = 0.027) but was down regulated over 5th instar (Mixed-Effects Model, t = 2.36, P = 0.03)
- msGaPI expression decreased from 4th to 5th instar (Mixed-Effects Model, t = 5.52, P = 0.0001) and remained stable over the 5th instar.
- msGPI expression decreased across the 5th instar (Mixed-Effects Model, t = 4.03, P = 0.0008).
- msGAPDH expression decreased from 24 HPM 5th to 72 HPM 5th but there was no overall trend toward decreased expression over 5th instar (Mixed-Effects Model, t = 3.06, P = 0.0068).

Discussion

- Increased actin expression from 4th to 5th instar may compensate for increased strain on individual cells as total mass of the organism increases.
- The decrease in 18s expression over the 5th instar can be attributed to decreased protein synthesis throughout the instar.
- Decreased msGPI expression across the 5th instar may support a shift from glycolysis to the PPP as larvae prepare to pupate.
- Conversely, decreased msGaPI expression from 4th to 5th instar could signal a shift from the PPP to glycolysis (Berg et al., 2010)
- There is a trend toward increased expression of msGAPDH in 5th instar, consistent with Gibellato and Chamberlain (1994).

Future Work

- Compare gene expression levels in the posterior midgut of 4th and 5th instar larvae.
- Study other metabolic genes involved in cholesterol transport and fatty acid synthesis.
- Find a better method for normalization of qRT-PCR data over the 5th instar.

Acknowledgements

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