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ORIGINAL ARTICLE

Shotgun proteomic analysis of the fat body during metamorphosis of domesticated silkworm (*Bombyx mori*)

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Abstract Protein expression profiles in the fat bodies of larval, pupal, and moth stages of silkworm were determined using shotgun proteomics and MS sequencing. We identified 138, 217, and 86 proteins from the larval, pupal and moth stages, respectively, of which 12 were shared by the 3 stages. There were 92, 150, and 45 specific proteins identified in the larval, pupal and moth stages, respectively, of which 17, 68, and 9 had functional annotations. Among the specific proteins identified in moth fat body, sex-specific storage-protein 1 precursor and chorion protein B8 were unique to the moth stage, indicating that the moth stage fat body is more important for adult sexual characteristics. Many ribosomal proteins (L23, L4, L5, P2, S10, S11, S15A and S3) were found in pupal fat bodies, whereas only three (L14, S20, and S7) and none were identified in larval and moth fat bodies, respectively. Twenty-three metabolic enzymes were identified in the pupal stage, while only four and two were identified in the larval and moth stages, respectively. In addition, an important protein, gloverin2, was only identified in larval fat bodies. Gene ontology (GO) analysis of the proteins specific to the three stages linked them to the cellular component, molecular function, and biological process categories. The most diverse GO functional classes were involved by the relatively less specific proteins identified in larva. GO analysis of the proteins shared among the three stages showed that the pupa and moth stages shared the most similar protein functions in the fat body.

Keywords Fat body · Silkworm · Shotgun · Proteome · Metamorphosis

Introduction

The fat body is a key organ in insects that is responsible for metabolic process, including energy storage and synthesis of carbohydrates, proteins, and lipids. Likewise, the fat body in the silkworm, *Bombyx mori*, also plays two well-defined functions: storage and intermediary metabolism (Beament et al. 1963). In addition, the fat body also plays a role in immune system, in that most of the characterized immune proteins, including antibacterial or antifungal peptides, induced by microbial infection are predominantly synthesized in the fat body and are then released into the hemolymph (Aggarwal and Silverman 2008).

The domesticated silkworm is economically important for the silk production and has been identified as the best model organism for lepidopteran biochemical, molecular genetic, and genomic studies (Nagaraju and Goldsmith 2002; Tomita et al. 2003; Goldsmith et al. 2005; Reumer et al. 2008). The whole life cycle of the silkworm can be described as the larval, pupal, and moth stages. Metamorphosis, which begins from larva to pupa and then to the adult moth, initiates after the fifth instar period. Previous

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studies have indicated that different diet conditions can change the protein profiles in silkworm suggesting that the fat body proteins can respond to external stimuli (Zhou et al. 2008). The proteomic profiles of fat bodies throughout metamorphosis will, therefore, allow the identification of key proteins in the control of energy metabolism and pivotal intracellular signaling pathways that are involved in the metamorphic process.

Following the completion of draft sequences of the genomes of several model organisms, including silkworm, proteomics has become the focal point in recent entomological research. As an effective tool for proteomics, shotgun proteomics, which is based on the in-gel or gel-free digestion of protein mixtures followed by liquid chromatography (LC) separation, MS detection and database searching, provides a highly sensitive and high throughput approach to determine the proteome components in a complex biological sample. This approach has been implemented in model insects, such as *Bombyx* (Li et al. 2009a, b), *Drosophila* (Li et al. 2007; Baggerman et al. 2005) and *Anopheles* (Kalume et al. 2005).

In the present study, we utilized the shotgun multidimensional Liquid Chromatography LTQ-Orbitrap mass spectrometry (LC–MS/MS) approach, combined with bioinformatics analysis to illuminate the differences among protein expression profiles of the fat bodies in the larval, pupal, and moth stages of the silkworm and to find valuable clues regarding energy metabolism and signaling mechanisms during metamorphosis of the silkworm.

Materials and methods

Silkworm rearing and fat body isolation

Silkworm strain P50 was reared on fresh mulberry leaves under an environment of 12 h light/12 h dark photoperiod, 26 ± 1°C and 70–85% relative humidity. The developmental stages were synchronized at each molt by collecting new larvae. On the fifth day of the fifth instar, the first day of the pupal stage and the first day of the moth stage, ten animals were killed and their fat bodies were collected. The tissue samples were stored at −20°C for the further use.

Sample preparation and sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) separation

Fat body tissue from the larva, pupa, and moth were mechanically homogenized on ice for 10 min in 10-μL lysis buffer [comprising 2.5% SDS, 10% glycerol, 5% β-mercaptoethanol and 62.5 mM Tris–HCl (pH 6.8)] per 1 mg tissue. The samples were then sonicated in an ice-bath for 30 s and then every 30 s, four times. The samples were then centrifuged at 20,000×*g* at 25°C for 10 min. The supernatants were then collected and were centrifuged again and the resultant supernatants were stored at −20°C for further use. The concentrations of protein samples were determined using 2-D Quant Kit (Amersham Biosciences, USA). The samples were boiled for 2 min and centrifuged at 20,000×*g*, for 10 min before being subjected to SDS-PAGE separation, using a 5% stacking gel and a 12.5% resolving gel. For each sample, a total amount 500 μg of protein was separated using SDS-PAGE on four lanes. The gels were stained with Coomassie Brilliant Blue R250 (CBB, Sigma, USA) after electrophoresis.

In-gel digestion

The CBB-stained SDS-PAGE 4 gel lanes were manually cut into 12 slices, depending on the quality of the protein bands (Fig. 1). Each slice was further sliced into 1 × 1 mm pieces and subjected to in-gel tryptic digestion, as described previously (Wilm et al. 1996; Li et al. 2009a). Briefly, the proteins were reduced with 50 mM Tris[2-carboxyethyl]phosphine (TCEP, Sigma) in 25 mM NH_4HCO_3 at 56°C for 1 h and alkylated with 100 mM iodoacetamide (IAA, Amersham) in 25 mM NH_4HCO_3 at room temperature in the dark for 0.5 h. The digestion was performed with 20 ng/μl porcine trypsin (modified proteomics grade, Sigma) overnight at 37°C.

Analysis of shotgun LC–MS/MS

All digested peptide mixtures were separated by online reversed-phase (RP) nano LC using the Ettan MDLC nanoflow/capillary LC system (GE Healthcare, Pittsburgh, PA, USA) and analyzed by a Thermo Finnigan Linear Orbitrap mass spectrometer (LTQ-Orbitrap MS) equipped with an electrospray ionization (ESI) source (Proxeon Biosystems, Odense, Denmark). An external standard solution with *m*/*z* range from 195.00 to 1,922.00 was used to calibrate the mass spectrometer. Monoisotopic peak selection was applied. The +2 and +3 charge states were selected for fragmentation and +1 and ≥+4 charge states were excluded. Samples were automatically injected into a 10 μl sample loop and delivered at 15 μl/min on the trapping column (Dionex/LC Packings μ-Precolumn Cartridge P/N 160454 C18 PepMap 100, 5 μm, 100 Å, 300 μm i.d., ×5 mm, Sunnyvale, CA, USA) for desalting. After flow-splitting down to approximately 250 nl/min, peptides were transferred to the analytical column (Dionex/LC Packings P/N 160321 150 × 0.075 mm i.d., C18 PepMap, 3 μm, 100Å, Sunnyvale, CA USA) and eluted using buffer B (95% ACN, 0.1% methanoic acid in water) at a flow rate of

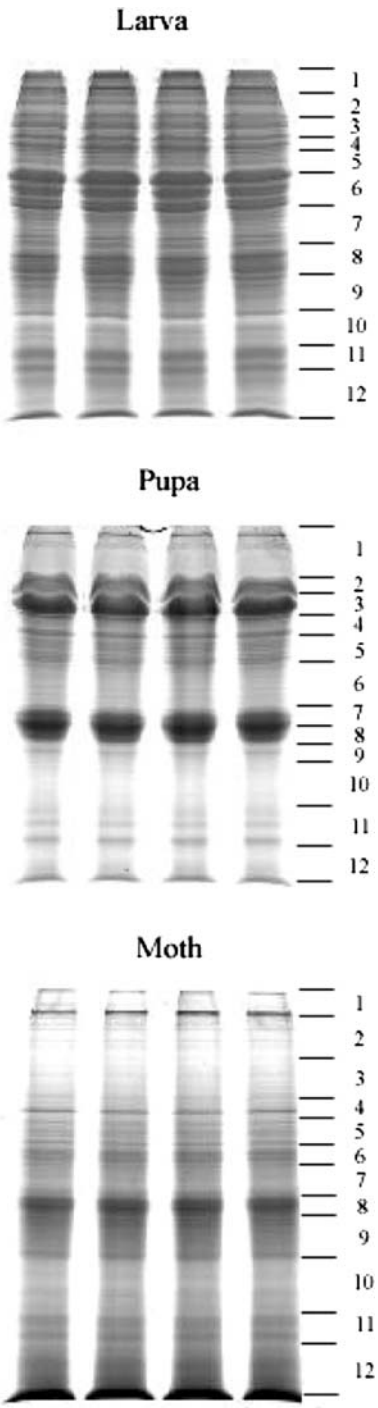

Fig. 1 One-dimensional SDS-PAGE gel separation of the three groups of fat body protein samples from larva, pupa and moth, respectively. The *numbers* indicated the 12 bands according to the slicing pattern used for sample fractionation prior to in-gel digestion

approximately 250 nl/min with 70 min gradients from 5 to 45% and 20 min gradients from 45 to 95%. The analytical column was regenerated for 20 min with 5% buffer A (5% ACN, 0.1% methanoic acid in water) at 250 nl/min before loading the next sample. Data-dependent acquisition was performed on the LTQ-Orbitrap mass spectrometer in the positive ion mode. The temperature of the ion transfer tube was set at 200°C. The spray voltage was set at 1.8 kV and normalized collision energy at 35% for MS2. The MS scan range was 300–2,000 *m*/*z* with a resolution $R = 60{,}000$ at *m*/*z* 400. The MS analysis was performed with one full MS scan followed by five MS/MS scans on the five most intense ions from the MS spectrum with the dynamic exclusion for 180 s. The experiments were repeated twice and the results were combined into the final result.

Database search

Database search was carried out against the in-house database we previously constructed (Li et al. 2009b) which contains a total of 25,325 proteins including the sequences of the domesticated silkworm (*B. mori*) and wild silkworm (*Bombyx mandarina*). The raw MS/MS spectra were interpreted by Biowork 3.0 (ThermoFinnigan, San Jose, CA, USA) and the database searches were performed with SEQUEST algorithm, which is a module of Biowork 3.0 on a local server. The peptide mass tolerance was 10.0 ppm and the fragment ions tolerance was 1.0. The trypsin enzyme and partial enzymatic cleavage of the amino acids bonds at both ends of protein were chosen. Two miss-cleavage sites were allowed. Only *b* and *y* fragment ions were taken into account. Fixed modification (Carboxamidomethyl) on cysteine and variable modification (Oxidation) on methionine were set.

Validation processes with the *trans*-proteomic pipeline (TPP)

Validation of the identified peptides and proteins was carried out according to the manual of the TPP software 3.4, which was downloaded from the website (http://tools.proteomecenter.org/TPP.php) and installed with the default options (Keller et al. 2002, 2005; Nesvizhskii et al. 2003). Validation of the identified proteins and peptides was carried using previously described methods (Li et al. 2009b). Protein probability threshold for running ProteinProphet was set at 0.9.

InterPro annotation and gene ontology (GO) categories

InterProScan software was used to carry out protein sequence searches against the InterPro member databases to identify signatures (Zdobnov and Apweiler 2001). The compiled RAW outputs were subjected to GO category analysis using the Web Gene Ontology Annotation Plot (WEGO) (Ye et al. 2006). The three groups of datasets

were simultaneously subjected to online analysis (http://wego.genomics.org.cn/cgi-bin/wego/index.pl) and the *P*-values were calculated by the Pearson Chi-square test.

Results and discussion

Commonly identified proteins among the fat bodies of the three developmental stages

We combined the shotgun proteomics strategy (based on the proteolytic digestion of complex protein mixtures, peptides LC separation and tandem MS sequencing) with searching against our in-house database, to obtain the protein expression profiles of the larval, pupal, and moth developmental stages. All proteins were identified by SEQUEST algorithms and were further validated by TPP under stringent criteria. We identified 138, 217, and 86 proteins from the larval, pupal, and moth stages, respectively (Fig. 2, supplementary Tables 1, 2, 3). Due to the limitations of the silkworm protein database, not all of the proteins had complete functional annotations. In our results, the number of proteins identified in the pupal fat bodies was the highest and that of the moth stage was the lowest. There were 12 common proteins, including 4 annotated proteins that were shared by the 3 stages: calreticulin, H^+ transporting ATP synthase beta subunit, actin and 90-kDa heat shock protein. Among them, calreticulin, which is a calcium binding chaperone molecule, is located in the endoplasmic reticulum, responds to endoplasmic reticulum stress and is especially highly expressed in silkworm fat body tissues (Goo et al. 2005). Our results also verified that in the larva, pupa, and moth developmental stages calreticulin was always identified in the fat body tissue. The function of calreticulin is also related to energy metabolism in fat body tissue. Previous research showed that the absence of calreticulin function could induce glucose uptake and the up-regulation of the insulin receptor (Jalali et al. 2008). The expression of calreticulin in all three stages indicates the importance of the insulin-signaling pathway in the fat body tissue during metamorphosis of the silkworm.

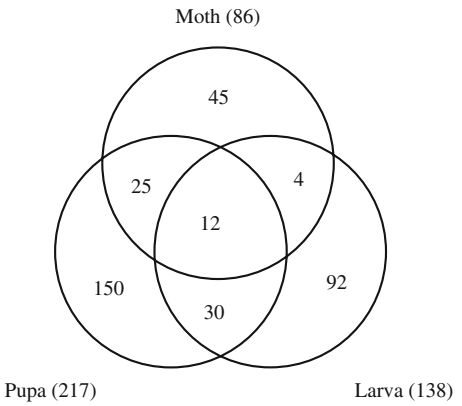

Fig. 2 Venn diagram shows the numbers of identified proteins in the fat body tissues from larva, pupa, and moth of the silkworms. Each *number* with *no overlap* of circles shows the number of proteins uniquely observed in that fat sample, while *overlapping circles* shows the numbers of identified proteins common to two or to three of the analyzes

Specific proteins identified in the fat bodies of the three developmental stages

Although few proteins were common to all three stages, there were many proteins expressed specifically in the larval, pupal, and moth stages (92, 150, and 45, respectively), of which only 17, 68, and 9 proteins had functional annotations.

The characteristic proteins specific to the moth are shown in Table 1. Among the moth specific proteins, sex-specific storage-protein 1 precursor (SP1) is a sex-related protein that can be expressed in both sexes of the silkworm, but is predominantly expressed in females (Suzuki et al. 2003). SP1 can also be transcribed with high efficiency in the fat body nuclear extract of fifth instar larva (Mine et al. 1995). Chorion protein B8 is a silk moth chorion protein belonging to the functional amyloid family and was also only identified in the moth fat body tissue. As a natural protective amyloid, silk moth chorion protein is the major component of the eggshell, a structure with extraordinary physiological and mechanical properties (Iconomidou and Hamodrakas 2008). These two proteins were not identified

Table 1 Functional annotations of specific proteins identified in the fat bodies of moth

Bmb/gi number	Functional annotation
gil225153	Chorion protein B8
gil114052462	Glutamate dehydrogenase [*B. mori*]
gil2696388	Histone H2b [*B. mori*]
Bmb012386, gil114052589	Phosphate transport protein [*B. mori*]
Bmb037755, gil112983366	Protein disulfide isomerase like protein ERp57 [*B. mori*]
Bmb011627, gil1335609	SP1, Sex-specific storage-protein 1 precursor (SP 1) (Methionine-rich storage protein) [*B. mori*]
Bmb018509, gil107953774	Transport protein Sec61 alpha subunit [*B. mori*]
Bmb003848, gil148298829	Vacuolar H + ATP synthase 16 kDa proteolipid subunit [*B. mori*]
Bmb025429, gil112984100	Yellow5 [*B. mori*], Yellow-b [*B. mori*]

in the fat bodies of the larva or pupa, indicating that the fat body in the moth is important to the sexual differentiation of the adult stage. Another important protein identified in the moth stage was ERp57, which is a protein disulfide isomerase-related polypeptide and is thought to catalyze the isomerization of non-native disulfide bonds formed in glycoproteins with unstructured disulfide rich domains (Mishra et al. 2005). ERp57 belongs to the endoplasmic reticulum oxidoreductases and its specificity requires accessory factors like calreticulin (Jessop et al. 2009). The co-translocation of ERp57 and calreticulin determines the immunogenicity of cell death (Panaretakis et al. 2008). In the present study, calreticulin and ERp57 were identified in the fat bodies of the moth stage, suggesting that fat body proteins might be involved in the immune function of the moth.

Compared with the larval and moth stage, the specific proteins in the fat bodies of the pupal stage were more diverse (Table 2). Many ribosomal proteins (L23, L4, L5, P2, S10, S11, S15A and S3) were found in the pupal fat bodies, whereas only three (L14, S20, S7) were identified in larvae and none in moth fat bodies. This abundance of ribosomal proteins in the pupal stage might be closely related to protein synthesis in the pupal fat bodies. Furthermore, among the pupal-specific proteins, 23 metabolic enzymes were identified (15% of pupal-specific proteins), whereas only 4 and 2 metabolic enzymes were specific to the larval and moth stages, representing 4.3 and 4.4% of their specific proteins. The metabolic enzymes include 6-phosphogluconate dehydrogenase, alcohol dehydrogenase, carboxylesterase, Cu/Zn-superoxide dismutase, enolase, glyceraldehyde-3-phosphate dehydrogenase, phosphoserine aminotransferase, and zinc-containing alcohol dehydrogenase. The identification of these essential metabolic enzymes indicated that during metamorphosis in the pupal stage the metabolism was most active.

The 30 K protein was also found in pupal fat bodies. The metabolism of 30 K proteins is a prerequisite for normal embryonic development (Zhong et al. 2005) and it might also be important for the fat body characteristics at different developmental stages. The 30 K protein also has the role of inhibiting hemolymph apoptosis in the silkworm and it might also display a similar function in pupal fat bodies (Kim et al. 2003). We also identified elongation factor 1 gamma subunit (EF-1γ) and elongation factor 1alpha subunit (EF-1α) in the pupal fat bodies. EF-1γ belongs to a subunit of silk gland EF-1L (the lighter form) and can facilitate the exchange of EF-1α bound GDP for GTP (Kamiie et al. 2002). Elongation factor 1 subunits were only identified in pupal fat bodies, suggesting that more active molecular changes were happening at the pupal stage than at the larva and moth stages. Other important proteins identified in the pupal fat bodies were heat shock cognate protein, profiling protein, serpin-2, and serpin-5.

In the fat bodies of the larval stage, some ribosomal proteins, such as L14, S20 and S7, were identified (Table 3), which was much fewer than ribosomal proteins identified in pupal stage. The four metabolic enzymes identified in larvae were ADP/ATP translocase, cytochrome *C* oxidase polypeptide Vb, transaldolase, and transitional endoplasmic reticulum ATPase. An important antibacterial peptide, gloverin2, was also expressed in larval fat bodies. Gloverin isoform genes, including gloverin2, were mainly observed in the fat body of fifth instar silkworm larvae in response to injection with *Escherichia coli* (Kaneko et al. 2007). The four gloverin-like genes in the silkworm *B. mori*, named BmGlov1-4, can be induced in the larval fat body after an immune challenge (Kawaoka et al. 2008). The larval stage is the only stage at which silkworms feed and absorb nutrients from the outside, and therefore it is probable that those antibacterial peptides are largely expressed during the larval stage and that the fat body plays a pivotal role in the innate immunity of the silkworm. Our results, showing that gloverin2 was specifically expressed in the larval fat bodies, support this hypothesis.

As far as the proteins shared between each pair of developmental stages are concerned, there are 42, 37, and 16 proteins commonly expressed between larva and pupa, pupa and moth, and moth and larva, respectively. The larva and pupa (42) and the pupa and moth (37) shared the highest number of common proteins, while the larva and moth (16) shared few common proteins. Thus, the protein profile of the larva was similar to that of the pupa and the protein profile of the pupa was similar to that of the moth. The protein profile of the larva was not very similar to that of the moth. Thus, our results suggest that, during metamorphosis, the developmental changes to protein expression were time-dependent and non-reversible.

Gene ontology (GO) analysis of the functional categories

GO analysis of the 92, 150, and 45 specific proteins identified in fat bodies of larva, pupa, and moth, respectively, linked these proteins to the cellular component, molecular function, and biological process categories (Fig. 3). Although they are uniquely identified in the three developmental stages, most of them were categorized into the same functional classes. These classes included: cell, cell part, macromolecular complex, organelle, organelle part in cellular component; binding, catalytic, structural molecular, and transporter in molecular function; biological regulation, cellular process, developmental process, establishment of localization, localization, metabolic process, and pigmentation in biological process. The three

Table 2 Functional annotations of specific proteins identified in the fat bodies of pupa

Bmb/gi number	Functional annotation
Bmb038426, gil114051770	26S proteasome non-ATPase regulatory subunit 13 [*B. mori*]
Bmb000199, gil114050901	Tyrosine 3-monooxygenase protein zeta polypeptide [*B. mori*]
gil112983548	27 kDa glycoprotein precursor (P27 K) [*B. mori*]
Bmb019518, gil10907	30 K protein [*B. mori*]
Bmb011479, gil153791847	Abnormal wing disk-like protein [*B. mori*]
gil112983501	Alpha-tubulin [*B. mori*]
Bmb017073, gil10801568	Annexin IX-A,B,C [*B. mori*]
gil112983471	Antichymotrypsin precursor [*B. mori*]
gil112983770	Antitrypsin precursor [*B. mori*]
Bmb013502, gil124430725	Arylphorin [*B. mori*]
Bmb030882, gil112984452	Beta-tubulin [*B. mori*]
Bmb018730, gil114053173	Bmp-2 [*B. mori*]
gil112982685	Cadherin-like membrane protein [*B. mori*]
Bmb027017, gil114050749	Chaperonin subunit 6a zeta [*B. mori*]
Bmb003962, gil87248481	Cytochrome b5 [*B. mori*]
Bmb009022, gil112983898	Elongation factor 1 gamma [*B. mori*]
Bmb015183, gil112984390	Elongation factor 1-alpha (EF-1-alpha) [*B. mori*]
Bmb004096, gil112982932	Ferritin [*Bombyx mori*], Ferritin subunit precursor [*B. mori*],
Bmb016869, gil114051243	FK506-binding protein [*B. mori*]
Bmb027040, gil114053313	GTP binding protein [*B. mori*]
gil112807176	H2A histone family member V [*B. mori*]
Bmb009360, gil112982828	Heat shock cognate protein [*B. mori*]
Bmb002839, gil11120618	Heat shock protein hsp20.8 [*B. mori*], Heat shock protein hsp20.4 [*B. mori*]
Bmb034335, gil1094393	Hemocytin,Hemocytin precursor (Humoral lectin), Humoral lectin prepropeptide [*B. mori*]
Bmb019659, gil112983264	Lipophorin receptor [*B. mori*]
Bmb021419	Low molecular 30 kDa lipoprotein PBMHPC-23 precursor
gil112984340	P270 [*B. mori*]
Bmb005383, gil114051003	Perilipin [*B. mori*]
gil3721631	Pol polyprotein [*B. mori*]
Bmb013674, gil112982865	Profilin [*B. mori*]
gil112983210	Serpin-2 [*B. mori*]
Bmb010729, gil112984548	Serpin-5 [*B. mori*]
Bmb011047, gil112983262	Small GTP binding protein RAB5 [*B. mori*]
gil114052645	Thymosin isoform 1 and 2 [*B. mori*]
gil112983240	Transferrin [*B. mori*]
Bmb013675, gil112983736	Translation initiation factor 4A [*B. mori*]
Bmb010905, gil112983746	Vitellogenin precursor [*B. mori*]
Bmb014252, gil112984274	Ribosomal protein L23 [*B. mori*]
Bmb020135, gil112982800	Ribosomal protein L4 [*B. mori*]
Bmb013316, gil112983276	Ribosomal protein L5 [*B. mori*]
Bmb010411, gil112984336	Ribosomal protein P2 [*B. mori*]
gil112983505	Ribosomal protein S10 [*B. mori*]
Bmb031584, gil112982861	Ribosomal protein S11 [*B. mori*]
Bmb006919, gil112982855	Ribosomal protein S15A [*B. mori*]
Bmb026411, gil112984112	Ribosomal protein S3 [*B. mori*]
Bmb007278, gil112983786	3-ketoacyl-CoA thiolase [*B. mori*], Sterol carrier protein x [*B. mori*]
Bmb022309, gil114053253	6-phosphogluconate dehydrogenase [*B. mori*]
Bmb021504, gil146424692	Acetoacetyl-CoA thiolase [*B. mori*]

Table 2 continued

Bmb/gi number	Functional annotation
Bmb032144, gil114052488	Alcohol dehydrogenase [*B. mori*]
Bmb013826, gil114052306	Carboxylesterase [*B. mori*]
Bmb008297, gil112983576	Cathepsin D [*B. mori*], Aspartic protease [*B. mori*]
Bmb007516, gil112982998	Cu/Zn-superoxide dismutase [*B. mandarina*]
Bmb022689, gil114051239	Cystathionine gamma-lyase [*B. mori*]
gil119381542	Enolase [*B. mori*]
gil148298746	Glucose-6-phosphate isomerase [*B. mori*]
Bmb008291, gil112361467	Glutathione S-transferase 2 [*B. mori*]
Bmb006175, gil109119903	Glyceraldehyde-3-phosphate dehydrogenase [*B. mori*]
gil114051866	Isocitrate dehydrogenase [*B. mori*]
Bmb022329, gil112983178	Juvenile hormone esterase [*B. mori*]
Bmb021298, gil153792270	Malate dehydrogenase [*B. mori*]
Bmb024179, gil114052408	Mitochondrial aldehyde dehydrogenase [*B. mori*]
Bmb032684, gil151301209	NADPH-specific isocitrate dehydrogenase [*B. mori*]
Bmb033292, gil114052472	Peptidylprolyl isomerase B [*B. mori*]
gil114052677	Phosphoserine aminotransferase [*B. mori*]
Bmb018693, gil153791817	*S*-adenosyl-L-homocysteine hydrolase [*B. mori*]
Bmb022208, gil112984224	Transfer RNA-Ala synthetase [*B. mori*]
Bmb011628, gil118500417	Vacuolar ATP synthase subunit B [*B. mori*]
Bmb019893, gil114051702	Zinc-containing alcohol dehydrogenase [*B. mori*]

Table 3 Functional annotations of specific proteins identified in the fat bodies of larva

Bmb/gi number	Functional annotation
Bmb013008, gil158631166	ADP\ATP translocase [*B. mori*]
Bmb010353	ADP-ribosylation factor [*B. mori*]
Bmb012754, gil112982697	Antibacterial peptide, gloverin2 [*B. mori*]
Bmb013733, gil110796922	Broad-complex isoform Z2/3 [*B. mori*]
gil95102860	Cytochrome *c* oxidase polypeptide Vb [*B. mori*]
gil145843755	GABA-gated chlorine channel alpha subunit [*B. mori*]
Bmb021314, gil157367283	Nicotinic acetylcholine receptor subunit beta 3 [*B. mori*], nicotinic acetylcholine receptor beta 2 subunit [*B. mori*]
Bmb006471, gil114053251	Peroxisomal membrane protein PMP22 [*B. mori*]
Bmb031937, gil112984376	Ribosomal protein L14 [*B. mori*]
Bmb028177, gil148298732	Ribosomal protein S20 [*B. mori*]
Bmb005653, gil112984058	Ribosomal protein S7 [*B. mori*]
Bmb028789, gil148298654	Small nuclear ribonucleoprotein polypeptide [*B. mori*]
Bmb017849, gil160333889	TFIIB-related factor [*B. mori*]
Bmb002691, gil112982996	Thiol peroxiredoxin [*B. mori*]
Bmb023533, gil114052613	Transaldolase [*B. mori*]
gil112983322	Transitional endoplasmic reticulum ATPase TER94 [*B. mori*]
Bmb004930, gil112983481	Tubulin alpha chain [*B. mori*]

stages shared the most common functional class in the biological process category. The differences in the functional classes among the three groups of proteins indicated that some of them were unique to that stage. Some proteins only identified in the larval stage were involved in extracellular region part, extracellular matrix part, membrane-enclosed lumen, synapse and synapse part classes in the cellular component category; and antioxidant, molecular

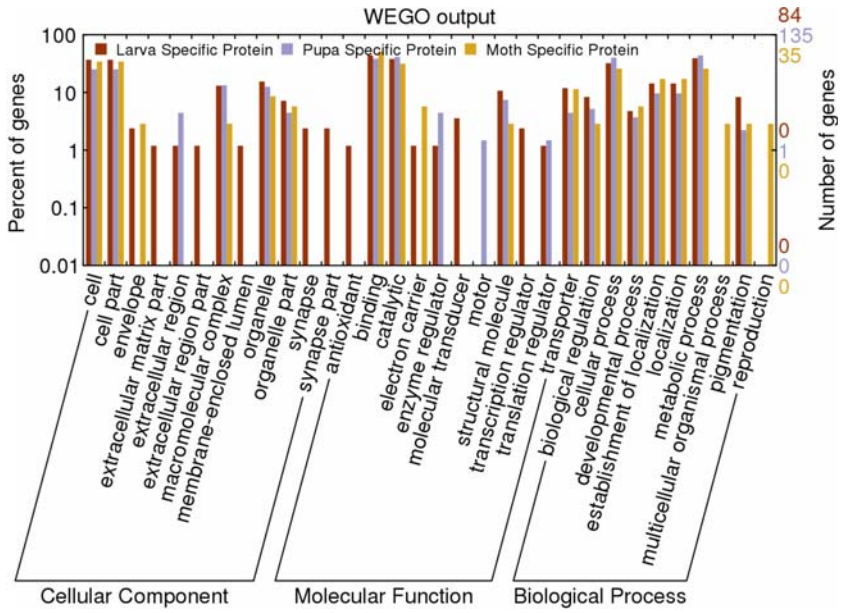

Fig. 3 Gene ontology categories for the specific proteins in fat bodies of larva and pupa, and moth. The *number* of genes shows the amount of the genes with available GO terms in each group. The *percent* of genes shows its proportion in total genes

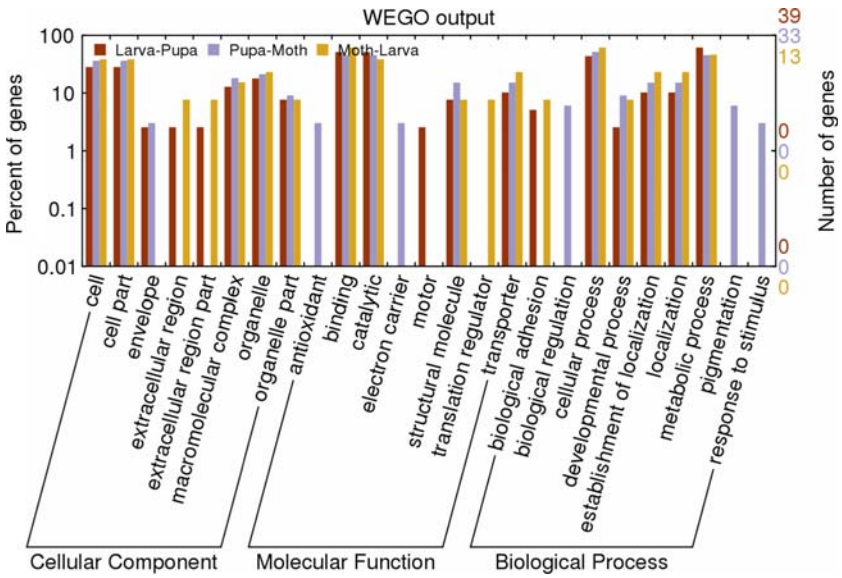

Fig. 4 Gene ontology categories for the proteins shared by larva and pupa, pupa and moth, and moth and larva. The *number* of genes shows the amount of the genes with available GO terms in each group. The *percent* of genes shows its proportion in total genes

transducer, and transcription regulator classes in the molecular function category. Only one class, motor in the molecular function category, was unique to the pupal stage. Some proteins specific to the moth stage were involved in multicellular organismal process and reproduction classes in the biological process category. From these results, we can see that, among the three stages, although more specific proteins were identified in the pupa, the most diverse GO functional classes were identified in the relatively few proteins specifically expressed in the larva. This might reflect the fact that feeding, growing, and complex metabolic activities mainly happen during the larval stage. Compared with the larval stage, there were few metabolic activities identified during the pupal and moth stages because during these two stages silkworms stop feeding. Of the GO functional classes involving proteins identified in fat bodies of the moth, the reproduction class was the only one unique to the moth stage, reflecting the predominant task of the moth stage, i.e. reproduction. GO analysis of these proteins provided us with a global view of their functions, allowing an understanding of fat body changes during the three developmental stages of metamorphosis of the silkworm.

The 42, 37, and 16 proteins shared by the larval and pupal, pupal and moth, and moth and larval stages, respectively, were also analyzed by GO (Fig. 4). Most of the categories related to the three groups of proteins were the same. The special functional classes involving

common proteins between the pupa and moth were antioxidant, electron carrier, biological regulation, pigmentation, and response to stimulus categories. There was only one special functional class involving a common protein between the larva and pupa and the moth and larva, respectively. The results showed that the pupa and moth shared the most similar protein functions in the fat body.

Conclusion

We observed the protein expression profiles of the fat bodies in the larval, pupal and moth stages during metamorphosis of the silkworm by shotgun proteomic analysis. Specific proteins identified in the three stages and common proteins shared by each pair of stages were identified and analyzed. Some interesting proteins were identified. GO analysis of these proteins also provided us with a global view of their functions. Our results indicated that during metamorphosis of domesticated silkworms, in the pupal stage, the fat body might perform the most active metabolic process compared with the larval and moth stage. These results will also help further research on the functions of the fat body proteins during metamorphosis of domesticated silkworm.

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