

Bombyx mori

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Protein expression profiles in the fat bodies of larval, pupal, and moth stages of silk worm 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, several 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. Ten of 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 · Silk worm · Shotgun · Proteome · Metamorphosis

studies have indicated that different diet conditions can change the protein profiles in silk worm 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 silk worm, 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 silk worm and to find valuable clues regarding energy metabolism and signaling mechanisms during metamorphosis of the silk worm.

Silk worm rearing and fat body isolation

Silk worm 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 dodecyl sulfate-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 NH₄HCO₃ at 56 °C for 1 h and alkylated with 100 mM iodoacetamide (IAA, Amersham) in 25 mM NH₄HCO₃ 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 nano online/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 on-line 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, 100 Å, Sunnyvale, CA, USA) and eluted using buffer B (95% ACN, 0.1% methanoic acid in water) at a flow rate of

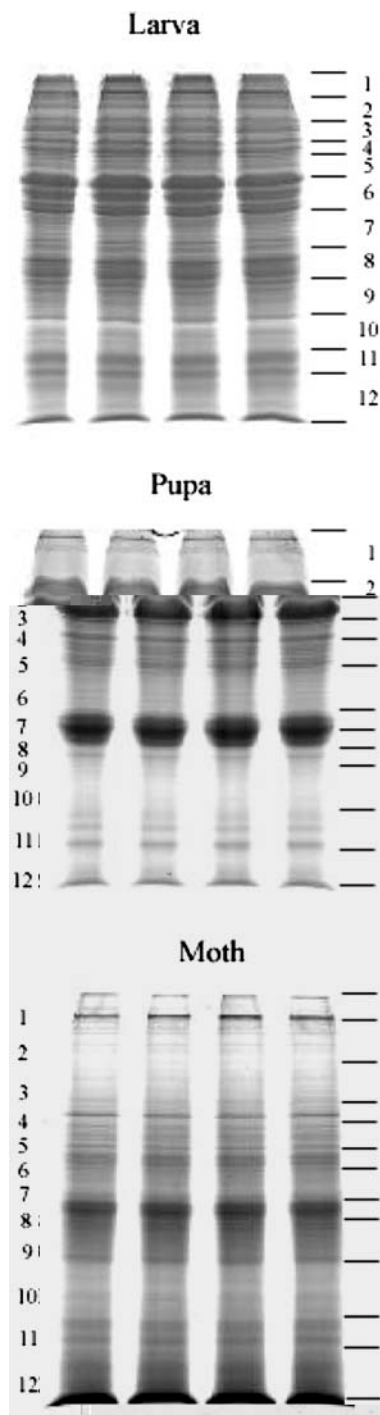


Fig. 1 One-dimensional SDS-PAGE gel separation of the three groups of fat body protein samples from larva, pupa and moth, respectively. The numbers indicated the 12 bands according to the slicing pattern used for sample fractionation prior to in-gel digestion

approimately 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 previously constructed (Li et al. 2009b) which contains a total of 25,325 proteins including the sequences of the domesticated silk worm (*B. mori*) and wild silk worm (*Bombyx mandarina*). The raw MS/MS spectra were interpreted by BioSpectrum 3.0 (ThermoFinnigan, San Jose, CA, USA) and the database searches were performed with SEQUEST algorithm, which is a module of BioSpectrum 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 acid bonds at both ends of protein were chosen. To miss-cleavage sites were allowed. Only b and y fragment ions were taken into account. Fixed modification (Carbamidomethylation) 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

ere simultaneousl subjected to online anal sis (<http://ego.genomics.org.cn/cgi-bin/ego/index.pl>) and the P -values ere calculated b the Pearson Chi-square test.

Commonl identi ed proteins among the fat bodies of the three developmental stages

We combined the shotgun proteomics strateg (based on the proteolitic digestion of comple x protein mixtures, peptides LC separation and tandem MS sequencing) ith searching against our in-house database, to obtain the protein e pression profiles of the larval, pupal, and moth developmental stages. All proteins ere identi ed b SEQUEST algorithms and ere further validated b TPP under stringent criteria. We identi ed 138, 217, and 86 proteins from the larval, pupal, and moth stages, respectively (Fig. 2, supplementar Tables 1, 2, 3). Due to the limitations of the silk orm protein database, not all of the proteins had complete functional annotations. In our results, the number of proteins identi ed in the pupal fat bodies as the highest and that of the moth stage as the lowest. There ere 12 common proteins, including 4 annotated proteins that ere shared b the 3 stages: calreticulin, H^+ transporting ATP synthase beta subunit, actin and 90-kDa heat shock protein. Among them, calreticulin, hich is a calcium binding chaperone molecule, is located in the endoplasmic reticulum, responds to endoplasmic reticulum stress and is especiall highl e pressed in silk orm fat bod tissues (Goo et al. 2005). Our results

also veri ed that in the larva, pupa, and moth developmental stages calreticulin as al a s identi ed in the fat bod tissue. The function of calreticulin is also related to energ metabolism in fat bod tissue. Previous research sho ed that the absence of calreticulin function could induce glucose uptake and the up-regulation of the insulin receptor (Jalali et al. 2008). The e pression of calreticulin in all three stages indicates the importance of the insulin-signaling path a in the fat bod tissue during metamorphosis of the silk orm.

Speci c proteins identi ed in the fat bodies of the three developmental stages

Although fe proteins ere common to all three stages, there ere man proteins e pressed speci call in the larval, pupal, and moth stages (92, 150, and 45, respectively), of hich onl 17, 68, and 9 proteins had functional annotations.

The characteristic proteins speci c to the moth are sho n in Table 1. Among the moth speci c proteins, se x speci c storage-protein 1 precursor (SP1) is a se x-related protein that can be e pressed in both se xes of the silk orm, but is predominantl e pressed in females (Sä uki et al. 2003). SP1 can also be transcribed ith high ef ienc in the fat bod nuclear e tract of fth instar larva (Mine et al. 1995). Chorion protein B8 is a silk moth chorion protein belonging to the functional am loid famil and as also onl identi ed in the moth fat bod tissue. As a natural protective am loid, silk moth chorion protein is the major component of the eggshell, a structure ith e traordinary biological and mechanical properties (Iconomidou and Hamodrakas 2008). These t o proteins ere not identi ed

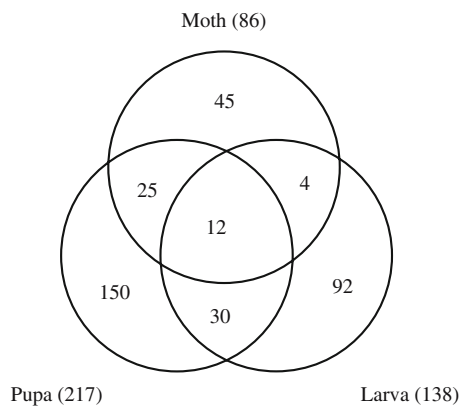


Fig. 2 Venn diagram sho s the numbers of identi ed proteins in the fat bod tissues from larva, pupa, and moth of the silk orms. Each *number* ith *no overlap* of circles sho s the number of proteins uniuell observed in that fat sample, hile *overlapping circles* sho s the numbers of identi ed proteins common to t o or to three of the anal es

Table 1 Functional annotations of speci c proteins identi ed in the fat bodies of moth

Bmb/gi number	Functional annotation
gi 225153	Chorion protein B8
gi 114052462	Glutamate deh drogenase [<i>B. mori</i>]
gi 2696388	Histone H2b [<i>B. mori</i>]
Bmb012386, gi 114052589	Phosphate transport protein [<i>B. mori</i>]
Bmb037755, gi 112983366	Protein disul de isomerase like protein ERp57 [<i>B. mori</i>]
Bmb011627, gi 1335609	SP1, Se x speci c storage-protein 1 precursor (SP 1) (Methionine-rich storage protein) [<i>B. mori</i>]
Bmb018509, gi 107953774	Transport protein Sec61 alpha subunit [<i>B. mori</i>]
Bmb003848, gi 148298829	Vacuolar H^+ ATP synthase 16 kDa proteolipid subunit [<i>B. mori</i>]
Bmb025429, gi 112984100	Yello 5 [<i>B. mori</i>], Yello -b [<i>B. mori</i>]

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-1 γ) and elongation factor 1 alpha subunit (EF-1 α) in the pupal fat bodies. EF-1 γ belongs to a subunit of silk gland EF-1L (the lighter form) and can facilitate the exchange of EF-1 α 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, proline 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

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

Bmb/gi number	Functional annotation
Bmb038426, gi 114051770	26S proteasome non-ATPase regulator subunit 13 [<i>B. mori</i>]
Bmb000199, gi 114050901	Tyrosine 3-monooxygenase protein eta polypeptide [<i>B. mori</i>]
gi 112983548	27 kDa gl coprotein precursor (P27 K) [<i>B. mori</i>]
Bmb019518, gi 10907	30 K protein [<i>B. mori</i>]
Bmb011479, gi 153791847	Abnormal ring disk-like protein [<i>B. mori</i>]
gi 112983501	Alpha-tubulin [<i>B. mori</i>]
Bmb017073, gi 10801568	Anne in IX-A,B,C [<i>B. mori</i>]
gi 112983471	Antichymotrypsin precursor [<i>B. mori</i>]
gi 112983770	Antitrypsin precursor [<i>B. mori</i>]
Bmb013502, gi 124430725	Arthropodin [<i>B. mori</i>]
Bmb030882, gi 112984452	Beta-tubulin [<i>B. mori</i>]
Bmb018730, gi 114053173	Bmp-2 [<i>B. mori</i>]
gi 112982685	Cadherin-like membrane protein [<i>B. mori</i>]
Bmb027017, gi 114050749	Chaperonin subunit 6 alpha [<i>B. mori</i>]
Bmb003962, gi 87248481	Cytochrome b5 [<i>B. mori</i>]
Bmb009022, gi 112983898	Elongation factor 1 gamma [<i>B. mori</i>]
Bmb015183, gi 112984390	Elongation factor 1-alpha (EF-1-alpha) [<i>B. mori</i>]
Bmb004096, gi 112982932	Ferritin [<i>Bombyx mori</i>], Ferritin subunit precursor [<i>B. mori</i>],
Bmb016869, gi 114051243	FK506-binding protein [<i>B. mori</i>]
Bmb027040, gi 114053313	GTP binding protein [<i>B. mori</i>]
gi 112807176	H2A histone family member V [<i>B. mori</i>]
Bmb009360, gi 112982828	Heat shock cognate protein [<i>B. mori</i>]
Bmb002839, gi 11120618	Heat shock protein hsp20.8 [<i>B. mori</i>], Heat shock protein hsp20.4 [<i>B. mori</i>]
Bmb034335, gi 1094393	Hemochitin, Hemochitin precursor (Humoral lectin), Humoral lectin prepropeptide [<i>B. mori</i>]
Bmb019659, gi 112983264	Lipophorin receptor [<i>B. mori</i>]
Bmb021419	Low molecular 30 kDa lipoprotein PBMHPC-23 precursor
gi 112984340	P270 [<i>B. mori</i>]
Bmb005383, gi 114051003	Perilipin [<i>B. mori</i>]
gi 3721631	Polypeptide protein [<i>B. mori</i>]
Bmb013674, gi 112982865	Profilin [<i>B. mori</i>]
gi 112983210	Serpin-2 [<i>B. mori</i>]
Bmb010729, gi 112984548	Serpin-5 [<i>B. mori</i>]
Bmb011047, gi 112983262	Small GTP binding protein RAB5 [<i>B. mori</i>]
gi 114052645	Thrombosin isoform 1 and 2 [<i>B. mori</i>]
gi 112983240	Transferrin [<i>B. mori</i>]
Bmb013675, gi 112983736	Translation initiation factor 4A [<i>B. mori</i>]
Bmb010905, gi 112983746	Vitellogenin precursor [<i>B. mori</i>]
Bmb014252, gi 112984274	Ribosomal protein L23 [<i>B. mori</i>]
Bmb020135, gi 112982800	Ribosomal protein L4 [<i>B. mori</i>]
Bmb013316, gi 112983276	Ribosomal protein L5 [<i>B. mori</i>]
Bmb010411, gi 112984336	Ribosomal protein P2 [<i>B. mori</i>]
gi 112983505	Ribosomal protein S10 [<i>B. mori</i>]
Bmb031584, gi 112982861	Ribosomal protein S11 [<i>B. mori</i>]
Bmb006919, gi 112982855	Ribosomal protein S15A [<i>B. mori</i>]
Bmb026411, gi 112984112	Ribosomal protein S3 [<i>B. mori</i>]
Bmb007278, gi 112983786	3-ketoacyl-CoA thiolase [<i>B. mori</i>], Sterol carrier protein alpha [<i>B. mori</i>]
Bmb022309, gi 114053253	6-phosphogluconate dehydrogenase [<i>B. mori</i>]
Bmb021504, gi 146424692	Acetoacetyl-CoA thiolase [<i>B. mori</i>]

continued

Bmb/gi number	Functional annotation
Bmb032144, gi 114052488	Alcohol dehydrogenase [<i>B. mori</i>]
Bmb013826, gi 114052306	Carboxylesterase [<i>B. mori</i>]
Bmb008297, gi 112983576	Cathepsin D [<i>B. mori</i>], Aspartic protease [<i>B. mori</i>]
Bmb007516, gi 112982998	Cu/Zn-superoxide dismutase [<i>B. mandarina</i>]
Bmb022689, gi 114051239	Cystathionine gamma-lyase [<i>B. mori</i>]
gi 119381542	Enolase [<i>B. mori</i>]
gi 148298746	Glucose-6-phosphate isomerase [<i>B. mori</i>]
Bmb008291, gi 112361467	Glutathione S-transferase 2 [<i>B. mori</i>]
Bmb006175, gi 109119903	Gluceraldehyde-3-phosphate dehydrogenase [<i>B. mori</i>]
gi 114051866	Isocitrate dehydrogenase [<i>B. mori</i>]
Bmb022329, gi 112983178	Juvenile hormone esterase [<i>B. mori</i>]
Bmb021298, gi 153792270	Malate dehydrogenase [<i>B. mori</i>]
Bmb024179, gi 114052408	Mitochondrial aldehyde dehydrogenase [<i>B. mori</i>]
Bmb032684, gi 151301209	NADPH-specific isocitrate dehydrogenase [<i>B. mori</i>]
Bmb033292, gi 114052472	Peptidyl prolyl isomerase B [<i>B. mori</i>]
gi 114052677	Phosphoserine aminotransferase [<i>B. mori</i>]
Bmb018693, gi 153791817	S-adenosyl-L-homocysteine hydrolase [<i>B. mori</i>]
Bmb022208, gi 112984224	Transfer RNA-Alaninynthetase [<i>B. mori</i>]
Bmb011628, gi 118500417	Vacuolar ATP synthase subunit B [<i>B. mori</i>]
Bmb019893, gi 114051702	Zinc-containing alcohol dehydrogenase [<i>B. mori</i>]

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

Bmb/gi number	Functional annotation
Bmb013008, gi 158631166	ADP/ATP translocase [<i>B. mori</i>]
Bmb010353	ADP-ribosylation factor [<i>B. mori</i>]
Bmb012754, gi 112982697	Antibacterial peptide, gloverin2 [<i>B. mori</i>]
Bmb013733, gi 110796922	Broad-complex isoform Z2/3 [<i>B. mori</i>]
gi 95102860	Cytochrome c oxidase polypeptide Vb [<i>B. mori</i>]
gi 145843755	GABA-gated chloride channel alpha subunit [<i>B. mori</i>]
Bmb021314, gi 157367283	Nicotinic acetylcholine receptor subunit beta 3 [<i>B. mori</i>], nicotinic acetylcholine receptor beta 2 subunit [<i>B. mori</i>]
Bmb006471, gi 114053251	Peroxisomal membrane protein PMP22 [<i>B. mori</i>]
Bmb031937, gi 112984376	Ribosomal protein L14 [<i>B. mori</i>]
Bmb028177, gi 148298732	Ribosomal protein S20 [<i>B. mori</i>]
Bmb005653, gi 112984058	Ribosomal protein S7 [<i>B. mori</i>]
Bmb028789, gi 148298654	Small nuclear ribonucleoprotein polypeptide [<i>B. mori</i>]
Bmb017849, gi 160333889	TFIIB-related factor [<i>B. mori</i>]
Bmb002691, gi 112982996	Thiol peroxidase [<i>B. mori</i>]
Bmb023533, gi 114052613	Transaldolase [<i>B. mori</i>]
gi 112983322	Transitional endoplasmic reticulum ATPase TER94 [<i>B. mori</i>]
Bmb004930, gi 112983481	Tubulin alpha chain [<i>B. mori</i>]

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

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

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.

We observed the protein expression profiles of the fat bodies in the larval, pupal and moth stages during metamorphosis of the silk worm 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 silk worms, 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 silk worm.

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