ORIGINAL ARTICLE

Bombyx mori

Received: 14 June 2009 / Accepted: 21 August 2009

Protein e pression pro les in the fat bodies of larval, pupal, and moth stages of silk orm ere determined using shotgun proteomics and MS sequencing. We identi ed 138, 217, and 86 proteins from the larval, pupal and moth stages, respectivel , of hich 12 ere shared b the 3 stages. There ere 92, 150, and 45 speci c proteins identi ed in the larval, pupal and moth stages, respectivel , of hich 17, 68, and 9 had functional annotations. Among the speci c proteins identi ed in moth fat bod , se -speci c storage-protein 1 precursor and chorion protein B8 ere unique to the moth stage, indicating that the moth stage fat bod is more important for adult se ual characteristics. Man ribosomal proteins (L23, L4, L5, P2, S10, S11, S15A and S3) ere found in pupal fat bodies, hereas onl three (L14, S20, and S7) and none ere identi ed in larval and moth fat bodies, respectivel . T ent -three metabolic en mes ere identi ed in the pupal stage, hile onl four and t o ere identi ed in the larval and moth stages, respectivel . In addition, an important protein, gloverin2, as onl identi ed in larval fat bodies. Gene ontolog (GO) anal sis of the proteins speci c to the three stages linked them to the cellular component, molecular function, and biological process categories. The most diverse GO functional classes ere involved b the relativel less speci c proteins identi ed in larva. GO anal sis of the proteins shared among the three stages sho ed that the pupa and moth stages shared the most similar protein functions in the fat bod .

Fat bod · Silk orm · Shotgun · Proteome · Metamorphosis

studies have indicated that different diet conditions can change the protein pro les in silk orm suggesting that the fat bod proteins can respond to e ternal stimuli (Zhou et al. 2008). The proteomic pro les of fat bodies throughout metamorphosis ill, therefore, allo the identi cation of ke proteins in the control of energ metabolism and pivotal intracellular signaling path a s that are involved in the metamorphic process.

Follo ing the completion of draft sequences of the genomes of several model organisms, including silk orm, proteomics has become the focal point in recent entomological research. As an effective tool for proteomics, shotgun proteomics, hich is based on the in-gel or gel-free digestion of protein mi tures follo ed b liquid chromatograph (LC) separation, MS detection and database searching, provides a highl sensitive and high throughput approach to determine the proteome components in a comple 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 stud , e utili ed the shotgun multidimensional Liquid Chromatograph LTQ-Orbitrap mass spectrometr (LC MS/MS) approach, combined ith bioinformatics anal sis to illuminate the differences among protein e pression pro les of the fat bodies in the larval, pupal, and moth stages of the silk orm and to nd valuable clues regarding energ metabolism and signaling mechanisms during metamorphosis of the silk orm.

Silk orm rearing and fat bod isolation

Silk orm strain P50 as reared on fresh mulberr leaves under an environment of 12 h light/12 h dark photoperiod, 26 1 C and 70 85% relative humidit . The developmental stages ere s nchroni ed at each molt b collecting ne larvae. On the fth da of the fth instar, the rst da of the pupal stage and the rst da of the moth stage, ten animals ere killed and their fat bodies ere collected. The tissue samples ere stored at −20 C for the further use.

Sample preparation and sodium dodec lsulfate-pol acr lamide gel electrophoresis (SDS-PAGE) separation

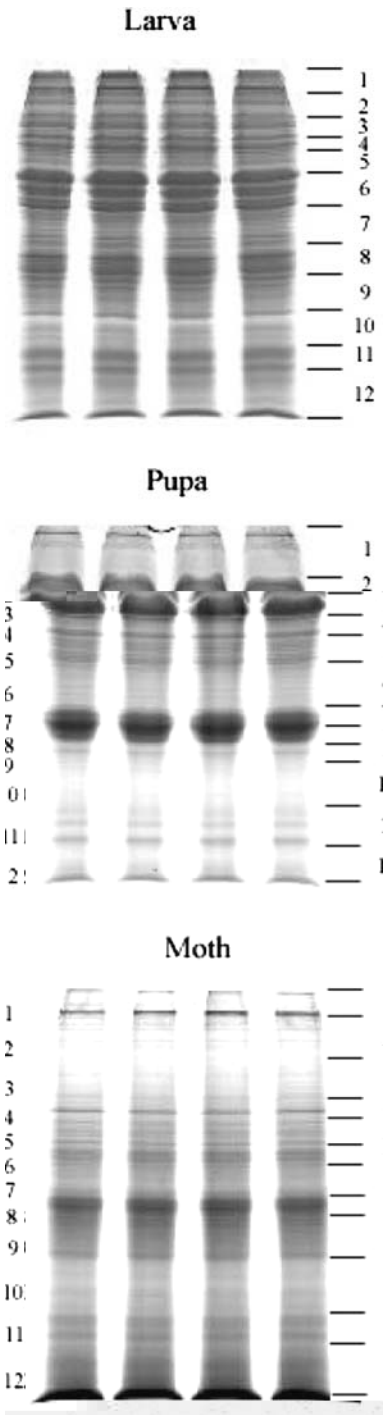
Fat bod tissue from the larva, pupa, and moth ere mechanicall homogeni ed on ice for 10 min in 10-µL l sis buffer [comprising 2.5% SDS, 10% gl cerol, 5% β-mercaptoethanol and 62.5 mM Tris HCl (pH 6.8)] per 1 mg tissue. The samples ere then sonicated in an ice-bath for 30 s and then ever 30 s, four times. The samples ere then centrifuged at 20,000×*g* at 25 C for 10 min. The supernatants ere then collected and ere centrifuged again and the resultant supernatants ere stored at −20 C for further use. The concentrations of protein samples ere determined using 2-D Quant Kit (Amersham Biosciences, USA). The samples ere 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 as separated using SDS-PAGE on four lanes. The gels ere stained ith Coomassie Brilliant Blue R250 (CBB, Sigma, USA) after electrophoresis.

In-gel digestion

The CBB-stained SDS-PAGE 4 gel lanes ere manuall cut into 12 slices, depending on the qualit of the protein bands (Fig. 1). Each slice as further sliced into 1 × 1 mm pieces and subjected to in-gel tr ptic digestion, as described previousl (Wilm et al. 1996; Li et al. 2009a). Brie , the proteins ere reduced ith 50 mM Tris[2-carbo eth l]phosphine (TCEP, Sigma) in 25 mM NH_4HCO_3 at 56 C for 1 h and alk lated ith 100 mM iodoacetamide (IAA, Amersham) in 25 mM NH_4HCO_3 at room temperature in the dark for 0.5 h. The digestion as performed ith 20 ng/µl porcine tr psin (modi ed proteomics grade, Sigma) overnight at 37 C.

Anal sis of shotgun LC MS/MS

All digested peptide mi tures ere separated b online reversed-phase (RP) nano LC using the Ettan MDLC nano o /capillar LC s stem (GE Healthcare, Pittsburgh, PA, USA) and anal- ed b a Thermo Finnigan Linear Orbitrap mass spectrometer (LTQ-Orbitrap MS) equipped ith an electrospra ioni ation (ESI) source (Pro eon Bios stems, Odense, Denmark). An e ternal standard solution ith *m/z* range from 195.00 to 1,922.00 as used to calibrate the mass spectrometer. Monoisotopic peak selection as applied. The +2 and +3 charge states ere selected for fragmentation and +1 and ≥+4 charge states ere e cluded. Samples ere automaticall injected into a 10 µl sample loop and delivered at 15 µl/min on the trapping column (Dione /LC Packings μ-Precolumn Cartridge P/N 160454 C18 PepMap 100, 5 µm, 100 A, 300 µm i.d., ×5 mm, Sunn vale, CA, USA) for desalting. After o - splitting do n to appro imatel 250 nl/min, peptides ere transferred to the anal tical column (Dione /LC Packings P/N 160321 150 × 0.075 mm i.d., C18 PepMap, 3 µm, 100A, Sunn vale, CA USA) and eluted using buffer B (95% ACN, 0.1% methanoic acid in ater) at a o rate of



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

appro imatel 250 nl/min ith 70 min gradients from 5 to 45% and 20 min gradients from 45 to 95%. The anal tical column as regenerated for 20 min ith 5% buffer A (5% ACN, 0.1% methanoic acid in ater) at 250 nl/min before loading the ne t sample. Data-dependent acquisition as performed on the LTQ-Orbitrap mass spectrometer in the positive ion mode. The temperature of the ion transfer tube as set at 200 C. The spra voltage as set at 1.8 kV and normali ed collision energ at 35% for MS2. The MS scan range as 300 2,000 *m/z* ith a resolution $R = 60{,}000$ at *m/z* 400. The MS anal sis as performed ith one full MS scan follo ed b ve MS/MS scans on the ve most intense ions from the MS spectrum ith the d namic e clusion for 180 s. The e periments ere repeated t ice and the results ere combined into the nal result.

Database search

Database search as carried out against the in-house database e previousl constructed (Li et al. 2009b) hich contains a total of 25,325 proteins including the sequences of the domesticated silk orm (*B. mori*) and ild silk orm (*Bombyx mandarina*). The ra MS/MS spectra ere interpreted b Bio ork 3.0 (ThermoFinnigan, San Jose, CA, USA) and the database searches ere performed ith SEQUEST algorithm, hich is a module of Bio ork 3.0 on a local server. The peptide mass tolerance as 10.0 ppm and the fragment ions tolerance as 1.0. The tr psin en me and partial en matic cleavage of the amino acids bonds at both ends of protein ere chosen. T o miss-cleavage sites ere allo ed. Onl *b* and *y* fragment ions ere taken into account. Fi ed modi cation (Carbo amidometh l) on c steine and variable modi cation (O idation) on methionine ere set.

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

Validation of the identi ed peptides and proteins as carried out according to the manual of the TPP soft are 3.4, hich as do nloaded from the ebsite (http://tools.proteomecenter.org/TPP.php) and installed ith the default options (Keller et al. 2002, 2005; Nesvi hskii et al. 2003). Validation of the identi ed proteins and peptides as carried using previousl described methods (Li et al. 2009b). Protein probabilit threshold for running ProteinProphet as set at 0.9.

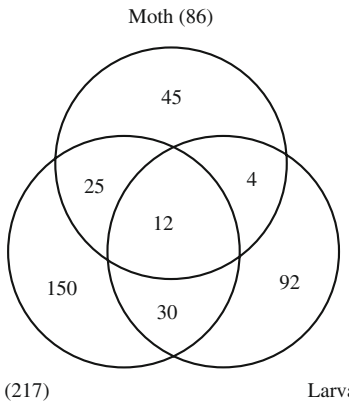
InterPro annotation and gene ontolog (GO) categories

InterProScan soft are as used to carr out protein sequence searches against the InterPro member databases to identif signatures (Zdobnov and Ap eiler 2001). The compiled RAW outputs ere subjected to GO categor anal sis using the Web Gene Ontolog 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/inde .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 proteol tic digestion of comple protein mi tures, peptides LC separation and tandem MS sequencing) ith searching against our in-house database, to obtain the protein e pression pro les 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, respectivel (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 lo est. There ere 12 common proteins, including 4 annotated proteins that ere shared b the 3 stages: calreticulin, H^+ transporting ATP s nthase 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.



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 uniquel 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 z es

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, respectivel), 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 -speci c storage-protein 1 precursor (SP1) is a se -related protein that can be e pressed in both se es of the silk orm, but is predominantl e pressed in females (Su uki et al. 2003). SP1 can also be transcribed ith high ef cienc 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 traordinar ph siological and mechanical properties (Iconomidou and Hamodrakas 2008). These t o proteins ere not identi ed

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 [*B. mori*]
gi 2696388	Histone H2b [*B. mori*]
Bmb012386, gi 114052589	Phosphate transport protein [*B. mori*]
Bmb037755, gi 112983366	Protein disul de isomerase like protein ERp57 [*B. mori*]
Bmb011627, gi 1335609	SP1, Se -speci c storage-protein 1 precursor (SP 1) (Methionine-rich storage protein) [*B. mori*]
Bmb018509, gi 107953774	Transport protein Sec61 alpha subunit [*B. mori*]
Bmb003848, gi 148298829	Vacuolar H + ATP s nthase 16 kDa proteolipid subunit [*B. mori*]
Bmb025429, gi 112984100	Yello 5 [*B. mori*], Yello -b [*B. mori*]

in the fat bodies of the larva or pupa, indicating that the fat bod in the moth is important to the se ual differentiation of the adult stage. Another important protein identi ed in the moth stage as ERp57, hich is a protein disul de isomerase-related pol peptide and is thought to catal- e the isomeri ation of non-native disul de bonds formed in gl coproteins ith unstructured disul de rich domains (Mishra et al. 2005). ERp57 belongs to the endoplasmic reticulum o idoreductases and its speci cit requires accessor factors like calreticulin (Jessop et al. 2009). The co-translocation of ERp57 and calreticulin determines the immunogenicit of cell death (Panaretakis et al. 2008). In the present stud , calreticulin and ERp57 ere identi ed in the fat bodies of the moth stage, suggesting that fat bod proteins might be involved in the immune function of the moth.

Compared ith the larval and moth stage, the speci c proteins in the fat bodies of the pupal stage ere more diverse (Table 2). Man ribosomal proteins (L23, L4, L5, P2, S10, S11, S15A and S3) ere found in the pupal fat bodies, hereas onl three (L14, S20, S7) ere identi ed in larvae and none in moth fat bodies. This abundance of ribosomal proteins in the pupal stage might be closel related to protein s nthesis in the pupal fat bodies. Furthermore, among the pupal-speci c proteins, 23 metabolic en mes ere identi ed (15% of pupal-speci c proteins), hereas onl 4 and 2 metabolic en mes ere speci c to the larval and moth stages, representing 4.3 and 4.4% of their speci c proteins. The metabolic en mes include 6-phosphogluconate deh drogenase, alcohol deh drogenase, carbo lesterase, Cu/Zn-supero ide dismutase, enolase, gl ceraldeh de-3-phosphate deh drogenase, phosphoserine aminotransferase, and- inc-containing alcohol deh - drogenase. The identi cation of these essential metabolic en mes indicated that during metamorphosis in the pupal stage the metabolism as most active.

The 30 K protein as also found in pupal fat bodies. The metabolism of 30 K proteins is a prerequisite for normal embr onic development (Zhong et al. 2005) and it might also be important for the fat bod characteristics at different developmental stages. The 30 K protein also has the role of inhibiting hemol mph apoptosis in the silk orm and it might also displa a similar function in pupal fat bodies (Kim et al. 2003). We also identi ed 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 e change of EF-1α bound GDP for GTP (Kamiie et al. 2002). Elongation factor 1 subunits ere onl identi ed in pupal fat bodies, suggesting that more active molecular changes ere happening at the pupal stage than at the larva and moth stages. Other important proteins identi ed in the pupal fat bodies ere heat shock cognate protein, pro ling protein, serpin-2, and serpin-5.

In the fat bodies of the larval stage, some ribosomal proteins, such as L14, S20 and S7, ere identi ed (Table 3), hich as much fe er than ribosomal proteins identi ed in pupal stage. The four metabolic en mes

Functional annotations of speci c proteins identi ed in the fat bodies of pupa

Bmb/gi number	Functional annotation
Bmb038426, gi 114051770	26S proteasome non-ATPase regulator subunit 13 [*B. mori*]
Bmb000199, gi 114050901	T rosine 3-monoo genase protein: eta pol peptide [*B. mori*]
gi 112983548	27 kDa gl coprotein precursor (P27 K) [*B. mori*]
Bmb019518, gi 10907	30 K protein [*B. mori*]
Bmb011479, gi 153791847	Abnormal ing disk-like protein [*B. mori*]
gi 112983501	Alpha-tubulin [*B. mori*]
Bmb017073, gi 10801568	Anne in IX-A,B,C [*B. mori*]
gi 112983471	Antich motr psin precursor [*B. mori*]
gi 112983770	Antitr psin precursor [*B. mori*]
Bmb013502, gi 124430725	Ar lphorin [*B. mori*]
Bmb030882, gi 112984452	Beta-tubulin [*B. mori*]
Bmb018730, gi 114053173	Bmp-2 [*B. mori*]
gi 112982685	Cadherin-like membrane protein [*B. mori*]
Bmb027017, gi 114050749	Chaperonin subunit 6a: eta [*B. mori*]
Bmb003962, gi 87248481	C tochrome b5 [*B. mori*]
Bmb009022, gi 112983898	Elongation factor 1 gamma [*B. mori*]
Bmb015183, gi 112984390	Elongation factor 1-alpha (EF-1-alpha) [*B. mori*]
Bmb004096, gi 112982932	Ferritin [*Bombyx mori*], Ferritin subunit precursor [*B. mori*],
Bmb016869, gi 114051243	FK506-binding protein [*B. mori*]
Bmb027040, gi 114053313	GTP binding protein [*B. mori*]
gi 112807176	H2A histone famil member V [*B. mori*]
Bmb009360, gi 112982828	Heat shock cognate protein [*B. mori*]
Bmb002839, gi 11120618	Heat shock protein hsp20.8 [*B. mori*], Heat shock protein hsp20.4 [*B. mori*]
Bmb034335, gi 1094393	Hemoc tin,Hemoc tin precursor (Humoral lectin), Humoral lectin prepropeptide [*B. mori*]
Bmb019659, gi 112983264	Lipophorin receptor [*B. mori*]
Bmb021419	Lo molecular 30 kDa lipoprotein PBMHPC-23 precursor
gi 112984340	P270 [*B. mori*]
Bmb005383, gi 114051003	Perilipin [*B. mori*]
gi 3721631	Pol pol protein [*B. mori*]
Bmb013674, gi 112982865	Pro lin [*B. mori*]
gi 112983210	Serpin-2 [*B. mori*]
Bmb010729, gi 112984548	Serpin-5 [*B. mori*]
Bmb011047, gi 112983262	Small GTP binding protein RAB5 [*B. mori*]
gi 114052645	Th mosin isoform 1 and 2 [*B. mori*]
gi 112983240	Transferrin [*B. mori*]
Bmb013675, gi 112983736	Translation initiation factor 4A [*B. mori*]
Bmb010905, gi 112983746	Vitellogenin precursor [*B. mori*]
Bmb014252, gi 112984274	Ribosomal protein L23 [*B. mori*]
Bmb020135, gi 112982800	Ribosomal protein L4 [*B. mori*]
Bmb013316, gi 112983276	Ribosomal protein L5 [*B. mori*]
Bmb010411, gi 112984336	Ribosomal protein P2 [*B. mori*]
gi 112983505	Ribosomal protein S10 [*B. mori*]
Bmb031584, gi 112982861	Ribosomal protein S11 [*B. mori*]
Bmb006919, gi 112982855	Ribosomal protein S15A [*B. mori*]
Bmb026411, gi 112984112	Ribosomal protein S3 [*B. mori*]
Bmb007278, gi 112983786	3-ketoac l-CoA thiolase [*B. mori*], Sterol carrier protein [*B. mori*]
Bmb022309, gi 114053253	6-phosphogluconate deh drogenase [*B. mori*]
Bmb021504, gi 146424692	Acetoacet l-CoA thiolase [*B. mori*]

continued

Bmb/gi number	Functional annotation
Bmb032144, gi 114052488	Alcohol deh drogenase [*B. mori*]
Bmb013826, gi 114052306	Carbo lesterase [*B. mori*]
Bmb008297, gi 112983576	Cathepsin D [*B. mori*], Aspartic protease [*B. mori*]
Bmb007516, gi 112982998	Cu/Zn-supero ide dismutase [*B. mandarina*]
Bmb022689, gi 114051239	C stathionine gamma-l ase [*B. mori*]
gi 119381542	Enolase [*B. mori*]
gi 148298746	Glucose-6-phosphate isomerase [*B. mori*]
Bmb008291, gi 112361467	Glutathione S-transferase 2 [*B. mori*]
Bmb006175, gi 109119903	Gl ceraldeh de-3-phosphate deh drogenase [*B. mori*]
gi 114051866	Isocitrate deh drogenase [*B. mori*]
Bmb022329, gi 112983178	Juvenile hormone esterase [*B. mori*]
Bmb021298, gi 153792270	Malate deh drogenase [*B. mori*]
Bmb024179, gi 114052408	Mitochondrial aldeh de deh drogenase [*B. mori*]
Bmb032684, gi 151301209	NADPH-speci c isocitrate deh drogenase [*B. mori*]
Bmb033292, gi 114052472	Peptid lprol l isomerase B [*B. mori*]
gi 114052677	Phosphoserine aminotransferase [*B. mori*]
Bmb018693, gi 153791817	*S*-adenos l-L-homoc steine h drolase [*B. mori*]
Bmb022208, gi 112984224	Transfer RNA-Ala s nthetase [*B. mori*]
Bmb011628, gi 118500417	Vacuolar ATP s nthase subunit B [*B. mori*]
Bmb019893, gi 114051702	Zinc-containing alcohol deh drogenase [*B. mori*]

Functional annotations of speci c proteins identi ed in the fat bodies of larva

Bmb/gi number	Functional annotation
Bmb013008, gi 158631166	ADP\ATP translocase [*B. mori*]
Bmb010353	ADP-ribos lation factor [*B. mori*]
Bmb012754, gi 112982697	Antibacterial peptide, gloverin2 [*B. mori*]
Bmb013733, gi 110796922	Broad-comple isoform Z2/3 [*B. mori*]
gi 95102860	C tochrome *c* o idase pol peptide Vb [*B. mori*]
gi 145843755	GABA-gated chlorine channel alpha subunit [*B. mori*]
Bmb021314, gi 157367283	Nicotinic acet lcholine receptor subunit beta 3 [*B. mori*], nicotinic acet lcholine receptor beta 2 subunit [*B. mori*]
Bmb006471, gi 114053251	Pero isomal membrane protein PMP22 [*B. mori*]
Bmb031937, gi 112984376	Ribosomal protein L14 [*B. mori*]
Bmb028177, gi 148298732	Ribosomal protein S20 [*B. mori*]
Bmb005653, gi 112984058	Ribosomal protein S7 [*B. mori*]
Bmb028789, gi 148298654	Small nuclear ribonucleoprotein pol peptide [*B. mori*]
Bmb017849, gi 160333889	TFIIB-related factor [*B. mori*]
Bmb002691, gi 112982996	Thiol pero iredo in [*B. mori*]
Bmb023533, gi 114052613	Transaldolase [*B. mori*]
gi 112983322	Transitional endoplasmic reticulum ATPase TER94 [*B. mori*]
Bmb004930, gi 112983481	Tubulin alpha chain [*B. mori*]

stages shared the most common functional class in the biological process categor . The differences in the functional classes among the three groups of proteins indicated that some of them ere unique to that stage. Some proteins onl identi ed in the larval stage ere involved in e tracellular region part, e tracellular matri part, membrane-enclosed lumen, s napse and s napse part classes in the cellular component categor ; and antio idant, molecular

transducer, and transcription regulator classes in the molecular function categor . Onl one class, motor in the molecular function categor , as unique to the pupal stage. Some proteins speci c to the moth stage ere involved in multicellular organismal process and reproduction classes in the biological process categor . From these results, e can see that, among the three stages, although more speci c proteins ere identi ed in the pupa, the most diverse GO

common proteins bet een the pupa and moth ere antio idant, electron carrier, biological regulation, pigmentation, and response to stimulus categories. There as onl one special functional class involving a common protein bet een the larva and pupa and the moth and larva, respectivel . The results sho ed that the pupa and moth shared the most similar protein functions in the fat bod .

We observed the protein e pression pro les of the fat bodies in the larval, pupal and moth stages during metamorphosis of the silk orm b shotgun proteomic anal sis. Speci c proteins identi ed in the three stages and common proteins shared b each pair of stages ere identi ed and anal- ed. Some interesting proteins ere identi ed. GO anal sis of these proteins also provided us ith a global vie of their functions. Our results indicated that during metamorphosis of domesticated silk orms, in the pupal stage, the fat bod might perform the most active metabolic process compared ith the larval and moth stage. These results ill also help further research on the functions of the fat bod proteins during metamorphosis of domesticated silk orm.

This ork as supported b the National Basic Research Program of China (Grant no. 2005CB121003), the National Hi-Tech Research and Development Program of China (Grant no. 2006AA10A118), the Doctoral Fund of Ministr of Education of China (Grant no. 20070335148), and b the Program for Ne Centur E cellent Talents of Universit .

Aggar al K, Silverman N (2008) Positive and negative regulation of the *Drosophila* immune response. BMB Rep 41:267 277

Baggerman G, Boonen K, Verle en P, De Loof A, Schoofs L (2005) Peptidomic anal sis of the larval *Drosophila melanogaster* central nervous s stem b t o-dimensional capillar liquid chromatograph quadrupole time-of- ight mass spectrometr . J Mass Spectrom 40:250 260

Beament JWL, Treherne JOE, Wiggles orth VB (1963) Advances in insect ph siolog . Academic Press, London, pp 163 165

Goldsmith MR, Shimada T, Abe H (2005) The genetics and genomics of the silk orm, *Bombyx mori*. Annu Rev Entomol 50:71 100

Goo TW, Park S, Jin BR, Yun EY, Kim I, Nho SK, Kang SW, K on OY (2005) Endoplasmic reticulum stress response of *Bombyx mori* calreticulin. Mol Biol Rep 32:133 139

Iconomidou VA, Hamodrakas SJ (2008) Natural protective am loids. Curr Protein Pept Sci 9:291 309

Jalali S, Aghasi M, Yeganeh B, Mesaeli N (2008) Calreticulin regulates insulin receptor e pression and its do nstream PI3 Kinase/Akt signalling path a . Biochim Bioph s Acta 1783:2344 2351

Jessop CE, Tavender TJ, Watkins RH, Chambers JE, Bulleid NJ (2009) Substrate speci cit of the o idoreductase ERp57 is determined primaril b its interaction ith calne in and calreticulin. J Biol Chem 284:2194 2202

Kalume DE, Okulate M, Zhong J, Redd R, Suresh S, Deshpande N, Kumar N, Pande AA (2005) Proteomic anal sis of salivar glands of female *Anopheles gambiae* mosquito. Proteomics 5:3765 3777

Kamiie K, Nomura Y, Koba ashi S, Taira H, Koba ashi K, Yamashita T, Kidou SI, Ejiri SI (2002) Cloning and e pression of Bomb mori silk gland elongation factor 1 γ in *Escherichia coli*. Biosci Biotechnol Biochem 66:558 565

Kaneko Y, Furuka a S, Tanaka H, Yamaka a M (2007) E pression of antimicrobial peptide genes encoding enbocin and gloverin isoforms in the silk orm, *Bombyx mori*. Biosci Biotechnol Biochem 71:2233 2241

Ka aoka S, Katsuma S, Daimon T, Isono R, Omuro N, Mita K, Shimada T (2008) Functional anal sis of four gloverin-like genes in the silk orm, *Bombyx mori*. Arch Insect Biochem Ph siol 67:87 96

Keller A, Nesvi hskii AI, Kolker E, Aebersold R (2002) Empirical statistical model to estimate the accurac of peptide identi cations made b MS/MS and database search. Anal Chem 74:5383 5392

Keller A, Eng J, Zhang N, Li XJ, Aebersold R (2005) A uniform proteomics MS/MS anal sis platform utili ing open XML le formats. Mol S st Biol 1:2005.0017

Kim EJ, Park HJ, Park TH (2003) Inhibition of apoptosis b recombinant 30 K protein originating from silk orm hemol mph. Biochem Bioph s Res Commun 29308:523 528

Li AQ, Popova-Butler A, Dean DH, Denlinger DL (2007) Proteomics of the esh brain reveals an abundance of upregulated heat shock proteins during pupal diapause. J Insect Ph siol 53:385 391

Li JY, Chen X, Hosseini Moghaddam SH, Chen M, Wei H, Zhong BX (2009a) Shotgun proteomics approach to characteri ing the embr onic proteome of the silk orm, *Bombyx mori*, at labrum appearance stage. Insect Mol Biol. doi:10.1111/j.1365-2583.2009.00903.

Li JY, Chen X, Fan W, Hosseini Moghaddam SH, Chen M, Zhou ZH, Yang HJ, Chen JE, Zhong BX (2009b) Proteomic and bioinformatic anal sis on endocrine organs of domesticated silk orm, *Bombyx mori* L. for a comprehensive understanding of their roles and relations. J Proteome Res 8:2620 2632

Mine E, Sakurai H, umi S, Tomino S (1995) The fat bod cell-free s stem for tissue-speci c transcription of plasma protein gene of *Bombyx mori*. Nucleic Acids Res 2523:2648 2653

Mishra S, Murph LC, N omba BL, Murph LJ (2005) Prohibitin: a potential target for ne therapeutics. Trends Mol Med 11:192 197

Nagaraju J, Goldsmith MR (2002) Silk orm genomics progress and prospects. Curr Sci 83:415 425

Nesvi hskii AI, Keller A, Kolker E, Aebersold R (2003) A statistical model for identif ing proteins b tandem mass spectrometr . Anal Chem 75:4646 4658

Panaretakis T, Jo a N, Modjtahedi N, Tesniere A, Vitale I, Durchschlag M, Fimia GM, Kepp O, Piacentini M, Froehlich KU, van Endert P, Zitvogel L, Madeo F, Kroemer G (2008) The co-translocation of ERp57 and calreticulin determines the immunogenicit of cell death. Cell Death Differ 15:1499 1509

Reumer A, Van Lo T, Cl nen E, Schoofs L (2008) Ho functional genomics and genetics complements insect endocrinolog . Gen Comp Endocrinol 155:22 30

Su uki MG, Funaguma S, Kanda T, Tamura T, Shimada T (2003) Anal sis of the biological functions of a doublese homologue in *Bombyx mori*. Dev Genes Evol 213:345 354

Tomita M, Munetsuna H, Sato T, Adachi T, Hino R, Ha ashi M, Shimi u K, Nakamura N, Tamura T, Yoshi ato K (2003) Transgenic silk orms produce recombinant human t pe III procollagen in cocoons. Nat Biotechnol 21:52 56

Wilm M, Shevchenko A, Houthaeve T, Breit S, Sch eigerer L, Fotsis T, Mann M (1996) Femtomole sequencing of proteins from pol acr lamide gels b nano-electrospra mass spectrometr . Nature 379:466 469

Ye J, Fang L, Zheng H, Zhang Y, Chen J, Zhang Z, Wang J, Li S, Li R, Bolund L, Wang J (2006) WEGO: a eb tool for plotting GO annotations. Nucleic Acids Res 34(Web Server issue):W293 W297

Zdobnov EM, Ap eiler R (2001) InterProScan an integration platform for the signature-recognition methods in InterPro. Bioinformatics 17:847 848

Zhong BX, Li JK, Lin JR, Liang JS, Su SK, Xu HS, Yan HY, Zhang PB, Fujii H (2005) Possible effect of 30 K proteins in embr onic development of silk orm *Bombyx mori*. Acta Biochim Bioph s Sin (Shanghai) 37:355 361

Zhou ZH, Yang HJ, Chen M, Lou CF, Zhang YZ, Chen KP, Wang Y, Yu ML, Yu F, Li JY, Zhong BX (2008) Comparative proteomic anal sis bet een the domesticated silk orm (*Bombyx mori*) reared on fresh mulberr leaves and on arti cial diet. J Proteome Res 7:5103 5111