Characterization and identification of the *Integrin* family in Silkworm, *Bombyx mori*

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Abstract

The silkworm, *Bombyx mori*, is an important economic insect as well as a model organism for lepidopteran insect. The *integrins* are evolutionarily conserved from sponges to humans and play vital roles in many physiological and pathological. To explore the diverse functions of the insect *integrins*, eleven *integrins* including six α and five subunits were first identified from silkworm. Phylogenetic analysis showed that gene duplication events occurred during evolutionary history of the silkworm, which makes greatly increased the numbers of the *integrins* compared to other invertebrates. The silkworm *integrin* α were clustered into three groups: PS1, PS2 and PS3. The β were mainly gathered in insect β and insect βν in invertebrates. However, β4 has a great difference in the sequences characteristics with other known insect *integrins* and was clustered into a novel phylogenetic branch. And expression profiles demonstrated that the *integrins* exhibits distinct patterns, but mainly expressed in hemocytes. α1 and β2 subunits were the predominant subunit both in the embryogenesis and larva stages. Five *integrins* specificly expressed in hemocyte with remarkably similar expression patterns. Interestingly, the *integrins* were significantly up-regulated by 20-hydroxyecdysone (20-E) in vivo. These results indicate that *integrins* play diverse function in hemocytes of silkworm. Overall, our results provide new insight into the function and evolutionary features of the *integrins*.

Keywords *Bombyx mori* • *integrin* family • evolutionary analysis • expression pattern

Introduction

Integrin is an important class of cell surface glycoprotein, which can mediate cell-to-cell and cell-to-extracellular matrix (ECM) interactions as well as transduce the bidirectional transmembrane signal through its unique signaling pathway[1]. The *integrins* are widely expressed in metazoans from sponges to humans and each is conserved composed of a large extracellular portion, a single transmembrane segment, and a short cytoplasmic domain (Except for β4, it owns a large cytoplasmic domain)[2]. In mammalians, there are 18 α and 8 β having been identified and they constitute at least 24 heterodimers[3]. They play crucial roles in many physiological and pathological processes including immune response, cell adhesion, migration,apoptosis, tissue organization and repair[4-8]. *Integrins* also widely express in cancer cells and contribute to cancer progression and metastasis by increasing cancer cell survival, proliferation, migration and invasion, moreover, several *integrins* are considered as potential targets for cancer therapy[9, 10].

In *Drosophila melanogaster*, essential roles of *integrins* in cell adhesion, migration, developmental, proliferation, apoptosis and innate immunity have been well studied[11-17]. *Drosophila integrins* are also important to anchoring the stem cell to their niche[18-20] and it is essential for intestinal stem cells maintenance and proliferation[20]. However, there are comparatively few works on the *integrins* of other insects, and the present limited research mainly

focus on the influence to insect cellular immunity. In *Manduca sexta*, α1, α2 and α3 play different roles in immune response and control different steps of celluar immunity [21]. Besides, β1 specificly expresses in plasmatocytes and is required for hemocytes encapsulation[22]. Three α and one β subunit have been identified from *Pseudoplusia includes*, and they are likely to regulate haemocyte adhesion during encapsulation[23, 24]. In *Ostrinia furnacalis*, β1 effects the spreading and encapsulation of plasmatocytes[25, 26]. RNA interference of β1 impairs the cellular immune response and larval development in *Spodoptera exigua*[27]. In *Anopheles gambiae* and *Ceratitis capitata* (medfly), β subunit also could regulate the bacterial phagocytosis[28, 29].

To extend the knowledge of the *integrin*s in insect, we have choosen the silkworm, *Bombyx mori*, which not only an economically important insect model for silk production, but also is an excellent model for fundamental research. In this study, we report the identification, characterization, expression analysis and functional study of the *integrins* in silkworm. Eleven *integrins*, including six α and five β subunits were identified in silkworm, and their gene phylogeny relationships, temporal and spatial expression profiles were investigated carefully, their responses to treatment of 20-ecdysone (20-E) were also surveyed.

Materials and methods

Biological materials

The Chinese silkworm strain Dazao (P50) was used in this study, maintained in State Key Laboratory of Silkworm Genome Biology. The larvae were feed with fresh mulberry leaves on an artificial diet under a temperature of 25°C, 60%-90% relative humidity and a 16 hours light/8 hours dark cycle. To obtain the expression profiles of the *integrin* in Silkworm, the sample from different embryonic stages and different tissues at day 3 of the fifth instar were isolated and stored in liquid nitrogen until use.

Identification of the *integrin* family in Silkworm

The *integrin* family was identified using a bioinformatic apprroach based on Silkworm genome. The databases of Silkworm including *Bombyx mori* 9× genomic sequencing database, *Bombyx mori* EST database, CDS database, and predicted protein database all from SilkDB (http://www.silkdb.org/silkdb/). The amino acid sequence of the *integrin* family genes from *H.Sapiens*, *D. Drosophila* and other insects were obtained from the GeneBank

(http://www.ncbi.nlm.nih.gov/). *Integrin* sequences from other species were used as queries to BLAST against the silkDB with an E-value threshold of 10^{-6}[30, 31]. Subsequently, each putative protein was futher validated by domain prediction using SMART (http://smart.embl-heidelberg.de/) and pfam(http://pfam.sanger.ac.uk/).

RNA extraction

Total RNA were extracted using TRIzol reagent (TaKaRa, China) basically according to the manufacturer's protocol. After digesting the residual genomic DNA using RNase-free DNase I (TaKaRa, China) for 30min at 37°C. First-strand cDNA was synthesized by using 2μg of total RNA in a 20μL reaction mixture using M-MLV Reverse Transcriptase (Promega, USA) according to the protocol provided by the manufacture and stored at -20°C.

Full-length cDNA cloning

According to the predicted CDS sequences and expressed sequence tags (EST) in the SilkDB(http://www.silkdb.org/silkdb/), primers were designed and the fragments of the *integrin* subunits were acquired by polymerase chain reaction (PCR). Subsequently, 3' and 5' RNA ligase-mediated rapid amplification of cDNA ends (RLM-RACE) were performed to obtain their full-length cDNA by using GeneRacer™ kit (Invitrogen) with the gene-specific primer. Finally, all of the open reading frames (ORF) deduced from the full-length cDNA sequences were confirmed by PCR. All PCR products were cloned into PMD19-T Simple vector (TaKaRa, China) and sequenced at invitrogen(Shanghai, China).

Bioinformatics and phylogeny analysis

The open reading frames (ORFs) of each *integrin* in Silkworm were determined with the ORF Finder software (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). The signal peptide predicted by SignalP 4.0 (http://www.cbs.dtu.dk/services/SignalP/). And the domain was predicted by using SMART (http://smart.embl-heidelberg.de/), Pfam (http://pfam.sanger.ac.uk/) and PROSITE (http://us.expasy.org/prosite). The deduced amino acid sequences of putative *integrins* were aligned using the ClustalX program, and two phylogentic trees on α and β subunit were constructed by the neighbor-joining method with 10,00 bootstrap

replicates using MEGA 4.0 program[32]. The sequences used for this analysis were shown in supplementary data Table S1.

RT-PCR

The semi-quantitative RT-PCR amplification was performed under the following condition: 94°C for 4 min, followed by 30 cycles of 94°C for 30s, 56°C for 30s and 72°C for 45s, finally, 72°C extension for 10min. Gene-specific primers for *integrins* used in this experiment are listed in supplementary data Table S3, and Bm*Actin3* gene was used as an internal control.

qRT-PCR

Quantitative Real-Time PCR (qRT-PCR) was performed with SYBR® *Premix Ex Taq*™ II (TaKaRa, China) with an StepOnePlus™ Real -Time PCR system (Applied Biosystems). The conditions for the PCR were 95°C for 30s, followed by 40 cycles of 95°C for 5s and 60°C for 30s. The primers for all genes used in this experiment are listed in supplementary data Table S4. The housekeeping sw22934 gene was used as indicator of the expression level of each gene and relative gene expression data was calculated with the $2^{-\Delta\Delta Ct}$ method[33]. Online t-test software Graphad Software (http://www.graphpad.com/quickcalcs/ttest1.cfm) was used to evaluate the statistical significance ($P<0.05$).

Injection of 20E

To investigate the effect of 20-hydroxyecdysone (20E) on *integrin* transcript levels, 1.5μg 20E (Sigma,USA) was injected to each larva on the second day of the 5th instar. 1×PBS containing corresponding amount of alcohol was used as a control. Then the silkworm were feed with fresh mulberry leaves under standard conditions. Hemocytes were collected 18 hours after the injection.

Results

Cloning and characterization of the *integrin* family

The completion of genome sequencing makes it possible to identify the *integrins* in the entire genome of silkworm, *Bombyx mori*[34-36]. The *integrins* from *H.Sapiens*, *D. Drosophila* and other insects were downloaded from NCBI as queries. Total of thirteen members, including eight α and five β subunits of the *integrin* family were identified through search of the silkworm genome

database (SilkDB) (Table.1). Complete cDNAs were acquired using PCR amplification and rapid-amplification of cDNA ends (RACE) , and then the results were confirmed through amplification of the complete ORFs. Finally, six α and five β members were obtained and confirmed. The results have been submitted to the Genebank and are available under the accession numbers given in table 1. Similar to other species, the *integrins* in silkworm consist of three domains, a large extracellular portion, a single transmembrane segment, and a short cytoplasmic domain[2].

Phylogenetic analysis

To analyze the relationships between silkworm *integrins* and other species, two phylogenetic trees were constructed by MEGA 4.0 using amino acid sequences from various species (Fig S1).

Hughes divided the *integrin* α subunits into four families based on evolutionary descent[4]. The I-DOM group, which have an "inserted" domain or "αI domain", not found in invertebrates (Fig 1). The PS1 and PS2 groups *integrins* have been found in various species, from invertebrates to vertebrates. In contrast, the PS3 group have only been found in insect so far[4, 24, 37] , which seems to be specific for insects[37]. The result showed that the six silkworm α *integrin* subunits clustered into PS1, PS2 and PS3 respectively (Fig 1). The silkworm α1 belongs to PS1 group, and it is related most closely to *P.includens* α1 (Identity: 76%) and *D.melanogaster* αPS1 (43%). α2 related most closely to *M.sexta* α2 (43%) and *D.melanogaster* αPS2 (24%), and clustered into PS2 group. α3, αPS1, αPS2 and αPS3 are all classified into PS3 group, which also contain *D.melanogaster* αPS3, αPS4 and αPS5. α3 is related most closely to *M.sexta* α3, they share 55% identity with each others. αPS1, αPS2 and αPS3 are related most closely to *M.sexta* α1, they share 35%, 33% and 23% identity with *M.sexta* α1, respectively. In the silkworm genome, four out of six α subunits were located on chromosome 10 (Table 1), interestingly, αPS1, αPS2 and αPS3 closely clustered on nscaf2855 with different transcriptional orientations (Fig 3).

The integrin β subunits can be classified into three major phylogenetic branches[4, 37-39]. The integrin β subunits from vertebrates were clustered into two groups, namely vertebrate A (including β1, β2, and β7 from human) and B (β3-β6 and β8 from human), the invertebrate sequences composed the third group, which seems to be specific for invertebrates [37, 38]. For this reason, forty-one different sequences of the integrin β subunits from invertebrate were used to

construct the phylogenetic tree in this study (Fig S1). The analysis showed that integrin β from insecta can be classified into three distinct groups in invertebrates (Fig 2). β1 is related most closed to *S.exigua* β1, and there are in turn related to other insecta β group subunits, which including the integrin β from coleoptera, lepidoptera, diptera and hymenoptera. Insecta βv group contain multiple integrin β subunits from silkworm, and the compositions of the βv group are as rich as β group. Two members (β2 and β3) from this group and β4 were all closely together on nscaf2847, which were located on chromosome 4 in silkworm genome (Fig 3). The third group only contain the integrin β subunits from coleopteran and hymenoptera. Unexpectedly, β4 from silkworm and βpat-3 separately cluster together, and are not belong to any one of the above three groups.

Expression profiles

The expression of the different *integrins* during embryogenesis stages were examined by RT-PCR (Fig 4). α1, α2 and β1 were highly expressed throughout development, the expression of α3 and β4 reached the peak from day 3 to day 7. The expression of β5 appeared to high at early stages and declined gradually thereafter. In contrast, other members were lowly expressed at early stages while expression levels increased gradually after day 7.

Microarray data from SilkDB database allowed us to analyze the tissue-expression profiles of the *integrins* in Silkworm[40]. All *integrins* have corresponding probes in the oligonucleotide chip (Table.1), and a heat map was created based on signal intensity value (Fig S2). Significantly, the majority of the *integrins* were mainly highly expressed in hemocytes compared to other tissues. β1 was widely expressed in various tissues on day 3 of the 5^{th} instar larvae.

The *integrins* expression profiles were verified by qRT-PCR in nine different tissues, including epidermis, head, testis, ovary, midgut, malpighian tube, silk gland, fat body and haemocyte, on day 3 of the 5^{th} instar larvae (Fig 5A-J). The results showed that almost all the members were highly expressed in hemocytes except α2 and β5. Besides, α1 and β2 were two dominant subunits in hemocytes (Fig 5L). Interestingly, five members (αPS1, αPS2, αPS 3, β2 and β3) were specifically expressed in hemocytes (We thought that the extremely low signal in head was caused by the pollution of small amount of hemocytes) (Fig 5D, E, F, H, I). α1, α2, β1 and β5 were also abundantly expressed in other tissues, but α3 had a low expression in several

organizations, such as fat body, head and ovary (Fig 5A, B, G, K).

Moreover, we also investigated the temporal expression patterns of the *integrins* in the hemocytes. Overall, the expression level of the *integrins* reached their lowest point at the wandering stage of the 4th instar, and then their expression level rised consistently. Interestingly, the hemocyte-specific *integrins* (αPS1, αPS2, αPS3, β2 and β3) have similar trends of expression (Fig 5D', E' , F', H', I'). α1 stayed at a high level with a small oscillation. The expression of α2 and β4 have remained at a relatively low level until the end of the 5th instar (Fig 5B', J'). And α3 and β1 transcript levels gone up steadily (Fig 5B', K').

Regulation of the *integrins*' expression by 20E

20-E was injected into the larvae to test whether ecdysone can regulate the expression of the integrins in hemocytes. The results showed that almost all the integrin members were significantly up-regulated by 20-E, except for α3 (Fig 6). α2 and β5 were not test because both of them were not or lowly expressed in hemocytes (Fig 5).

Discussion

The *integrins* are widely exist in metazoan, ranging from sponge to human. The *Integrins* are transmembrane receptors which function by forming heterodimers based on two distinct subunits. Actually, there are eighteen α and eight β subunits in *H.sapiens*, they constitute at least twenty-four heterodimers. The numbers may be fewer in lower organisms, but it is clear that there are at least two integrin αβ heterodimers since primitive bilateria[3]. *C.elegans* has two α and one β subunits, which compose two dimers. In *D.melanogaster*, there are five α subunits and three β subunits, and the βPS could form three different heterodimers by the combination of αPS1, αPS2, αPS3[41]. In our study, six α and five β subunits have been identified from silkworm genome (Table 1). The number more than other insects, the hypothesized reasons are: (1) There are relatively few studies on insect *integrins* (except *Drosophila*), additionally, the lack of genomic information makes it difficult to identify new members. (2) Gene duplication events occurred during evolution of silkworm. The *integrins* are necessary to form a heterodimer, which target cell membrane to perform functions. Thus, exploring combinations between α and β subunits will be required in the future.

The expression of the *integrins* shows obvious tissue specificity in lepidoptera. In *M.sexta* and *P.includens*,α1 and α3 are mainly expressed in hemocytes, α2 is highly expressed in fat body and lowly expressed in hemocytes, β1 is specificly expressed in hemocytes[21, 22, 24]. *O.furnacalis* β1 is mainly expressed in hemocytes[25]. In the present study, α1 is highly expressed in hemocytes, α2 showes very low expression level in hemocytes, α3 is mainly expressed in hemocytes, and β2 (homologue of β1 from *M.sexta*) specificly expressed in hemocytes (Fig 5). The results imply that these members may play conserved roles in lepidoptera.

Overall, according to their sequence feature and expression patterns (Fig 1 and Fig 5), six integrin α subunits from silkworm can be classified into three groups: PS1 (α1), PS2 (α2) and PS3 (α3, αPS1, αPS2 and αPS3). This suggested that integrin α subunits may play various functions in silkworm. α1 is widely expressed in various tissues containing hemocytes, which indicates that α1 may be relate to the development of multiple organs. α2 is probably an important subunit in regulating hemocytes adhesion[21, 24]. α3, αPS1, αPS2 and αPS3 were speculate linked to the development of hemocyte and the celluar immunity. Besides, duplicate events and analogous expression patterns of αPS1, αPS1 and αPS3 suggested that their functions are highly conserved in silkworm. The *integrin* β subunits can be classified into three groups: insect β (β1), insect βv (β2, β3 and β5) and β4 (Fig 2). β1 is likely to play diverse functions in various organs, and it may also have effects on the spreading of plasmatocytes[25]. β2 and β3 are related closely to β1 from *M.sexta*, it is may be essential for celluar innate immune response[22, 42]. β5 seems to be different with β2 and β3, and it is related closely to βv from *D.melanogaster*. Therefore, we speculates that it may be responsible for the clearance of apoptotic cells[17]. β4 and βpat-3 seem to form a novel phylogenetic branch (Fig 2.), the sequence and structure characteristics of β5 has a great difference with other integrins known in insect, as we know they may be related to BmNPV infection in *B.mori*[43]. Obviously, further surveys on identifying the functions of the *integrins* is required.

Integrin signaling is important for anchoring the stem cell to their respective niches[19] and they also play essential roles in intestinal stem cells maintenance and proliferation in *Drosophila*[20]. In our studies, majority of the *integrins* are highly or specificly expressed in hemocytes of the silkworm (Fig 5). These indicates that the *integrins* may have great influence

on regulating the hematopoiesis and hematopoietic stem cells, which had been identified in our previous studies[44]. Moreover, a part of *integrins* are specifically expressed in hemocytes, even in some single hemocyte lineage, for example, β1 in plasmatocytes of *M.sexta*[22], αPS3 in granulocytes and β2 in plasmatocytes of *B.mori* (Unpublished data). This property shows that integrin have the potential to be used as hemocyte or single hemocyte lineage label, which is an useful and urgently required tool in hematopoietic research[44].

In our study, eight *integrin* subunits were significantly induced by 20E (Fig 6). It has been reported that ecdysone is associated with hematopoiesis in silkworm, 20E may not only could control the hemocyte cell cycle event[45], but also stimulate hemocytes to be discharge from the hematopoietic organs in vitro[46]. It has not been reported that integrins can be regulated by ecdysone in hematopoietic system. Some published documents[47] and our preliminary experiments (data no shown) suggested that the density of hemocytes changed 12-18 hours after 20-E injection, and the *integrin* expression diversity peaked at 18 hours after injection. We speculate the *integrins* may paly a role in the development of hemocytes. However, it is not clear the *integrins* are directly or indirectly regulated by 20E, and the mechanisms remain unclear. Furthermore, Detection of the transcriptal level could not refulect real information sometimes, so more researches are required to identify the functions of the *integrins* in silkworm.

In summary, eleven *integrin* orthologs in silkworm have been been identified in our study. The sequence characteristics, express patterns were analyzed carefully. These results will provide fundamental knowledges and will be useful for further exploring the functions of the *integrins* in silkworm.

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References

1. Takada, Y., X. Ye, and S. Simon, *The integrins.* Genome Biol, 2007. **8**(5): p. 215.
2. Giancotti, F.G., *A structural view of integrin activation and signaling.* Developmental cell,

2003. **4**(2): p. 149-151.
3. Hynes, R.O., *Integrins: bidirectional, allosteric signaling machines.* Cell, 2002. **110**(6): p. 673-687.
4. Hughes, A.L., *Evolution of the integrin α and β protein families.* Journal of molecular evolution, 2001. **52**(1): p. 63-72.
5. Meredith, J.E. and M.A. Schwartz, *Integrins, adhesion and apoptosis.* Trends in cell biology, 1997. **7**(4): p. 146-150.
6. Hood, J.D. and D.A. Cheresh, *Role of integrins in cell invasion and migration.* Nature Reviews Cancer, 2002. **2**(2): p. 91-100.
7. Brooks, P.C., et al., *Integrin αvβ3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels.* Cell, 1994. **79**(7): p. 1157-1164.
8. Huttenlocher, A. and A.R. Horwitz, *Integrins in cell migration.* Cold Spring Harbor perspectives in biology, 2011. **3**(9).
9. Jin, H. and J. Varner, *Integrins: roles in cancer development and as treatment targets.* British Journal of Cancer, 2004. **90**(3): p. 561-565.
10. Desgrosellier, J.S. and D.A. Cheresh, *Integrins in cancer: biological implications and therapeutic opportunities.* Nature Reviews Cancer, 2010. **10**(1): p. 9-22.
11. Yee, G.H. and R.O. Hynes, *A novel, tissue-specific integrin subunit, beta nu, expressed in the midgut of Drosophila melanogaster.* Development, 1993. **118**(3): p. 845-858.
12. O'Reilly, A.M., H.-H. Lee, and M.A. Simon, *Integrins control the positioning and proliferation of follicle stem cells in the Drosophila ovary.* The Journal of cell biology, 2008. **182**(4): p. 801-815.
13. Dinkins, M.B., V.M. Fratto, and E.K. LeMosy, *Integrin alpha chains exhibit distinct temporal and spatial localization patterns in epithelial cells of the Drosophila ovary.* Developmental Dynamics, 2008. **237**(12): p. 3927-3939.
14. Bulgakova, N.A., B. Klapholz, and N.H. Brown, *Cell adhesion in< i> Drosophila</i>: versatility of cadherin and integrin complexes during development.* Current Opinion in Cell Biology, 2012.
15. McMahon, A., et al., *Mesoderm migration in Drosophila is a multi-step process requiring FGF signaling and integrin activity.* Development, 2010. **137**(13): p. 2167-2175.
16. Moreira, C.G., A. Jacinto, and S. Prag, *Drosophila integrin adhesion complexes are essential for hemocyte migration in vivo.* Biology open, 2013. **2**(8): p. 795-801.
17. Nagaosa, K., et al., *Integrin βv-mediated phagocytosis of apoptotic cells in Drosophila embryos.* Journal of Biological Chemistry, 2011. **286**(29): p. 25770-25777.
18. Van Doren, M., *Much HUBbub about stem-cell niches.* nature cell biology, 2007. **9**(12): p. 1344-1345.
19. Tanentzapf, G., et al., *Integrin-dependent anchoring of a stem-cell niche.* nature cell biology, 2007. **9**(12): p. 1413-1418.
20. Lin, G., et al., *Integrin signaling is required for maintenance and proliferation of intestinal stem cells in Drosophila.* Developmental biology, 2013.
21. Zhuang, S., et al., *Multiple α subunits of integrin are involved in cell-mediated responses of the Manduca immune system.* Developmental & Comparative Immunology, 2008. **32**(4): p. 365-379.
22. Levin, D.M., et al., *A hemocyte-specific integrin required for hemocytic encapsulation in the*

tobacco hornworm, Manduca sexta. Insect biochemistry and molecular biology, 2005. **35**(5): p. 369-380.

23. Pech, L.L. and M.R. Strand, *Encapsulation of foreign targets by hemocytes of the moth Pseudoplusia includens (Lepidoptera: Noctuidae) involves an RGD-dependent cell adhesion mechanism.* Journal of insect physiology, 1995. **41**(6): p. 481-488.
24. Lavine, M. and M. Strand, *Haemocytes from Pseudoplusia includens express multiple α and β integrin subunits.* Insect Molecular Biology, 2003. **12**(5): p. 441-452.
25. Hu, J., et al., *Integrin β1 subunit from Ostrinia furnacalis hemocytes: Molecular characterization, expression, and effects on the spreading of plasmatocytes.* Journal of Insect Physiology, 2010. **56**(12): p. 1846-1856.
26. Xu, Q., et al., *Ostrinia furnacalis integrin β1 may be involved in polymerization of actin to modulate spreading and encapsulation of plasmatocytes.* Developmental & Comparative Immunology, 2012. **37**(3): p. 438-445.
27. Surakasi, V.P., A.A. Mohamed, and Y. Kim, *RNA interference of β1 integrin subunit impairs development and immune responses of the beet armyworm, Spodoptera exigua.* Journal of Insect Physiology, 2011. **57**(11): p. 1537-1544.
28. Moita, L.F., et al., *Integrins of Anopheles gambiae and a putative role of a new β integrin, BINT2, in phagocytosis of E. coli.* Insect biochemistry and molecular biology, 2006. **36**(4): p. 282-290.
29. Mamali, I., et al., *A β integrin subunit regulates bacterial phagocytosis in medfly haemocytes.* Developmental & Comparative Immunology, 2009. **33**(7): p. 858-866.
30. Altschul, S.F., et al., *Gapped BLAST and PSI-BLAST: a new generation of protein database search programs.* Nucleic acids research, 1997. **25**(17): p. 3389-3402.
31. Duan, J., et al., *SilkDB v2. 0: a platform for silkworm (Bombyx mori) genome biology.* Nucleic acids research, 2010. **38**(suppl 1): p. D453-D456.
32. Tamura, K., et al., *MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0.* Molecular biology and evolution, 2007. **24**(8): p. 1596-1599.
33. Livak, K.J. and T.D. Schmittgen, *Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the* $2^{-\Delta\Delta CT}$ *Method.* methods, 2001. **25**(4): p. 402-408.
34. Xia, Q., et al., *A draft sequence for the genome of the domesticated silkworm (Bombyx mori).* Science, 2004. **306**(5703): p. 1937-1940.
35. Xia, Q., et al., *Complete resequencing of 40 genomes reveals domestication events and genes in silkworm (Bombyx).* Science, 2009. **326**(5951): p. 433-436.
36. Xia, Q., S. Li, and Q. Feng, *Advances in Silkworm Studies Accelerated by the Genome Sequencing of Bombyx mori**. Annual review of entomology, 2014. **59**: p. 513-536.
37. Johnson, M.S., et al., *Integrins during evolution: evolutionary trees and model organisms.* Biochimica et Biophysica Acta (BBA)-Biomembranes, 2009. **1788**(4): p. 779-789.
38. Huhtala, M., et al., *Integrin evolution: insights from ascidian and teleost fish genomes.* Matrix biology, 2005. **24**(2): p. 83-95.
39. Lowell, C.A. and T.N. Mayadas, *Overview: studying integrins in vivo*, in *Integrin and Cell Adhesion Molecules*. 2012, Springer. p. 369-397.
40. Xia, Q., et al., *Microarray-based gene expression profiles in multiple tissues of the domesticated silkworm, Bombyx mori.* Genome Biology, 2007. **8**(8): p. R162.
41. Brown, N.H., *Cell–cell adhesion via the ECM: integrin genetics in fly and worm.* Matrix

Biology, 2000. **19**(3): p. 191-201.
42. Zhuang, S., et al., *An integrin-tetraspanin interaction required for cellular innate immune responses of an insect, Manduca sexta.* Journal of Biological Chemistry, 2007. **282**(31): p. 22563-22572.
43. Sagisaka, A., et al., *Genome-wide analysis of host gene expression in the silkworm cells infected with Bombyx mori nucleopolyhedrovirus.* Virus research, 2010. **147**(2): p. 166-175.
44. Tan, J., et al., *Characterization of hemocytes proliferation in larval silkworm, Bombyx mori.* J Insect Physiol, 2013.
45. Kiuchi, T., F. Aoki, and M. Nagata, *Effects of high temperature on the hemocyte cell cycle in silkworm larvae.* Journal of insect physiology, 2008. **54**(2): p. 454-461.
46. Nakahara, Y., et al., *In vitro studies of hematopoiesis in the silkworm: cell proliferation in and hemocyte discharge from the hematopoietic organ.* Journal of insect physiology, 2003. **49**(10): p. 907-916.
47. Ling, E., et al., *Why does hemocyte density rise during the wandering stage of the silkworm, Bombyx mori?* Journal of insect biotechnology and sericology, 2003. **72**(2): p. 101-109.

Legend

Table 1. Summary of the *integrin* family identified in the silkworm genome.

Fig 1. The phylogenetic tree of the *integrin* α family based on the full-length amino acid by the neighbor-joining method. Boostrap values (1000 replications) of > 60% were showed. The silkworm α subunits are labeled with red diamonds.

Fig 2. A neighbor-joining tree for *integrin* β subfamily in invertebrates based on the full-length amino acid. The number closed to individual branches represents the percentage of 1000 bootstrap iterations supporting the branch, and values below 60% were omitted. The silkworm β subunits are labeled with red circles.

Fig 3. The genomic locations of the *integrins* in silkworm. The red boxes represent the integrin gene localization and transcriptional oritation were indicated by black arrows.

Fig 4. Developmental expression patterns of the *integrin* family during embryogenesis in silkworm. *Actin3* was used as an internal control.

Fig 5. Analysis the relative transcript abundance of the *integrin* family in different larval tissues on day 3 of the fifth instar larvae and the hemocytes from day 3 of 4th instar to the whole 5th instar of silkworm by qRT-PCR. Abbreviation: Ep, epidermis; He, head; Te, testis; Ov, ovary; Mi, midgut; Ma , Malpighian tube; Si, silk gland; Fa, fat body; Ha, haemocyte; L, larval; M, molting. (A-K) Relative transcript abundance of the *integrin*, temporal expression profile of β5 not detected because it not expressed in hemocytes. (L) Relative transcript abundance of the *integrin*

family in hemocytes. sw22934 was used as an internal control.

Fig 5. Relative transcript levels of the *integrins* in response to 20-E treated 18 hours in vivo. The corresponding amount of alcohol was used as a control. The differences between the experimental and the control groups were analyzed by the Student's t test, **P<0.01, ***P<0.001. All experiments were repeated at least three times.

Fig S1. Species, proteins and genbank accession numbers of *integrins* used in phylogenetic reconstructions.

Fig S2. Microarray analysis of the expression of the putative *integrin* family genes on day 3 of the fifth instar larvae of Silkworm, *Bombyx mori*. Gene expression levels are represented by Green (lower expresson) and red (Higher expression) boxes. The columns represent ten dfferent tissues, namely, hemocyte, midgut, head, testis, ovary, integument, malpighian tubule, fat body, anterior/median silk gland (A/MSG) and posterior silk gland (PSG).

Fig S3. Primer sequences, sizes of PCR production and melting temperature for semi-quantitative RT-PCR.

Fig S4. Primer sequences, sizes of PCR production and melting temperature for qRT-PCR.