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RESEARCH REPORT

Differentially expressed genes in the midguts of BmNPV-susceptible and resistant silkworm strains determined using suppression subtractive hybridization

L Gao[1], Y Yang[2], Q Yao[2], K Chen[2]*

[1]School of Food and Biological engineering, Jiangsu University, Zhenjiang, Jiangsu Province, China, 212013
[2]Institute of Life Sciences, Jiangsu University, Zhenjiang, Jiangsu Province, China, 212013

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Abstract

Bombyx mori nuclear polyhedrosis virus (BmNPV) has caused great economic losses to sericulture and considerable effort has been made to identify disease resistance genes in *B. mori*. We constructed differential expression gene libraries of the resistant near-isogenic line (NIL) BC10 and susceptible strain 306 using the suppression subtractive hybridization technique. A total of 23 differentially expressed genes were obtained, of which 17 genes were upregulated in BC10, and 6 genes were upregulated in 306. These differentially expressed genes are involved in cell metabolism, transmembrane transport, cytoskeleton, protease, development and immunity. Six BC10 upregulated genes were verified by real-time quantitative PCR, and the results were consistent with those of subtractive hybridization. The resistance trait of NIL BC10 was inherited from the resistant strain NB, and its genetic background was 99.99% similar to that of recurrent parent 306. The upregulated genes in NIL BC10 may be associated with silkworm BmNPV resistance.

Key Words: *Bombyx mori*, nuclear polyhedrosis virus, suppression subtractive hybridization, antivirus

Introduction

The *Bombyx mori* nuclear polyhedrosis virus disease is a common problem in silkworm breeding, and it causes great economic losses every year. Sericultural scientists have focused their research on the pathogenesis, transmission routes, and control of the disease. Screening for resistant genes is a key research area.

In nature, a few strains of silkworm have natural antiviral ability to BmNPV infection. After screening 344 Chinese local silkworm strains, Chen *et al*. (1991) obtained a highly resistant strain NB, whose median lethal concentration was 1000 times higher than the susceptible strain 306. Silkworm's resistance to NPV disease was controlled both by dominant major genes on autosomes and modificator genes on sex chromosome Z (Zafar *et al*., 2013). Liu *et al*. (2004) used 500 random primers to screen genomic DNA of resistant strain NB, susceptible strain 306 and the NIL BC8. The marker OPF-072023 was found to be linked to the disease resistance gene. The segregation ratio of NPV resistance molecular marker (R736) in BC1 and F2 populations was highly consistent with the resistant trait (Feng *et al*., 2012). These results will contribute to the location of resistant genes.

It is considered that the difference of mRNA and protein levels between resistant and susceptible strains is related to the mechanism of BmNPV resistance. Using fluorescence differential display PCR (FDD-PCR), a high expression gene BmSOP2 was screened from resistant strain NB (Xu *et al*., 2010). Using proteomic analysis, Liu *et al*. (2010) found that the expression of beta-N-glucosidase in hemolymph of NB and BC8 was upregulated after oral administration of BmNPV. The high expression of this gene may interfere with the glycosylation of the budded virion (BV) GP64 protein that is essential for initiating secondary infections (Okada *et al*., 2007). Using proteomics, Qin *et al*. (2012) found that the expression levels of Caspase 1 and Serine protease in F1 reciprocal hybrids and resistant parents NB were all higher than those in susceptible strain 306. Using Suppression Subtractive Hybridization, Bao *et al*. (2009) analyzed the differential gene expression in the midgut and fat body of the BmNPV-resistant strain KN and susceptible strain 306 at 12-hour post-BmNPV infection. They obtained a number of upregulated genes in resistant strain. Using cDNA microarray, Zhou *et al*. (2013) analyzed the differential expression of genes in the midgut of NIL BC8 and

Corresponding author:
Keping Chen
Institute of Life Sciences
Jiangsu University
Zhenjiang, 212013 Jiangsu Province, China
E-mail: kpchen@ujs.edu.cn

306 after BMNPV infection. Compared with 306, the expression of 10 genes in BC8 midgut was upregulated. Using genome wide microarray in resistant race (Sarupat) and susceptible race (CSR-2), Lekha *et al*. (2015) found large number of differentially expressed protein. Among them, Sugar transporters were down regulated upon BmNPV infection, and strongly associated with BmNPV infection (Lekha *et al*., 2016). By comparing the proteomes of infected and non-infected susceptible P50 and resistant BC9 silkworms, Yu *et al*. (2017) identified 84 differentially expressed proteins potentially involved in resistance to BmNPV. Similarly, the functions of these identified differentially expressed genes involve cytoskeleton, immune response, apoptosis, ubiquitination, transmembrane transport, protease and so on. All of these results have added to our understanding of molecular mechanism of silkworm antivirus.

Even so, the BmNPV resistance genes of the silkworm have not yet been completely identified. In addition to resistance genes, the genetic background of NIL BC10 is exactly the same as 306. The use of NIL BC10 facilitates screening of resistant genes. In this study, the suppression subtractive hybridization (SSH) method was used to construct a differentially expressed gene library of resistant strain NIL BC10 and susceptible strain 306, and the genes were screened in relation to disease resistance.

Materials and Methods

Silkworm strains and viruses

BmNPV-resistant NIL BC10 and BmNPV-susceptible silkworm strain 306 were used in this study. Chen Keping's method (2003) was used to select NIL BC10. After eight generations of backcrossing and two generation self-crossing, the NIL BC10 was developed, which has the anti-BmNPV characteristics of resistant strain NB. BmNPV (T3 strain) preserved in our laboratory was used in this study.

Oral exposure to the virus

BmNPV (5×10^6 polyhedra/larva) was administrated to the newly exuviated 5^{th} fifth instar larvae by oral inoculation. The treated silkworms were reared at standard temperature and under a photoperiod of 12 h of light and 12 h of dark.

Sample preparation and extraction of RNA

After oral administration, silkworm larvae were dissected at 0, 12, 24, and 48 hour postinfection (h pi). At each time, midgut samples from each one of 15 larvae were collected and pooled for each strain. And their midguts were cleaned with PBS and stored at -70 °C. RNA was extracted by Trizol reagent (Invitrogen, USA) and RNA was quantified on a spectrophotometer (Nanodrop, USA). RNA purity was tested on A260/280 and A260/230 and the integrity of the RNA was tested using an agarose gel.

Construction of an SSH library

At 24 h pi, the midgut RNA was extracted and the mRNA was purified from total RNA using PolyA Tract mRNA Isolation Systems (Promega, USA). A total of 2 μg mRNA was transcribed to cDNA using SMARTer™ PCR cDNA Synthesis Kit (Clontech, USA). Two subtractive cDNA libraries were constructed using the PCR-Select cDNA Subtraction Kit (Clontech, USA). The forward cDNA library used 306 cDNA as the driver and BC10 cDNA as the tester to enrich the high expression gene in BC10. The reverse cDNA library used BC10 cDNA as the driver and 306 cDNA as the tester, enriched the high expression genes in 306. The PCR products of the differentially expressed genes obtained by SSH were cloned into the pMD18T vector (Takara, Dalian, China) respectively, and the positive clones were screened by blue-white selection.

Table 1 Primers used in real-time qPCR for confirmation of differentially expressed genes

Gene name	Length	Primer (5'-3')
Ubiquitin binding enzyme E2	192 bp	gaatctaccgaaaacatgcaacac cagggtttggttccaagaataaat
Death-related protein kinase	236 bp	accgacgaaagttcctctctgt tttttaatgttggccccaattc
Trypsin-like serine protease	277 bp	ggccgtcatttacctacccagtcc agggccaccggagtcaccac
Actin-binding protein	183 bp	gcgcggatggatgtaatgcctaac gagcccgtcgtttgagttcgtt
Lectin	289 bp	aggccgttgtgatgtcgtgctct gcgcggatggatgtaatgcctaac
Uncharacterized protein	254 bp	ccccagttccactaacagagc ggtgagtttatgaaccgaagagt
Bm GAPDH*	256 bp	tgcccccatgtttgttgtg agtagaggcaggaatgatgttttg

*GAPDH: glyceraldehyde-3-phosphate dehydrogenase

Dot-blot hybridization and sequencing

98 clear positive clones were selected from the forward and reverse SSH libraries for PCR. The Equal amounts of PCR products were spotted on two nylon membranes at the same position after denaturation respectively. BC10 and 306 cDNA were separately labeled using Biotin Random Prime DNA Labeling Kit (Beyotime, China). After hybridization with the two different cDNA probes, analysis of hybridization signals variance on the two membranes was performed to identify differentially expressed genes. We used actin A3 and GAPDH as the internal control.

The positive clones were sequenced using an ABI 3100 DNA Analyzer (Applied Biosystems, USA). The nucleic acid sequences obtained were searched in the GenBank database and then the BLASTX similarity was compared.

Efficiency of suppression subtractive hybridization

0.5 µl of cDNA present in the subtracted and unsubtracted cDNA pools were respectively used as templates. The efficiency of subtractive hybridization was verified by PCR and quantitative PCR using the GAPDH specific primers. The recombinant plasmids (pMD18T-GAPDH) were constructed and used as the standard plasmid for the efficiency analysis. Using TE buffer, 10-fold serial dilution series from 1.0×10^7 copies/µl to 1.0×10^1 copies/µl were prepared from the standard plasmid. The standard curve was prepared by using an ABI 7500 fluorescence quantitative PCR instrument (Applied Biosystems, CA, USA). The abundances of GAPDH in two samples were quantified by standard curve method (Sebastião *et al*., 2015). All the reactions were performed in triplicates.

Real-time quantitative PCR

Six differentially expressed genes were selected and verified using relative quantitative real-time PCR. Specific primers for the candidate genes were designed from EST sequences (see Table 1). RNA samples were extracted from the midgut of the silkworm at 0, 12, 24 and 48 h pi. Quantitative real-time PCR was performed using ABI 7500 fluorescence quantitative PCR instrument based on SYBR Green fluorescent label. Each sample was tested in triplicate. GAPDH was used as a housekeeping control gene to normalize data among the samples. Expression levels were analyzed using the $2^{-\Delta\Delta Ct}$ method (Livak *et al*., 2001). Student's t test was conducted using SPSS 20.0. A significant difference was accepted at $P< 0.05$.

Results

Efficiency evaluation of the subtractive library

To verify the efficiency of suppression subtraction, we used PCR to compare the abundances of the GAPDH housekeeping gene in both the subtracted and the non-subtracted cDNA pools. The results show that the GAPDH gene was detectable in the 10th cycle of amplification in the unsubtracted cDNA, but was still not detectable in the 25th cycle of amplification in the subtracted cDNA (Fig. 1A). The number of GAPDH copies in unsubtracted cDNA was 9.2×10^4 copies/µl by quantitative PCR. After subtractive hybridization, the number of GAPDH copies in the subtracted cDNA was 6.3×10^2 copies/µl (Fig.1B). Both results indicate successful subtraction efficiency.

Dot blot hybridization analysis

A total of 98 clear positive clones were screened by blue white selection in the forward and reverse SSH libraries. After dot blot hybridization using forward and reverse cDNAs probes, most clones showed the variations in the hybridization signals on the two membranes. The results showed that 20 clones had strong positive signals when hybridized with the BC10 cDNA probe (Fig. 2A) compared to their weak or no signals with the 306 cDNA probe. On the contrary, 13 clones had strong positive signals when hybridized with the 306 cDNA probe (Fig. 2B). The positive clones verified by dot blot hybridization indicated that the differentially expressed genes were identified and could be sequenced for function analysis.

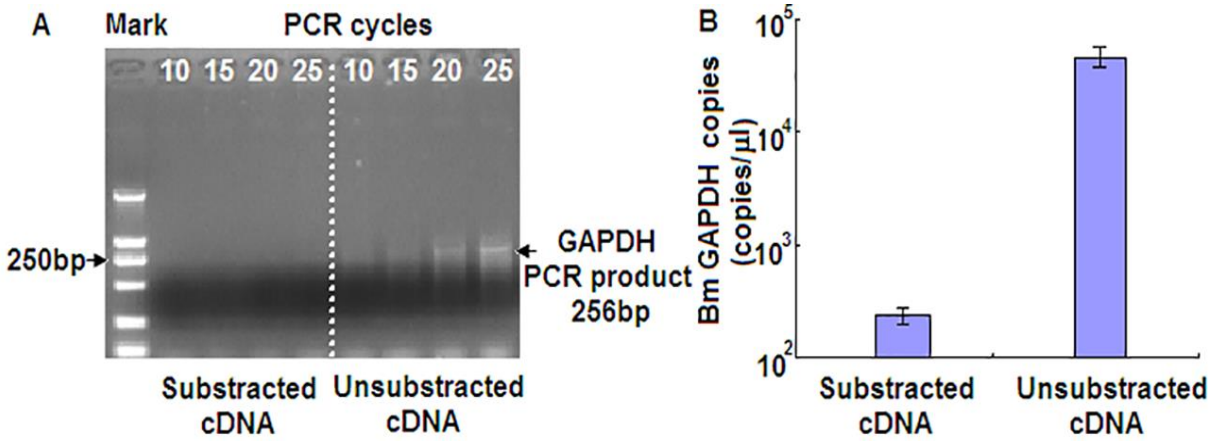

Fig. 1 Evaluation of the subtraction efficiency of subtracted cDNAs

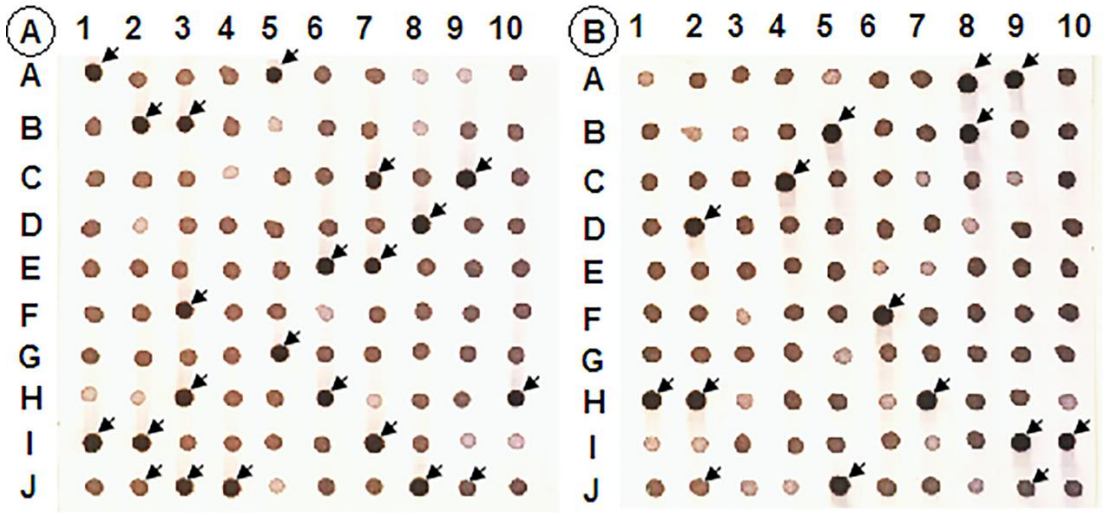

Fig. 2 Dot blot hybridization of EST clones from the forward and reverse SSH libraries. 98 colony PCR products from the forward and reverse SSH libraries were transferred to two nylon membranes A and B. A was hybridized with the Biotin-labeled cDNA probes from BC10. B was hybridized with the Biotin-labeled cDNA probes from 306. The arrows refer to clones with differential hybridization signals. The actin A3 (J2 point) and GAPDH (J9 point) of *B. mori* housekeeping gene were used as internal control

Sequencing and homology analysis

Based on above results, the 33 differentially expressed clones were sequenced by commercial sequencing services. All sequencing results were analyzed by using BLASTX to identify homologous sequences in the NCBI database sequences. By comparison, a total of 23 clones were matched homologous sequences, include 17 sequences from the forward cDNA library and 6 sequences from the reverse cDNA library respectively (Table 2). Of these, 21 clones were similar to silkworm genes, and 2 genes were similar to genes of other species. According to the annotation of gene function, differentially expressed genes were divided into eight main categories, including substance metabolism, development, transmembrane transport, apoptosis, cytoskeleton, protease and immunity. The distribution of gene function in the two subtractive libraries was different. In 306, the upregulated genes were mainly involved in protein metabolism, carbohydrate metabolism and transmembrane transport. The upregulated genes in BC10 were widely distributed and involved a variety of functions. In addition, two high expression genes were identified in BC10. At present, gene function is not yet clear (Fig. 3).

SSH results verified by real-time quantitative PCR

To verify the reliability of subtractive hybridization, we selected six genes from the forward subtractive library, including E2 ubiquitin ligase, long chain fatty acid CoA ligase, trypsin like serine protease, actin binding protein, sialic binding immunolectin, and an unknown functional gene. QPCR was performed to analyze expression patterns of these genes in the midgut. There was no significant difference in the expression pattern of these genes in 306 strain and NIL BC10 at 0 h pi. Following BmNPV infection, increased transcript levels were observed in the two silkworm strains. In susceptible 306 strain, the transcripts of these gene were consistently detected at low levels throughout the time course. However, the expression levels of these genes were consistently higher in the resistant NIL BC10 when compared to the susceptible 306 strain. Remarkably, the transcripts of an unknown functional gene were consistently detected at extremely low levels in 306. Conversely, the expression of this gene quickly increased and reached a peak in NIL BC10 at 24 h pi (Fig. 4). The results of quantitative PCR showed that the level of their expression in the resistant strain BC10 was significantly higher than that in 306, which verified the results of suppression subtractive hybridization ($P<0.01$).

Discussion

The resistance of silkworm strains to BmNPV is controlled by a dominant single gene. This has been verified by previous experiments (Feng *et al*., 2012). In Bao's subtractive experiments (2009), resistant strain KB and susceptible strain 306 were used for comparison. Due to the interference of other genetic traits, the differentially expressed genes may not be related to disease resistance. In order to eliminate the interference of other genes, we successfully bred 306 near isogenic lines through the 8 generation backcross and 2 generation selfing. Each generation was screened by exposure to 100% lethal dose BmNPV (2.5×10^8 polyhedral/larvae). In addition to resistance trait from NB, the genetic background of the NIL BC10 had 99.99% similarity to the recurrent parent 306. In theory, the difference in expression of

Table 2 Annotation of differentially expressed genes induced by BmNPV in the midgut from NIL BC8 and strain 306 isolated by SSH

SSH EST Number	Protein name	Strain	GenBank ID	E-Value
BC10 up-regulated gene				
SSH-1	Mitochondrial ribosomal protein L2	*Bombyx mori*	ABF71567.1	4.00E-77
SSH-2	Chromodomain helicase DNA binding protein	*Bombyx mori*	XP_004921642.1	1.00E-54
SSH-3	TATA binding protein	*Bombyx mori*	NP_001037059.1	4.00E-129
SSH-4	Reverse transcriptase	*Bombyx mori*	AAA17752.1	2.00E-43
SSH-5	Ubiquitin-conjugating enzyme E2M	*Bombyx mori*	NP_001040241.1	2.00E-89
SSH-6	Acyl-CoA dehydrogenase	*Aedes aegypti*	ABF18453.1	1.00E-167
SSH-7	Long-chain-fatty-acid-CoA ligase	*Bombyx mori*	XP_012550652.1	3.00E-59
SSH-8	Juvenile hormone binding protein	*Bombyx mori*	NP_001037074.2	2.00E-140
SSH-9	Juvenile hormone epoxide hydrolase	*Bombyx mori*	AAQ87024.1	5.00E-33
SSH-10	Serine carboxypeptidase	*Bombyx mori*	XP_004929002.1	5.00E-98
SSH-11	Trypsin-like serine protease	*Bombyx mori*	NP_001243950.1	3.00E-95
SSH-12	TPR repeat-containing protein	*Bombyx mori*	XP_004928023.1	4.00E-16
SSH-13	Actin-binding protein	*Bombyx mori*	XP_004922082.1	1.00E-08
SSH-14	Death-associated protein kinase	*Bombyx mori*	NP_001189466.1	1.00E-14
SSH-15	Sialic acid binding Ig-like lectin	*Homo sapiens*	EAX10517.1	4.00E-13
SSH-16	Uncharacterized protein	*Bombyx mori*	NP_001139709.1	1.00E-86
SSH-17	Uncharacterized protein	*Bombyx mori*	NP_001153678.1	3.00E-81
306 up-regulated gene				
SSH-18	Translation elongation factor 2	*Bombyx mori*	ABF71565.1	2.00E-54
SSH-19	60S Ribosomal protein L18	*Bombyx mori*	NP_001037217.1	8.00E-95
SSH-20	Ribosomal protein L13	*Bombyx mori*	NP_001037153.1	4.00E-117
SSH-21	Alpha-glucosidase	*Bombyx mori*	XP_004925073.2	4.00E-16
SSH-22	Ubiquinol-cytochrome c reductase complex 14 kDa protein	*Bombyx mori*	NP_001038957.1	2.00E-40
SSH-23	Aminopeptidase N	*Bombyx mori*	XP_012551671.1	0

mRNA between BC10 and 306 is correlated with BmNPV resistance. This could provide a basis for screening resistance-related genes.

The silkworm midgut is the frontline where silkworm initiates its defense against BmNPV infection. In this study, 23 differentially expressed genes were obtained from midgut cells. Of these, 17 genes were up-regulated in BC10. The other 6 genes were up regulated in 306, which were related to susceptibility of silkworm virus disease. The six genes selected for real-time quantitative analysis were BC10 up-regulated genes with significant difference in expression. According to the results of other whole genome gene expression studies, the differentially expressed genes mainly focus on the functions of cytoskeleton, immune response, apoptosis, ubiquitination, transmembrane transport, protease and so on. Of the six genes, 5 genes belong to these functions. Their differential expression might account for the resistance of *B. mori*.

The expression level of the ubiquitin-binding enzyme E2 in BC10 was higher than that in 306. Ubiquitin binding enzyme E2 is an important component of the ubiquitin proteasome system (UPS), which combines with ubiquitin ligase E3 for ubiquitination of target proteins and participates in the degradation of endogenous proteins (Ciechanover *et al.*, 2015). UPS can selectively degrade viral proteins and limit viral growth in host cells (Wang *et al.*, 2007). In addition, the UPS plays an important role in the regulation of signaling pathway during viral infection. It is generally accepted that the NF-κB pathway plays a critical role

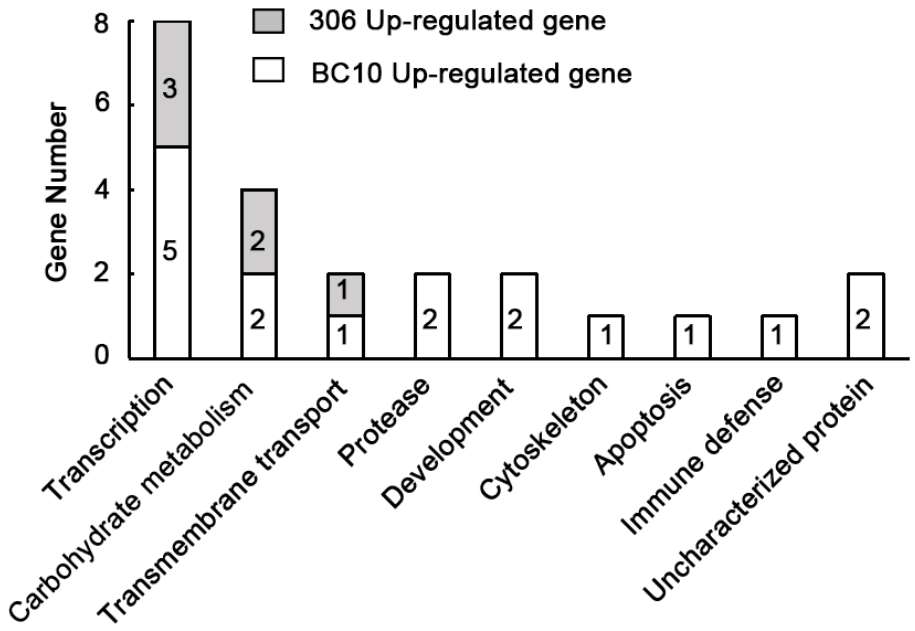

Fig. 3 Functional classification of differentially expressed genes identified in two subtractive libraries

in host anti-viral defenses. BmNPV invaded the midgut of the silkworm and stimulated NF-κB signaling pathway by interacting with the cell surface pattern recognition receptor. The activation of transcription factor NF-κB regulates downstream immune active substances, such as antimicrobial peptides and defensins (Yokoi *et al*., 2012). In this process, the ubiquitination of NF-κB inhibitor (IκB) can abolish the inhibition of NF-κB nuclear translocation (Kim *et al*., 2014). However, some viruses can also develop deubiquitination strategies to evade the innate immunity of the host. For example, ubiquitin-specific protease encoded by Herpes simplex virus deubiquitinates IκBα and limits activation of NF-κB (Ye *et al*., 2016). So, ubiquitination of proteins is an important mechanism used by insects to regulate immune responses.

Apoptosis is an important mechanism used by the Lepidoptera to defend against baculovirus infection. By initiating apoptosis, the host insect actively scavenges infected cells, and this improves its ability to resist viral infection. Apoptosis occurs through the activation of caspase through the apoptosis information pathway (Wang *et al*., 2016). We have used proteomics to demonstrate that caspase-1 was highly expressed in the midgut of BmNPV-resistant silkworm larvae (Qin *et al*., 2012). In the present study, we found high expression of the death-related protein kinase in the midgut of BC10. Death-related protein kinase is a positive regulator of apoptosis and plays an important role in the apoptosis pathway induced by p19ARF/p53. Phosphorylated p19ARF triggers the apoptosis pathway of p53 and induces the expression of specific apoptotic genes downstream, such as Bax and APAF (Kögel *et al*., 2003). In addition, death-related proteins kinase can also interact with the microtubule protein MAP1B without relying on the p53 mechanism to induce cell autolysis (Isshiki *et al*., 2015). Death-related protein kinase appears to be the upstream regulatory proteins of the apoptosis activation pathway.

Fibrous actin is the main component of the eukaryotic cytoskeleton. Baculovirus infection of Lepidoptera host cells requires the involvement of actin. After baculovirus enters the host cell, the actin of the host cell is aggregated to participate in the transport of the nucleocapsid from the cytoplasm to the nucleus (Wang *et al*., 2007). The migration of actin from the cytoplasm to the nucleus is essential for viral replication and nucleocapsid assembly (Newsome *et al*., 2015). Cytochalasin D and microfilament depolymerizing agent A can bind actin to inhibit actin polymerization. In this case, the baculovirus does not produce progeny in the host cell (Kasman *et al*., 2000). Actin-binding protein can regulate the function of fibrous actin and inhibit the polymerization of fibrous actin. In this study, we found that the expression of actin-binding protein in BC10 was higher than that in 306. Upregulation of actin-binding protein expression may alter the formation of F actin in cells, thereby affecting the transport and replication of baculovirus in the host cells.

Trypsin-like serine protease belongs to the serine protease family, and it is an important digestive enzyme in invertebrate digestive tract (Wu *et al*., 2005). Besides its digestive function, trypsin-like serine protease also participates in the activation of phenoloxidase-associated reaction (Liu *et al*., 2017) and in the activation of the antiseptic defensin precursor invertase (Hamilton *et al*., 2010). The expression of serine protease in the BmNPV-resistant strain NB was higher than that of

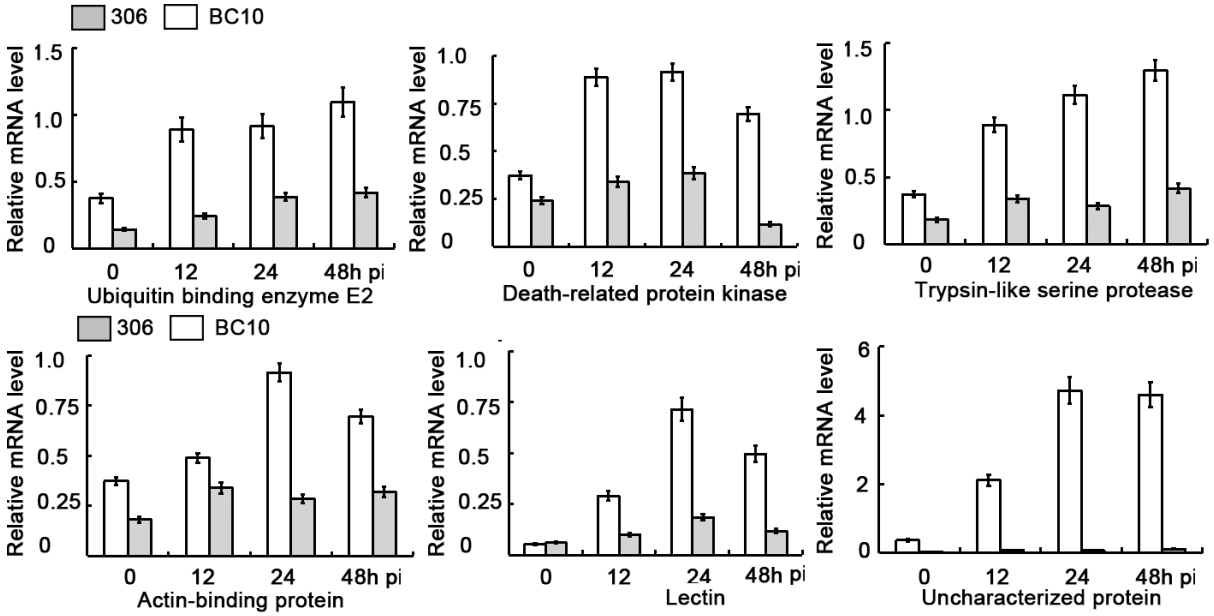

Fig. 4 Analysis of mRNA expression by real-time qPCR. Six genes were randomly selected for RT-qPCR verification of the SSH library. The relative expression levels of 6 genes at different time points were normalized to Bm GAPDH, the endogenous control. All samples were tested in triplicate. Results are expressed as means ± SD

strain 306 at the protein level (Qin *et al*., 2012). In Bao's study (2009), the expression of serine protease in midgut cells of resistant strain was also confirmed to be higher than that in 306. These results showed that serine protease was indeed involved in silkworm immune defense against BmNPV infection. A possible mechanism is that serine protease inactivated the occlusion-derived virus (ODV) at the initial infection site of the silkworm gut and prevented the ODV from adhering to the midgut epithelial cells and then spreading to the hemolymph (Nakazawa *et al*., 2004).

Lectin was highly expressed in NIL BC10. Two kinds of silkworm agglutinin have been identified, BmLBP and BmIML (Kim *et al*., 2003). They belong to the C-lectin family. There are two glycosyl recognition domains in the molecular structure, which have strong adsorption capacity for bacteria and nematode surface lipid polysaccharides (Zhou *et al*., 2017). *In vitro* experiments showed that the combination of recombinant silkworm agglutinin and inactivated *Micrococcus luteus* cells could cause their aggregation in the blood cells of the silkworm (Watanabe *et al*., 2006). Recombinant Immulectin were injected into the hemocoel of the fifth instar *Manduca sexta* larvae and triggered encapsulation and melanization responses (Yu *et al*., 2004). There are many genes similar to agglutinin in the silkworm genome. The newly discovered lectin proteins are likely to be involved in the early recognition of silkworm infection and activate the humoral immunity.

We found two unidentified genes that were highly expressed in NIL BC10. In susceptible strain 306, gene expression remained at a low level, which was 40 times different from that of BC10. Through homology analysis, the sequence contains the conservative domain of the transcription-activating factor (MBF-2). MBF-2 stabilizes the binding of the DNA-binding factor (Ftz-F1) to DNA and coregulates the transcription of the Ftz-F1-dependent gene with Ftz-F1 (Liu *et al*., 2000). FTZ-F1 is a nuclear receptor type transcription factor, which trigger gene expression and regulate molting of larvae. In *Drosophila melanogaster*, loss-of-function mutation in DmFTZ-F1 caused severe ecdysis failure (Cho *et al*., 2014). The enzyme encoded by the baculovirus *egt* gene result in the inactivation of molting hormone, which leads to the disruption of larval molting and pupation and facilitates the proliferation of the viral progeny (Zhang *et al*., 2012). Previous reports documented that sloughing of infected midgut cells prevented the virus from spreading beyond the midgut (Washburn *et al*., 1998). The highly expressed MBF-2 in NIL BC10 increased sensitivity to ecdysones and promoted intestinal developmental resistance against lethal baculovirus infection.

In susceptible strain 306, the upregulated genes were mainly involved in protein metabolism, energy metabolism, and transmembrane transport. These functions may be related to the susceptibility of silkworm to viruses. In previous study, SF21 cells were infected by recombinant baculovirus expressing vesicular stomatitis virus RNA dependent RNA polymerase. The results showed that the host SF21 cell translation elongation factor could be tightly bound to the expressed protein subunit (Das *et al*., 1998). The P6 protein of the cauliflower mosaic virus interacts with the host ribosomal proteins L13, L18, L24 and initial factor eIF3, which initiate the transcription and translation of the virus gene (Bureau *et al*., 2004). Aminopeptidase N (APN) is a transmembrane glycoprotein abundant on the

apical membrane of intestinal epithelial cells. Besides the functions of peptide metabolism, cell movement and adhesion, APN is also the receptor of the virus. In silkworm, there are few studies on the role of APN in NPV infection. In pig, aminopeptidase N (pAPN) can promote the infection of porcine epidemic diarrhea virus (PEDV) to host cells (Shirato *et al*., 2016). Overexpression of pAPN in mice confers its susceptibility to PEDV infection (Park et al., 2015). These studies indicate that BMNPV can completely embezzle proteins from host cells for viral entry and translation of their own components.

The resistant strain NIL BC10 is highly similar to the susceptible strain 306 in genetic background, but there are differences in BmNPV resistance traits. Using them as analysis materials for gene expression difference analysis can eliminate the interference of other trait genes and isolate the resistant genes more efficiently. The differentially expressed genes screened in this study will help clarify the resistance mechanism of *B. mori* to BMNPV infection.

Acknowledgments

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