Antiviral Mechanism of Serine Protease in Various Insects

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Abstract

During the last two decades, insects have had the self-ability to develop a vigorous and potent immune system that contests a huge diversity of pathogens and lead them to grow into the most distinct and efficient organisms in the world. Immune reactions against pathogens are basically characterized by invasion of their cellular and humoral response. In the present era of challenging environmental conditions, there is an urgent need for the prevention and control of viral diseases. Serine proteases (SPs) are a vast group of proteolytic enzymes that play an enormous role in anatomical systems (cell signaling, defenses, and movement, etc.); thus, they are crucial to the antiviral mechanism (hemolymph coagulation, activation of antimicrobial peptide, and melanin synthesis). They participate in various biochemical and physiochemical pathways and act as catalysts that break down the peptide bond in the protein. SPs are vital to numerous microorganisms and contribute to several structural and biochemical concerns, including a conserved catalytic triad (Ser, Asp, and His) that enacts the fundamental principle for the classification of a protein. SPs have diverse functions and play a vital role in cellular differentiation, digestion, complement activation, the immune response, and hemostasis. Recently, immunological responses in many insects such as *Bombyx mori*, Drosophila, Anopheles, etc., are maintained by circulatory hemocytes and performed a significant role in innate immune system, namely, the synthesis of antimicrobial proteins, encapsulation, and phenoloxidase. Most of the antimicrobial proteins such as cecropins, attacins, lebocin, moricin, gloverins, lysozyme, defensins, hemolin, etc., are effectively engaged in defense reactions against invading pathogens. For antiviral mechanisms, molecular and cell target-based analysis are valuable studies for identifying the genome

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and expression analysis of SPs, and their homologs in the silkworm, *Drosophila melanogaster*, *Apis mellifera*, and *Anopheles gambiae*, are generally considered to be model organisms for providing the relevant information regarding such biological functions. In this chapter, we devote our endeavors to the antiviral mechanism of SPs in various insects and critique the recent data on visualizing the role of antiviral pathways. Furthermore, the antiviral pathways may encounter the infectious virus towards the systemic and specific level.

8.1 Introduction

Insect resistance plays a relevant role in the synergism within the host–pathogen relationship, as a part of a survival strategy, along with physical impediments such as epithelial barriers, cuticle and peritrophic matrix, protease cascades leading to coagulation and melanization, and fundamental cellular responses (Choo et al. 2010; Feng et al. 2013; Wang et al. 2016; Lavin and Strand 2002; Lehane et al. 2004; Kotani et al. 1999). For many decades, the defense systems of insects, in contrast to various pathogens such as bacteria, fungus, and protozoa, have been well recognized, but articles on antiviral mechanism are scarce and surprising owing to a lack of understanding of the mechanism of virus invasion and host–virus response (Popham et al. 2004). In an earlier report, insect baculoviruses were accomplished in recombinant protein expression systems, but there are virtually no studies including the antiviral mechanisms against viruses. Thus, there is specific consideration of *Bombyx mori*, which is attracted to the area to regulate the gene and protein and to balance expression in genetically modified cell lines (Popham et al. 2004). Serine proteases (SPs) act as hydrolytic enzymes that are incorporated into the conserved catalogue of triad residue (His, Ser, and Asp) and are routinely integrated as idle zymogen with propeptide, which must be evacuated for their activation (Ross et al. 2003). Most studies have delineated that insect SPs perform a fundamental aspect of dietary protein digestion (Herrero et al. 2005; Soares et al. 2011), molting (Wei et al. 2007; Liu et al. 2009; He et al. 2009), metamorphosis (Danielli et al. 2000; Kaji et al. 2009), and the immune response (An et al. 2009), whereas SP homologs(SPHs) are identical to SPs in amino acid sequences; nevertheless, there is a scarcity in amidase activity because of mutation (Romualdi et al. 2003). In various reports, *Anopheles gambiae*, *Drosophila melanogaster* and *Bombyx mori*, and *Bombus ignitus* contain 305, 206, and 143 SP or SPH genes, 1,720 bp respectively (Choi et al. 2006; Romualdi et al. 2003; Zdobnov et al. 2002; Zhao et al. 2010) and are categorized into different families (Table 8.1). Insects have the ability to develop mechanisms that resist various pathogens, including viruses (Qin et al. 2012). Choo et al. (2010) investigated the bee venom SPs (Bi-VSPs), which promote the arthropods prophenoloxidase (proPO)-activating factors (PPAFs) via a melanization process and illustrated fibrin(ogen)

Table 8.1 List of gene number of serine proteases and serine protease homolog gene in different insects (adopted from Zhao et al. 2010)

Family	*D. melanogaster*	*A. gambiae*	*A. mellifera*	*B. mori*
SP_fam1	65	49	28	24
SP_fam2	12	37	4	9
SP_fam3	18	22	2	6
SP_fam4	17	19	4	5
SP_fam5	2	29	–	4
SP_fam6	9	9	1	2
SP_fam7	3	4	2	1
SP_fam8	–	1	0	5
SP_fam9	1	1	1	2
SP_fam10	1	1	1	1
SP_fam11	1	1	1	1
SP_fam12	–	49	28	15
SP_fam13	–	37	4	7
SP_fam14	–	22	2	6
SP_fam15	–	19	4	5
SP_fam16	–	29	–	3
SP_fam17	–	9	1	3

activity by eradication of insects. Furthermore, they reported that SP constitutes of multifunctional enzymes that stimulate prothrombin without any deviation, degenerating the fibrinogen into fibrin-like products. In this chapter, we emphasize the immune response against viral infection and how to consider the aspects of SP via different mechanisms.

For 300 billion years, *Bombyx mori* has represented great economic value from an industrial and a pharmaceutical and medicinal point of view. In the current scenario, there are major economic resources found in various countries, e.g., India, China, Vietnam, and Thailand. (Qin et al. 2012) and are used as bioreactors to produce clinically significant biomolecules such as human granulocyte macrophage colony-stimulating factor (Xia et al. 2004; Chen et al. 2006). Certain immune mechanisms also exist in *Bombyx mori* and they are susceptible to *B. mori* nuclear polyhedrosis virus virus (BmNPV) infection (Qin et al. 2012). Various researchers have also reported that BmNPV acts as a primary pathogen of the domestic silkworm and induces large commercial losses (Cheng et al. 2014; Miao et al. 2005). Most silkworm strains are highly sensitive to BmNPV, but rarely show high resistance to BmNPV (Bao et al. 2009). Recently, some studies on insect resistance to BmNPV, i.e., *Bombyx mori* SP-2, lipase-1, and alkaline trypsin protein purified from the digestive juice of *B. mori* larvae, have demonstrated strong antiviral activity to BmNPV in vitro (Nakazawa et al. 2004; Ponnuvel et al. 2003; Ponnuvel et al. 2012). Here, we attempt to consolidate the antiviral mechanism of SP in different insects (Figs. 8.1 and 8.2).

Fig. 8.1 Life cycle of baculovirus in an antiviral mechanism

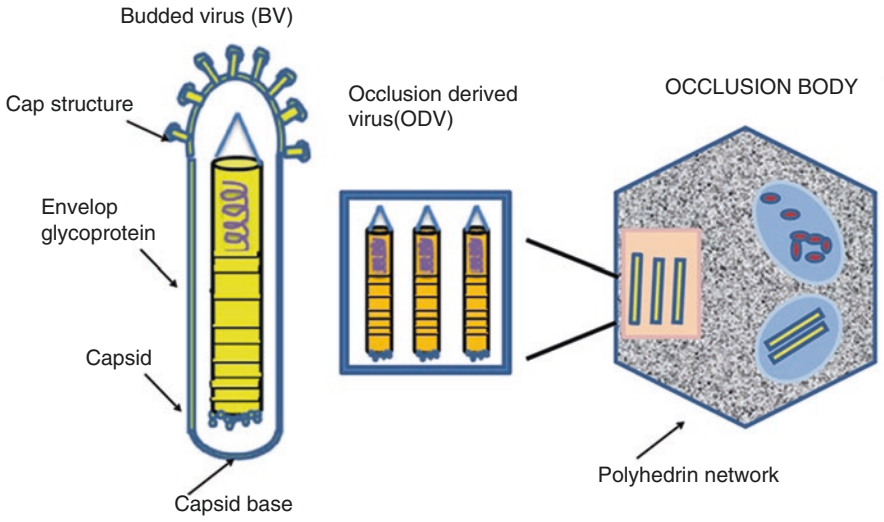

Fig. 8.2 Detailed structure of budded virus (BV) and occlusion-derived virus (ODV)

8.2 What Is Serine Protease?

Proteases are the largest group of enzymes, ubiquitous in nature, that hydrolyze proteins by adjoining water across peptide bonds (Saleem et al. 2012) and catalyze peptide construction in an organic solvent with a low water content (Soares et al. 2011). According to Verma et al. (2011) proteases are subdivided into diverse groups based on their catalytic activity with reference to reaction medium and can be identified as acidic, alkaline, neutral, and in active site groups. Proteases are also classified according to three major criteria: (1) the type of reaction catalyzed, (2) the chemical nature of the catalytic site, and (3) the evolutionary relationship with regard to structure (Barett 1994). Proteases are also categorized into exo- and endopeptidases depending on their action at or away from termini respectively and subdivided based on the nature of their functional active site groups, i.e., SPs, aspartic proteases, cysteine proteases, and metalloproteases (Hartley 1960). Of these functions, SPs act as mediators among the immune systems of different insects and determine the defense mechanisms of various pathogens via antimicrobial peptide synthesis, hemolymph coagulation, and melanization of pathogen surfaces (Gorman and Paskewitz 2001). Previously, a protein was demonstrated to have a significant antiviral activity against BmNPV from the digestive juice of *Bombyx mori* and was termed *B. mori* SP (BMSP-2) (Nakazawa et al. 2004). According to Kotani et al. (1999), BmSp-2 exhibited 94% amino acid sequence identity with SP. Recently, Lin et al. (2017) reported that SP inhibitors (SPIs) were present in all living animals and performed a vital role in development, digestion, and innate immunity. They studied the genome-wide characterization and expression profiling of the *SPI* gene in *Plutella xylostella* and noted that the *SPI* gene was categorized into serpins, canonical inhibitors, and alpha-2-macroglobulins. Of these, serpins demonstrated an association with the regulation of innate immunity of insects, whereas according Zhao et al. (2010), the upregulation and downregulation of different SP inhibitor genes may be participating in the combat with pathogenic microorganisms such as *Escherichia coli*, *Bacillus bombysepticus*, *Beauveria bassiana*, or BmNPV.

8.3 Role of Serine Protease in National and International Industries

In earlier studies, a diverse group of proteins and SPs played a very significant role in insect immunity for the mechanism of antiviral infection and were referred to as "serpocidins" (Kim et al. 2009). Of the various proteases, SPs have a zymogen-like structure that comprises the catalytic domain of the C-terminal and the regulatory domain of the N-terminal, are generally activated by His/Asp/Ser from the catalytic site, and participate in the regulatory mechanism of melanization, coagulation, and antimicrobial peptide production, through protease cascades (Kim et al. 2009; Gabay 1994; Gorman and Paskewitz 2001). In addition, SP homologs possess the catalytic triad and play a vital role in the antibacterial mechanism of human azurocidin (Gabay and Almeid 1993) and horseshoe crab factor D (Iwanaga 2002); cell

adhesion, *Drosophila* masquerade (Murugasu-Oei et al. 1995); *Pacifastacus* masquerade-like protein (Huang et al. 2000); prophenoloxidase activation, *Holotrichia* masquerade-like protein (Kwon et al. 2000); and immune function, *Anopheles* SP15 (Dimopoulos et al. 1997) and human hepatocyte growth factor (Nakamura et al. 1989).

In several regions of the world, arbovirus (arthropod-borne viruses) has been shown to be a risk to animal and human health and there is scanty knowledge about arbovirus infection (Fragkoudis et al. 2009). Previously, vertebrate immunity to viral infection could be regulated by the JAK/STAT signaling pathway, virus interference, and the virus nucleic acid sensor pathway (Randall and Goodbourn 2008). However, during the last few years, there has been a vast increase in knowledge on mosquito genetics and immunity-related genes, mainly through the *Anopheles gambiae* and *Aedes aegypti* sequencing projects (Christophides et al. 2002; Holt et al. 2002). Recently, Fragkoudis et al. (2009) reported that most arboviruses are RNA virus families such as Bunyaviridae, Togaviridae, and Flaviviridae, although the bluetongue virus is from the double-stranded RNA (dsRNA) family Reoviridae, an arbovirus family of great veterinary importance. The immune pathway involved in arbovirus–mosquito interactions has largely relied on genomic studies to identify differentially regulated genes (Ross et al. 2003; Wang et al. 2008). In the midguts of *Anopheles gambiae* and *Aedes aegypti* infected with SINV, upregulation of the Toll pathways is followed by activation of JNK signaling and is probably preceded by IMD activation (both pathways are linked in *D. melanogaster*) (Ross et al. 2003). In addition, other immune molecules such as SPs are upregulated and play an important role in innate immunity (Lemaitre and Hoffmann 2007); however, their role in the response to viral infections remains unclear. In the case of another alphavirus, SFV, if activated before infection, not Toll- but Gram-negative-mediated signaling (JAK/STAT or IMD/JNK) can inhibit virus replication in mosquito cell cultures (Fragkoudis et al. 2008; Jiang et al. 2009).

Jiang et al. (2009) and Gorman et al. (2000b) reported that SP plays a crucial role in an insect immune pathway for the synthesis of melanin, but in the process of melanization it acts as activator in mosquitoes and *Drosophila*-like insects. In this regard, the Sp22D protein sequence is determined by quantitative northern blot analysis and in-situ hybridization. Gorman et al. (2000a) also reported that Sp22D codes for a 1322 amino acid polypeptide with a complex domain organization in *Anopheles gambiae*. In addition to the SP catalytic domain, Sp22D contains two putative chitin-binding domains, a mucin-like domain, two low-density lipoprotein receptor class A domains, and two scavenger receptor cysteine-rich domains, and participates in translational upregulation. A few years earlier, Choo et al. (2007) described an SP characterized based on cDNA cloning, expression, and enzyme activity in the midgut of the *Bombus ignitus* and consisting of four introns and five exons coding for 250 amino acid residues. Wang et al. (2008) also suggested that infection of mosquitoes with recombinant arboviruses expressing activators or inhibitors of apoptosis might be used in the regulation of antiviral mechanisms.

Recently, the SP homolog SPH-3 (an insect non-clip domain-containing SPH) played a central role in insect immunity in *Manduca sexta* infection, with a virulent,

insect-specific, Gram-negative bacterium *Photorhabdus luminescens* (Felfoldi et al. 2011). Felfoldi et al. (2011) reported that RNA interference suppression of bacteria induced SPH-3 synthesis severely compromises the insect's ability to defend itself against infection by preventing the transcription of multiple antimicrobial effector genes, but, surprisingly, not the transcription of immune recognition genes. After that, gene encoding prophenoloxidase was performed by upregulation and the activity of the phenoloxidase enzyme are among the antimicrobial responses that are severely attenuated on SPH-3 knockdown, which concluded that SPH-3 regulates the genes encoding pattern in signaling pathways and controls the infection. As a nonfunctional serine proteinase homolog, SPH-3 cannot be an enzymatically active proteolytic component in a signaling cascade. However, the signaling pathway akin to the Toll family receptor in *Drosophila*, which may play a key adaptor in signal-mediating upstream toward the receptor. This finding indicates that study of viral and microbial infections is important for conferring the immune response in different insects, such as *Bombyx mori*, *Drosophila*, *Anopheles*, *Manduca sexta*, along with its prevention technology.

8.4 Serine Protease Mechanism and Specificity

The process of catalysis and specificity are not quietly inhibited by a scant residue, nevertheless effects on the whole protein frameworks disciplined via the rationing of the hydrogen bond charges, as it may be linking of domain drift to the chemical conversion (Hedstrom 2002). The specificity of SP is basically deliberated the physiography of the substrate binding sites which can adjoin the catalytic site of cleft (Polgar 2005). However, in an earlier report, SP specificity has produced a lot of information regarding biological function and the establishment of efforts (Perona and Craik 1995). Of the industrial enzymes, about 75% of microbial proteas belong to SPs and serve the nucleophilic Ser residue at their functioning site (Rao et al. 1998). SPs are classified as the catalytic triad with the presence of Asp, His, and Ser, and these are designated as catalytic machinery, which is subdivided into four separate groups. In an earlier report, these four groups of SPs are described as chymotrypsin, subtilisin, carboxypeptidase Y, and Clp protease (Rawlings and Barrett 2000).

8.5 Silkworm: Mode of Infection and Mechanism of Serine Protease as an Antiviral Factor

Feng et al. (2013) reported that insects get participated in different ways to defend themselves against different pathogen such as fungi, bacteria, nematodes but scanty knowledge about the insect immune response against viruses. Among them, one of the most important viruses is *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV), which spreads almost 30 lepidopteron species, whereas baculoviruses have a very narrow host range that contains a circular double-stranded genome ranging from 80 to 180 kbp and moderated in the form of larvae of *Bombyx mori* (An et al. 2009; Rahman and Gopinathan 2004). The life cycle of *Bombyx mori* contains two

specific forms of virus, occlusion-derived virus (ODV) and the budded virus (BV). Both forms play a different role in the interim pathogenesis in Fig. 8.1 (Katsuma et al. 2006). Antiviral immunity in other insects is well known, but there is not much more information on the silkworm, *B. mori*. In addition, other factors such as hemolin, receptors in midgut epithelial cells, phenoloxidase, and apoptosis also played a significant role in the antiviral mechanism (Ponnuvel et al. 2012). Recently, a protein RNAse III (dicer) was identified that showed the antiviral mechanism against infectious flu virus (IFV) (Ponnuvel et al. 2008; Ponnuvel et al. 2012). Nakazawa et al. (2004) reported that *Bombyx mori* possesses the two forms of virus particles, BmNPV and AcMNPV, which showed that they are phenotypically different, but genetically identical, and complied with their life cycle at the time of pathogenesis. The two forms play different roles. During this process, infection begins and the ODV fuses with the microvillar membrane (Monsma et al. 1996). This virus particle is enclosed with proteinaceous occlusions, which are discharged into the midgut of the larvae with the combination of alkaline gut pH and protease (Engelhard and Volkman 1995). The cell is infected and produces many primary single nucleocapsids through the basal membrane. This BV gained an envelope studded with glycoprotein GP-64, which infected the neighboring host and tissues (Monsma et al. 1996). The detailed structure of budded virus (BV) and occlusion-derived virus (ODV) is summarized in Fig. 8.2.

Various researchers reported that SP played a significant role in antiviral activity against BmNPV, which is designated as BmSP-2 and analyses their gene expression in the midgut of *Bombyx mori* (Nakazawa et al. 2004; Ponnuvel et al. 2008), whereas Zhao et al. (2010) presumed that potential SPI genes based on the genome sequences of the silkworm are susceptible to the antiviral mechanism. He reported that these SPI genes may be responsible for defenses to pathogenic microorganisms through microarray and qRT-PCR assay. This report highlights the upregulation and downregulation of several SPI genes subsequently infected by *Escherichia coli*, *Bacillus bombysepticus*, *Beauveria bassiana*, or BmNPV. Recently, Liu et al. (2016) investigated the roles of SP for antiviral mechanisms and identified SP gene BmSP36, which has a 292-residue protein and is cloned. Liu et al. (2016) also defined that the BmSP36 consists of an intact catalytic triad (H57, D102, and S195) and a conserved substrate binding site (G189, H216, and G226), which is responsible for chymotrypsin-like specificity. According to their reports, BmSP36 plays a significant role in the midgut of *B. mori* and they analyzed the transcriptional and translational expression using western blotting, immunofluorescence, and liquid chromatography-tandem mass spectrometry assay.

8.6 Anopheles: Mode of Infection and Mechanism of Serine Protease as an Antiviral Factor

Anopheles serves as an obligate intermediate parasite, which is harmful to humans. In a susceptible mode during blood feeding in the midgut, it encounters the multiple rhythms of reproduction and development before ascending to the salivary glands (Gorman et al. 2000b). Various numbers of parasites complete

their life cycle in susceptible mosquitoes, which is interrupted by various mechanisms and only a very small proportion succeeds (Beier 1998; Gorman et al. 2000b). Richman et al. (1996) also reported that the antimicrobial peptides function as defensins and cecropins against parasitic infection in the mosquito. Ashida and Brey (1997) reported that melanotic encapsulation and the production of antimicrobial peptides are types of innate, humoral immune responses regulated by SPs, mediated the cleavage of prophenoloxidase (proPO), and instigated the construction of reactive quinones, which cross-link to form melanin, whereas melanotic encapsulation generated the refractory lines of mosquitoes in the process of melanization and killed the parasites completely (Gorman et al. 2000b). For many decades, mosquito-borne arboviral disease (such as dengue), was responsible for an estimated 300 million infections annually (Bhatt et al. 2013). The replication cycle of the dengue virus is carried in the Ades mosquito for 7–14 days and can fluctuate with regard to virus performance and temperature (Alto and Bettinardi 2013). In an earlier study, Souza-Neto et al. (2009) reported that the Janus kinase/signal transducer and activator of the transcription (JAK/STAT) pathway regulated a conserved immune signaling pathway for the development of antiviral mechanisms in both mammals and insects, and previously hypothesized that the JAK/STAT pathway regimented dengue infection in *A. aegypti*. Recently, Jupatanakul et al. (2017) investigated the JAK/STAT pathway, which conserved the immune signaling pathway and regulated developmental processes and antiviral immunity in both mammals and insects with viral infection. In this method, activation of the JAK/STAT pathway through RNAi-mediated gene silencing of a protease inhibitor such as SP-activated STAT (PIAS) renders mosquitoes more resistant to DENV infection of the midgut, whereas silencing of the receptor Dome or the Janus kinase Hop renders the mosquitoes more susceptible to DENV infection (Souza-Neto et al. 2009). SPs activate the signaling pathways that accelerate the transcription of the genes and five new SP (Sp14A, Sp14D1, Sp14D2, Sp18D, and Sp22D) cDNAs from the hemolymph of the malaria vector (*Anopheles gambiae*) were identified, which significantly enhanced the melanotic encapsulation of plasmodium and antiviral immunity (Gorman et al. 2000b). Thus, there are urgent needs from the last few decades regarding dengue virus (DENV: *Flavivirus*), which poses a significant risk to human health , and the scarcity of the drugs required for the prevention and control of dengue disease.

8.7 *Drosophila*: Mode of Infection and Mechanism of Serine Protease as an Antiviral Factor

Recently, the mode of infection and mechanism of antiviral immunity were well documented through the Toll and IMD pathways (De Gregorio et al. 2002). According to An et al. (2013) melanization regulated the innate immunity via binding, eradicated the interfering organisms, and intervened using a SP cascade that is regulated by serpins in a similar manner. Zhao et al. (2012) demonstrated that serpins may be associated with the management of the innate immunity of different

insects on the basis of genome wide identification. The process of melanization combined with other immune activity, such as blood coagulation, phagocytosis, wound healing, and antimicrobial peptide expression (Kanost and Gorman 2008).

Drosophila melanogaster has been extensively used to study molecular mechanisms that are involved in the activation and regulation of innate immunity. However, scanty knowledge was found compared with the silkworm, *Bombyx mori*, the tobacco hornworm, *Manduca sexta*, the and *Tenebrio molitor* for the performance of the Toll signaling pathway (An et al. 2009; Lindsay and Wasserman 2014). In the melanization process, phenoloxidase (PO) catalyzes the hydroxylation of monophenols to o-diphenols and the oxidation of o-diphenols to quinones, which polymerize to form melanin (Nappi et al. 2009) and inactive zymogens called prophenoloxidase (PPO) (Cerenius et al. 2008). Other researchers reported that PPO activation is mediated by a SP cascade (serpin superfamily), which contains 400 amino acid residues with an exposed reactive center loop near their carboxyl terminus and functions as suicide substrate inhibitors by forming irreversible complexes with target proteases after the cleavage of a scissile bond (designated P1–P1′) in the reactive center loop (Gettins 2002; Jiang et al. 2009; An et al. 2013). Recently, An et al. (2013) reported the functional role of SP inhibitors (SPn27A, MP2) in *Drosophila* and stated that both are used in the prevention of prophenoloxidase-1. Thus, they concluded that molecular and biochemical analyses play a vital role in understanding the PPO-activating cascade in insects and expect to shed some light on the action of protease, which could be beneficial in targeting biochemical pathways that are potentially applicable to control of the viral infection.

8.8 Molecular and Cell Target-Based Analysis of Antiviral Protein (Silkworm, *Drosophila*, Anopheles)

8.8.1 Genome-Wide Identification and Expression

For the study of genome-wide identification and expression analysis, there is a current need to understand the genetics and regulatory mechanisms of immune responses of insects that have developed the effective biological control system (You et al. 2013). Recently, Xia et al. (2015) investigated the genome-wide identification, characterization, and expression analysis in *Plutella xylostella* by using Toll, IMD, and JAK-STAT signaling pathways. Previously, genome-wide identification and expression were reported by Zhao et al. (2010) in *Bombyx mori* who compared the expression analysis of SPs and their homologs (Table 8.1). SP homologs (SPHs) are similar to SPs in amino acid sequences, but they have no protease activity because of the loss of one or more of the catalytic residues (Zou et al. 2006). In insects, SPHs participate in the innate immune response to regulate the mechanism insect immunity (Dimopoulos et al. 1997; Kim et al. 2008). Genome-wide characterization was also performed in SP and SP homologs of *Drosophila melanogaster* (Ross et al. 2003). Besides these insects, *Anopheles gambiae* and

Table 8.2 List of serine proteases and serine protease inhibitors which upregulated and downregulated the transcripts region and their gene expression analysis (adopted from Riddell et al. 2014)

	Upregulated	Downregulated
Serine proteases		
cSp3	BTT35293_1	BTT10579_1, BTT10912_1, BTT25711_1
Sp18	BTT20808_1	BTT20808_1
Sp27	BTT40251_1	BTT40251_1
Sp28		BTT20637_1
Sp35	BTT05300_1	BTT10155_1
Sp40	BTT15256_1	
Sp23	BTT01709_1, BTT05886_1, BTT09081_1, BTT20661_1, BTT20725_1, BTT24359_1, BTT25071_1	
Serine protease homologs		
cSPH39		BTT21868_1
Sph54		BTT27769_1
Sph56	BTT17814_1	
Serine protease inhibitors		
Kunitz serprotease inhibitor		BTT14993_1
Necrotic (nec)	BTT35742_1	
Spn 4	BTT04130_1	BTT04130_1
SRPN10		BTT02607_1, BTT4508_1, BTT20259_1

Apis mellifera also had immunity-related SPs and SPH characteristics (Christophides et al. 2002; Zou et al. 2006). There is little information about these proteins in *Bombyx mori* against SPs and SPHs, but it emphasizes the biological functions such as digestion, immune response, development (Nakazawa et al. 2004). Furthermore, Tanaka et al. (2008) identified the potential immunity of SPs and SPH-related geneswhile modifications to the mRNA level that were considered to be involved in the antiviral mechanism. According to (Zhao et al. 2010; Xia et al. 2007; and Riddell et al. 2014) the SPs and SPH genes were quietly used in upregulation and downregulation after pathogen induction by using microarray and real-time quantitative experiments (Table 8.2).

8.8.2 cDNA Microarray-Based Assay and Two-Dimensional Gel Electrophoresis

In recent years, Chang et al. (2011) studied the comparative analysis of gene expression techniques and determined that cDNA microarray and two-dimensional gel electrophoresis have become part of routine in checking the changes in gene

expression. Among them, such methodologies are implemented to identify differently expressed transcripts by virtue of many genes being examined simultaneously. Recently, several studies have been conducted on insect resistance such as SP-2, lipase-1, and alkaline trypsin protein purified from the digestive juice of *B. mori* larvae showed strong antiviral activity to BmNPV in vitro (Nakazawa et al. 2004; Ponnuvel et al. 2003, 2012).

In an earlier report, the SP gene in *Bombus ignites* was cloned to provide some valuable knowledge on determining the possible function/role(s) of SPs (Choo et al. 2010). In this report, Choo et al. first isolated the genomic DNA and PCR of the *BiSP* gene and southern blot analysis, then expression of recombinant BiSP protein, preparation of polyclonal antibody, and western blot analysis; further, cDNA encoding BiSP was expressed as a 28-kDa polypeptide in baculovirus-infected insect cells, and the recombinant BiSP showed activity in a protease enzyme assay. BiSP was specifically expressed in the midgut of *B. ignitus* queens, males, and workers, suggesting that the BiSP is a gut enzyme involved in the digestion of dietary proteins, i.e., SP of *B. ignitus*, but the involvement of BiSP in the defense from microbial infection. Choi et al. (2006) constructed the cDNA library by screening, sequencing, and generating the expressed sequence tags (ESTs). Altschul et al. (1997) also compared the sequence by using DNASIS and BLAST software and used this to align the amino acid sequences of SP. In accordance with this hypothesis, we also interpreted our data by using a sequence comparison program and identified the pathogen that was responsible for the infection. Using the fluorescent differential display (FDD) technique, Bmsop2 and Bms3a were identified (Xu et al. 2012).

8.8.3 Polyclonal Antibody-Based Preparation and Western Blot Analysis

Polyclonal antibody-based analysis is used to identify immune mechanisms in insects, as SPs act as a pro-PO cascade, which is involved in superoxide generation, melanin synthesis, and subsequent sequestration of foreign matter entering the hemocoel of the insect (Ashida and Brey 1998). Another SP cascade in insects is involved in the establishment of the dorso-ventral pattern in the *Drosophila* embryo (Morisato 1995). Choo et al. (2007) reported that the *B. ignitus* SP (BiSP) gene functioned as a gut enzyme involved in the digestion of dietary proteins by polyclonal antibody-based preparation and western blotting. The recombinant proteins fused with an N-term GST tag were overexpressed in *E. coli* and further purified to near homogeneity to prepare mouse antibodies. The western blot analysis showed that these proteins were expressed in various tissues and organs, and in different developmental stages. Amazingly, the expression of BmTHY2 was hugely increased during the pupae stage, indicating a specialized role during this period. The expression of these proteins was gradually decreased in BmN cells infected by BmNPV, suggesting that they might play different roles in virus infection (Ma et al. 2015).

8.9 (In) Multigene Expression

In the present scenario, multiple gene expression techniques used on the basis of three potential criteria:

1. The functions of targeted genes exclusively related to multiple genes.
2. Break down of the co-expression of multiple genes or multi-functional protein complex in silk gland bioreactor research.
3. Construction of multiresistant transgenic *B. mori* strains such as BmNPV and cytoplasmic polyhedrosis virus (BmCPV).
4. For the production of silk material genetically modified techniques are used, which improve the middle and posterior silk gland in *B. mori*. (Wang et al. 2017). Previously, an applied aspect of silkworm on genome sequencing projects resulted in a higher requirement of functional genomic research, which is providing a suitable platform for the study of multiple genes (Xia et al. 2009). Recently, Wang et al. (2017) reported 2A self-cleaving peptide-based multiple gene expression system in the *B. mori* and concluded that the multiple gene expression would be an efficient tool of the functional genomic era. In the process, he investigated that the gene regulated the simultaneous expression and cleavage of multiple gene targets in the silk gland of transgenic silkworms. First, a glycine-serine-glycine spacer (GSG) was found to significantly improve the cleavage efficiency of 2A. This study enhances the functional knowledge of genes and proteins, and potentially advances innovative research into various functional silk materials in medicine, cosmetics, and other biomedical areas.

8.10 Differentially Expressed Genes Analysis

In recent years, there has been a need to analyze gene efficiency by comparing the transcriptome of two strains, which is used to improve the efficiency and gene discovery of host–pathogen responses. A new technique was developed by scientists and used for comparative analysis of two strains with a transcriptome (Wang et al. 2016). According to their hypothesis, they reported that molecular changes in *B. mori* occurred during BmNPV infection and were examined by transcriptome sequencing in the isogenic line BC9 and the recurrent parent P50. These genes were related to transport, virus replication, intracellular innate immune, and apoptosis (Wang et al. 2016). After normalizing gene expression levels, DEGs were obtained by pair-wise comparison of the four transcriptome libraries using IDEG6 software (Romualdi et al. 2003). Genes participating in innate immunity pathways were identified and analyzed with regard to their potential role in BmNPV infection in silkworm, which could be classified into the Toll pathway, the IMD pathway, the PPO pathway, the pattern recognition receptor, and the antimicrobial peptide. Wang et al. (2016) further reported that the SP inhibitor performed a significant role in the PPO pathway and it appears that there was downregulation in the gene of 47% and 57% respectively, whereas 30% and 20% were upregulated after infection with

BmNPV, and 57% were downregulated and 20% were upregulated in P50 after BmNPV infection. By this comparative analysis, a total of 869 DEGs were obtained, which included many genes potentially related to BmNPV resistance. After that, Wang et al. (2016) predicted that reliable evidence may be produced to clarify the molecular mechanism of the silkworm. Hu et al. (2014) also reported analysis of several differentially expressed genes (DEGS), which is involved in metabolism, immunity, and inflammatory responses in *Microtus fortis* following infection with *Schistosoma japonicum* based on comparative transcriptome analysis.

Conclusion

In the current scenario, wonderful progress has been made in the antiviral mechanism of SP against viral infection. The synergism of host and pathogen played a pivotal role in viral infections, and various mechanisms such as genome-wide identification and expression, microarray-based expression, recombinant-based expression, polyclonal antibody-based expression, multiple gene-based expression, etc., were performed using the SP pathway. The discovery of Molecular and cellular analysis - Genome-Wide Identification and Expression, cDNA Microarray-based Assay, Polyclonal Antibody-Based Preparation and Western Blot, Multigene Expression, Differentially expressed genes analysis of anti-protein detection plays a vital role in fighting viral infection, but recently, antimicrobial target-based pathways, immensely used, such as siRNA, Toll, JAK-STAT, IMD, etc., is used to determine the host–pathogen-specific immune response to the fascinating antiviral mechanism. In this study, a new technology was developed for understanding the fundamental and applied aspects of insect immunity against viral infection in the functional genomic era, and potentially improving the antiviral mechanism.

References

Alto BW, Bettinardi D (2013) Temperature and dengue virus infection in mosquitoes: independent effects on the immature and adult stages. Am J Trop Med Hyg 88:497–505

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSIBLAST: a new generation of protein database search programs. Nucl Acids Res 25:3389–3402

An C, Ishibashi J, Ragan EJ, Jiang H, Kanost MR (2009) Functions of Manduca sexta hemolymph proteinases HP6 and HP8 in two innate immune pathways. J Biol Chem 284:19716–19726

An C, Zhang M, Chu Y, Zhangwu Zhao Z (2013) Serine protease MP2 activates prophenoloxidase in the melanization immune response of drosophila. PLoS One 8(11):1–10

Ashida M, Brey PT (1997) Recent advances in research on the insect prophenoloxidase cascade. In: Brey PT, Hultmark D (eds) Molecular mechanisms of immune responses in insects. Chapman and Hall, London, pp 135–172

Ashida M, Brey PT (1998) In: Brey PT, Hultmark D (eds) Molecular mechanisms of immune responses in insects. Chapman & Hall, London, pp 135–172

Bao YY, Tang XD, Lv ZY, Wang XY, Tian CH, YP X, Zhang CX (2009) Gene expression profiling of resistant and susceptible Bombyx mori strains reveals nucleopolyhedrovirus-associated variations in host gene transcript levels. Genomics 94(2):138–145

Barett AJ (1994) Proteolytic enzymes: serine and cysteine peptidases. Methods Enzymol 244:1–15
Beier JC (1998) Malaria parasite development in mosquitoes. Annu Rev Entomol 43:519–543
Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL et al (2013) The global distribution and burden of dengue. Nature 496:504–507
Cerenius L, Lee BL, Soderhall K (2008) The proPO-system: pros and cons for its role in invertebrate immunity. Trends Immunol 29:263
Chang C, Liu X, Chen K (2011) Molecular cloning, expression and characterization of a novel gene**β**-N-acetylglucosaminidase from Bombyx mori. Adv Biosci Biotechnol 2(2):123–127
Chen J, X-F W, Zhang YZ (2006) Expression, purification and characterization of human GM-CSF using silkworm pupae (Bombyx morie) as a bioreactor. J Biotechnol 123:236–247
Cheng Y, Wang X-Y, Hao H, Killiny N, Jia-Ping X (2014) A hypothetical model of crossing Bombyx mori nucleopolyhedrovirus through Its host midgut physical barrier. PLoS One 9(12):e115032
Choi YS, Lee KS, Yoon HJ, Kim I, Sohn HD, Jin BR (2006) A bumblebee thioredoxin-like protein gene that is upregulated by a temperature stimulus and lipopolysaccharide injection. Eur J Entomol 103:291–296
Choo YM, Lee KS, Yoon HJ, Lee SB, Kim JH, Sohn HD, Jin BR (2007) A serine protease from the midgut of the bumblebee, Bombus ignitus (hymenoptera: apidae): cDNA cloning, gene structure, expression and enzyme activity. Eur J Entomol 104:1–7
Choo YM, Lee KS, Yoon HJ, Kim BY, Sohn MR, Roh JY, Je YH, Kim NJ, Kim I, Woo SD, Sohn HD, Jin BR (2010) Dual function of a bee venom serine protease: prophenoloxidase-activating factor in arthropods and fibrin(ogen)olytic enzyme in mammals. PLoS One 5(5):1–10
Christophides GK, Zdobnov E, Barillas-Mury C, Birney E, Blandin S, Blass C, Brey PT, Collins FH, Danielli A, Dimopoulos G (2002) Immunity-related genes and gene families in Anopheles gambiae. Science 298(5591):159–165
Danielli A, Loukeris TG, Lagueux M, Muller HM, Richman A, Kafatos FC (2000) A modular chitin-binding protease associated with hemocytes and hemolymph in the mosquito Anopheles gambiae. Proc Natl Acad Sci U S A 97:7136–7141
De Gregorio E, Han SJ, Lee WJ, Baek MJ, Osaki T et al (2002) An immune-responsive Serpin regulates the melanization cascade in Drosophila. Dev Cell 3:581
Dimopoulos G, Richman A, Mullar HM, Kafatos FC (1997) Molecular immune responses of the mosquito Anopheles gambiae to bacteria and malaria parasites. Proc Natl Acad Sci U S A 94:11508–11513
Engelhard EK, Volkman LE (1995) Developmental resistance in fourth instar Trichoplusia ni orally inoculated with Autographa californica M nuclear polyhedrosis virus. Virology 209:384–389
Feng Fan, Hu Ping and Chen Keping (2013) Progress of antiviral mechanisms in the mulberry silkworm: A review 7(14):1173–1178
Felfoldi G, Eleftherianos I, Ffrench-Constant RH, Venekei I (2011) A serine proteinase homolog, SPH-3, plays a central role in insect immunity. J Immunol 186:4828–4834
Fragkoudis R, Chi Y, Siu RW, Barry G, Attarzadeh-Yazdi G, Merits A, Nash AA, Fazakerley JK, Kohl A (2008) Semliki forest virus strongly reduces mosquito host defense signaling. Insect Mol Biol 17:647–656
Fragkoudis R, Attarzadeh-Yazdi G, Nash AA, Fazakerley JK, Alain Kohl A (2009) Advances in dissecting mosquito innate immune responses to arbovirus infection. J Gen Virol 90:2061–2072
Gabay JE (1994) Antimicrobial proteins with homology to serine protease. Ciba Found Symp 186:237–247
Gabay JE, Almeid RP (1993) Antibiotic peptides and serine protease homologs in human polymorphonuclear leukocytes: defensins and azurocidin. Curr Opin Immunol 5:97–102
Gettins PG (2002) Serpin structure, mechanism, and function. Chem Rev 102:4751
Gorman MJ, Paskewitz SM (2001) Serine proteases as mediators of mosquito immune responses. Insect Mol Biol 31:257–262
Gorman MJ, Andreeva OV, Paskewitz SM (2000a) Sp22D: a multidomain serine protease with a putative role in insect immunity. Gene 251:9–17

Gorman MJ, Olga V, Andreeva OV, Susan M, Paskewitz SM (2000b) Molecular characterization of five serine protease genes cloned from Anopheles gambiae hemolymph. Insect Biochem Mol Biol 30:35–46

Hartley BS (1960) Proteolytic enzymes. Annu Rev Biochem 29:45–72

He WY, Zheng YP, Tang L, Zheng SC, Beliveau C, Doucet D, Cusson M, Feng QL (2009) Cloning, expression and localization of a trypsin-like serine protease in the spruce budworm, Choristoneura fumiferana. Insect Sci 16:455–464

Hedstrom L (2002) Serine protease mechanism and specificity. Chem Rev 102:4501–4524

Herrero S, Combes E, Oers V, Vlak MM, DeMaagd RA, Beekwilder J (2005) Identification and recombinant expression of a novel chymotrypsin from Spodoptera exigua. Insect Biochem Mol Biol 35:1073–1082

Holt RA, Subramanian GM, Halpern A, Sutton GG, Charlab R, Nusskern DR, Wincker P, Clark AG, Ribeiro JM, authors o (2002) The genome sequence of the malaria mosquito Anopheles gambiae. Science 298:129–149

Hu Y, YX X, WY L, Yuan ZY, Quan H, Shen YJ et al (2014) De novo assembly and transcriptome characterization: novel insights into the natural resistance mechanisms of Microtus fortis against Schistosoma japonicum. BMC Genomics 15(1):1–13

Huang HS, Wang H, Lee SY, Johansson WM, Soderhall K, Cerenius L (2000) A cell adhesion protein from the crayfish Pacifastacus leniusculus, a serine protease homologue similar to drosophila masquerade. J Biol Chem 275:9996–10001

Iwanaga S (2002) The molecular basis of innate immunity in the horseshoe crab. Curr Opin Immunol 14:87–95

Jiang R, Kim EH, Gong JH, Kwon HM, Kim CH et al (2009) Three pairs of protease-serpin complexes cooperatively regulate the insect innate immune responses. J Biol Chem 284:35652

Jupatanakul N, Shuzhen Sim S, Angleroa-Rodroaguez YI, Souza-Neto J, Das S, Poti KE, Rossi SL, Bergren N, Vasilakis N, Dimopoulos G (2017) Engineered *Aedes aegypti* JAK/STAT pathway-mediated immunity to dengue virus. PLoS Negl Trop Dis 1:24

Kaji K, Tomino S, Asano T (2009) A serine protease in the midgut of the silkworm, Bombyx mori: protein sequencing, identification of cDNA, demonstration of its synthesis as zymogen form and activation during midgut remodeling. Insect Biochem Mol Biol 39:207–217

Kanost MR, Gorman MJ (2008) Phenoloxidases in insect immunity. In: Beckage NE (ed) Insect immunology. Elsevier/Academic, Manhattan, pp 69–96

Katsuma S, Daimon T, Horie S, Kobayashi M, Shimada T (2006) N-linked glycans of Bombyx mori nucleopolyhedrovirus fibroblast growth factor are crucial for its secretion. Biochem Biophys Res Commun 350(4):1069–1075

Kim CH, Kim SJ, Kan H, Kwon HM, Roh KB et al (2008) A three-step proteolytic cascade mediates the activation of the peptidoglycan-induced toll pathway in an insect. J Biol Chem 283:7599–7607

Kim SY, Jeong EJ, Song J, Park KS (2009) Molecular cloning and characterization of a serine protease-like protein from silkworm (Bombyx Morie). Gene Genom 31(5):387–395

Kotani E, Niwa T, Tokizane M, Suga K, Sugimura Y, Oda K, Mori M, Furusawa T, (1999) Cloning and sequence of a cDNA for a highly basic protease from the digestive juice of the silkworm, *Bombyx mori.* Insect Mol. Biol. 8:299–304

Kwon TH, Kim MS, Choi HW, Joo CH, Cho MY, Lee BL (2000) A masquerade-like serine protease homologue necessary for phenoloxidase activity in the coleopteran insect Holotrichia diomphalia larvae. Eur J Biochem 267:6188–6196

Lavine MD, Strand MR (2002). Insect hemocytes and their role in immunity. Insect Biochem. Mol. Biol. 32(10):1295–1309

Lehane MJ, Aksoy S, Levashina E (2004) Immune responses and parasite transmission in blood-feeding insects. Trends Parasitol 20(9):433–439

Lemaitre B, Hoffmann J (2007) The host defense of Drosophila melanogaster. Annu Rev Immunol 25:697–743

Lin H, Lin X, Zhu J, XO Y, Xia X, Yao F, Guang Yang G, You M (2017) Characterization and expression profiling of serine protease inhibitors in the diamondback moth, Plutella xylostella (Lepidoptera: Plutellidae). BMC Genomics 18:162

Lindsay S, Wasserman SA (2014) Conventional and non-conventional Drosophila Toll signaling. Dev Comp Immunol 42(1):10.10–10.16

Liu HW, Li YS, Tang X, Guo PC, Wang DD, Chun-Yan Zhou CY, Xia QY, Zhao P (2016) A midgut-specific serine protease, BmSP36, is involved in dietary protein digestion in the silkworm, Bombyx mori. Insect Sci:1–15. https://doi.org/10.1111/1744-7917.12369

Liu Y, Sui YP, Wang JX, Zhao XF (2009) Characterization of the trypsin-like protease (Ha-TLP2) constitutively expressed in the integument of the cotton bollworm, Helicoverpa armigera. Arch Insect Biochem Physiol 72:74–87

Ma S, Kang Z, Lü P, Yang Y, Yao Q, Xia H, Chen K (2015) Molecular and physiological characterization of two novel multirepeat **β**-thymosins from silkworm, Bombyx mori. PLoS One 10(10):e0140182

Miao XX, SJ X, Li MH, Li MW, Huang JH, Dai FY, Marino SW, Mills DR, Zeng PY, Mita K, Jia H, Zhang Y, Liu WB, Xiang H, Guo QH, AY X, Kong XY, Lin HX, Shi YZ, Lu G, Zhang XL, Huang W, Yasukochi Y, Sugasaki T, Shimada T, Nagaraju J, Xiang ZH, Wang SY, Goldsmith MR, Lu C, Zhao GP, Huang YP (2005) Simple sequence repeat-based consensus linkage map of Bombyx mori. Proc Natl Acad Sci U S A 102:16303–16308

Monsma SA, Oomens AG, Blissard GW (1996) The GP64 envelope fusion protein is an essential baculovirus protein required for cell-to-cell transmission of infection. J Virol 70(7):4607–4616

Morisato D (1995) Signaling pathways that establish the dorsal-ventral pattern of the Drosophila embryo. Annu Rev Genet 29:371–399

Murugasu-Oei B, Rodrigues V, Yang X, Chia W (1995) Masquerade: a novel secreted serine protease-like molecule is required for somatic muscle attachment in the drosophila embryo. Genes Dev 9:139–154

Nakamura T, Nishizawa T, Hagiya M, Seki T, Shionishi M, Sugiura A, Tashiro K, Shiizu S (1989) Molecular cloning and expression of human hepatocyte growth factor. Nature 342:440–443

Nakazawa H, Tsuneishi E, Ponnuvel KM, Furukawa S, Asaoka A, Tanaka H, Ishibashi J, Yamakawa M (2004) Antiviral activity of a serine protease from the digestive juice of Bombyx mori larvae against nucleopolyhedrovirus. Virology 321(1):154–162

Nappi A, Poirie M, Carton Y (2009) The role of melanization and cytotoxic byproducts in the cellular immune responses of drosophila against parasitic wasps. Adv Parasitol 70:99–121

Perona JJ, Craik CS (1995) Structural basis of substrate specificity in the serine proteases. Protein Sci 4(3):337–360

Polgar L (2005) The catalytic triad of serine peptidases CMLS. Cell Mol Life Sci 62:2161–2172

Ponnuvel KM, Nakazawa H, Furukawa S, Asaoka A, Ishibashi J, Tanaka H, Yamakawa M (2003) A lipase isolated from the silkworm Bombyx mori shows antiviral activity against nucleopolyhedrovirus. J Virol 77(19):10725–10729

Ponnuvel KM, Koundinya PR, Sinha RK, Kamble CK (2008) Mechanism of viral resistance in mulberry silkworm, Bombyx mori L. Indian J Seric 47(1):1–6

Ponnuvel KM, Nithya K, Sirigineedi S, Awasthi AK, Yamakawa M (2012) In vitro antiviral activity of an alkaline trypsin from the digestive juice of Bombyx mori larvae against nucleopolyhedrovirus. Arch Insect Biochem 81(2):90–104

Popham HJ, Shelby KS, Brandt SL, Coudron TA (2004) Potent virucidal activity in larval Heliothis virescens plasma against Helicoverpa zea single capsid nucleopolyhedrovirus. J Gen Virol 85(8):2255–2261

Qin L, Xia H, Shi H, Zhou Y, Chen L, Yao Q, Liu X, Feng F, Yuan Y, Chen K (2012) Comparative proteomic analysis reveals that caspase-1 and serine protease may be involved in silkworm resistance to Bombyx mori nuclear polyhedrosis virus. J Proteome 75:3630–3638

Rahman MM, Gopinathan KP (2004) Systemic and in vitro infection process of Bombyx mori nucleopolyhedrovirus. Virus Res 101:109–118

Randall RE, Goodbourn S (2008) Interferons and viruses: interplay between induction, signaling, antiviral responses and virus countermeasures. J Gen Virol 89:1–47

Rao MB, Tanksale AM, Ghatge MS, Deshpande VV (1998) Molecular and biotechnological aspects of microbial protease. Microbial Mol Biol Rev 62:597–615

Rawlings ND, Barrett AJ (2000) MEROPS: the peptidase database. Nucleic Acids Res 28:323–325

Richman AM, Bulet P, Hetru C, Barillas-Mury C, Hoffmann JA et al (1996) Inducible immune factors of the vector mosquito Anopheles gambiae: biochemical purification of a defensin antibacterial peptide and molecular cloning of preprodefensin cDNA. Insect Mol Biol 5:203–210

Riddell CE, Garces JDL, Adams S, Barribeau SM, David Twell D, Eamonn B, Mallon EB (2014) Differential gene expression and alternative splicing in insect immune specificity. BMC Genomics 15(1):1031

Romualdi C, Bortoluzzi S, DAlessi F, Danieli GA (2003) IDEG6: a web tool for detection of differentially expressed genes in multiple tag sampling experiments. Physiol Genomics 12(2):159–162

Ross J, Jiang H, Kanost MR, Wang Y (2003) Serine proteases and their homologs in the Drosophila melanogaster genome: an initial analysis of sequence conservation and phylogenetic relationships. Gene 304:117–131

Saleem M, Rehman A, Yasmin R, Munir B (2012) Biochemical analysis and investigation on the prospective applications of alkaline protease from a Bacillus cereus strain. Mol Biol Rep 39(6):6399–6408

Soares TS, Watanabe RM, Lemos FJ, Tanaka AS (2011) Molecular characterization of genes encoding trypsin-like enzymes from Aedes aegypti larvae and identification of digestive enzymes. Gene 489:70–75

Souza-Neto JA, Sim S, Dimopoulos G (2009) An evolutionary conserved function of the JAK-STAT pathway in anti-dengue defense. Proc Natl Acad Sci U S A 106:17841–17846

Tanaka H, Ishibashi J, Fujita K, Nakajima Y, Sagisaka A, Tomimoto K, Suzuki N, Yoshiyama M, Kaneko Y, Iwasaki T (2008) A genome-wide analysis of genes and gene families involved in innate immunity of Bombyx mori. Insect Biochem Mol Biol 38(12):1087–1110

Verma J, Modi DR, Sharma R, Saxena S (2011) Vital role of alkaline protease in bio-industries: a review. Plant Archives 11(2):1083–1092

Wang H, Blair CD, Olson KE, Clem RJ (2008) Effects of inducing or inhibiting apoptosis on Sindbis virus replication in mosquito cells. J Gen Virol 89:2651–2661

Wang XY, HZ Y, Geng L, JP X, Yu D, Zhang SZ, Ma Y, Dong-Qiong Fei DQ (2016) Comparative transcriptome analysis of Bombyx mori (Lepidoptera) larval midgut response to BmNPV in susceptible and near-isogenic resistant strains. PLoS One 11(5):1–22

Wang Y, Wang F, Riyuan Wang R, Zhao P, Xia Q (2017) 2A self-cleaving peptide-based multi-gene expression system in the silkworm Bombyx mori. Sci Rep 5:16273

Wei Z, Yin YP, Zhang B, Wang ZK, Peng GX, Cao YQ, Xia YX (2007) Cloning of a novel protease required for the molting of Locusta migratoria manilensis. Develop Growth Differ 49:611–621

Xia Q, Zhou Z, Lu C, Cheng D, Dai F, Li B, Zhao P, Zha X, Cheng T, Chai C (2004) A draft sequence for the genome of the domesticated silkworm (Bombyx mori). Science 306(5703):1937–1940

Xia Q, Cheng D, Duan J, Wang G, Cheng T, Zha X, Liu C, Zhao P, Dai F, Zhang Z (2007) Microarray-based gene expression profiles in multiple tissues of the domesticated silkworm, Bombyx mori. Genome Biol 8(8):R162

Xia Q, Guo Y, Zhang Z, Li D, Xuan Z, Li Z (2009) Complete resequencing of 40 genomes reveals domestication events and genes in silkworm (Bombyx morie). Science 326:433–436

Xia X, Liying Yu l, Xue M, Yu X, Vasseur L, Gurr GM, Baxter SW, Lin H, Lin J, You M (2015) Genome-wide characterization and expression profiling of immune genes in the diamondback moth, Plutella xylostella (L.) Sci Rep 5:1–13

Xu QY, Lu AR, Xiao GH, Yang B, Zhang J, Li XQ, Guan JM, Shao QM, Beerntsen BT, Zhang P, Wang CS, Ling EJ (2012) Transcriptional profiling of midgut immunity response and degeneration in the wandering silkworm, Bombyx mori. PLoS One 7:43769

You M et al (2013) A heterozygous moth genome provides insights into herbivory and detoxification. Nat Genet 45:220–225

Zdobnov EM, Von Mering C, Letunic I, Torrents D, Suyama M, Copley RR, Christophides GK, Thomasova D, Holt RA, Subramanian GM, Mueller HM, Dimopoulos G, Law JH, Wells MA, Birney E, Charlab R, Halpern AL, Kokoza E, Kraft CL, Lai Z, Lewis S, Louis C, Barillas-Mury C, Nusskern D, Rubin GM, Salzberg SL, Sutton GG, Topalis P, Wides R, Wincker P, Yandell M, Collins FH, Ribeiro J, Gelbart WM, Kafatos FC, Bork P (2002) Comparative genome and proteome analysis of Anopheles gambiae and Drosophila melanogaster. Science 298:149–159

Zhao P, Wang GH, Dong ZM, Duan J, PZ X, Cheng TC, Xiang ZH, Xia QY (2010) Genome-wide identification and expression analysis of serine proteases and homologs in the silkworm Bombyx mori. BMC Genomics 11:405

Zhao P, Dong ZM, Duan J, Wang GH, Wang LY, Li YS, Xiang ZH, Xia QY (2012) Genome-wide identification and immune response analysis of serine protease inhibitor genes in the silkworm, Bombyx mori. PLoS One 7(2):e31168

Zou Z, Lopez DL, Kanost MR, Evans JD, Jiang H (2006) Comparative analysis of serine protease-related genes in the honey bee genome: possible involvement in embryonic development and innate immunity. Insect Mol Biol 15(5):603–614