Pattern recognition receptors from lepidopteran insects and their biological functions

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A R T I C L E I N F O
Keywords:
C-type lectin
Peptidoglycan recognition protein
Gram-negative binding protein
Galectin

A B S T R A C T
Lepidopteran insects have potent innate immunity to fight against the invading pathogens. As the initiation step, pattern recognition receptors (PRRs) recognize and bind to microbial surface configurations known as pathogen-associated molecular patterns (PAMPs). Afterward, they initiate both cellular and humoral immune responses, including phagocytosis, agglutination, nodulation, encapsulation, prophenoloxidase activation, and synthesis of antimicrobial peptides. In this review, we summarize the recent findings concerning PRRs in lepidopteran insects, mostly agriculture pests including \textit{Helicoverpa armigera}, \textit{Plutella xylostella}, and \textit{Spodoptera exigua}. We mainly focus on the function and phylogeny of C-type lectins (CTLs), peptidoglycan recognition proteins (PGRPs), β-1,3-glucan recognition proteins (βGRPs), and galectins (GALEs). It enriches our understanding of the immune system of lepidopteran insects and provides directions in the future research.

1. Introduction
Lepidoptera is the largest group of plant-feeding insects, playing important roles in natural ecosystems such as pollinators or as food for predators (Mitter et al., 2017). Lepidopteran larvae are often considered as serious agricultural pests. While many Lepidoptera seriously affect crop production, some species, such as the silkworm \textit{Bombyx mori} and the ghost moth \textit{Thitarodes xiaojinensis}, provide valuable economic resources. Lepidopteran insects provide important systems for studies of genetics, physiology, development, and innate immunity (Groot et al., 2016; Jones et al., 2019; Kanost et al., 2004; Triant et al., 2018).

Pattern recognition receptors (PRRs) are germline-encoded proteins, which play a crucial role in the initial sensing of nonself pathogen infection (Brubaker et al., 2015). In the innate immune systems of insects, PRRs recognize and bind to conserved molecules, which are called pathogen-associated molecular patterns (PAMPs) located on the pathogen surface (Stokes et al., 2015). PRRs trigger both cellular and humoral immune responses, including phagocytosis, agglutination, encapsulation, melanization activation, and induction of antimicrobial peptides (AMPs) (Dubovsky et al., 2016; Lavine and Strand, 2002; Lemaître and Hoffmann, 2007; Stokes et al., 2015). In lepidopteran insects, there are several types of PRRs, including peptidoglycan recognition proteins (PGRPs), β-1,3-glucan recognition proteins (βGRPs)/Gram-negative binding proteins (GNBPs), galectins (GALEs), C-type lectins (CTLs), fibrinogen-related proteins (FREPs), thiostrepton proteins (TEPs) and scavenger receptors (SCRs) (Meng et al., 2015; Tanaka et al., 2008; Vogel et al., 2011; Xia et al., 2015; Xiong et al., 2015; Zhang et al., 2015).

Based on biochemical and immunotranscriptomic analysis, the putative PRR genes have been identified in many lepidopteran species, including \textit{B. mori} (Hou et al., 2014; Tanaka et al., 2008; Wu and Yi, 2018), \textit{Manduca sexta} (Cao et al., 2015; Zhang et al., 2015), \textit{Helicoverpa armigera} (Wang et al., 2010; Xiong et al., 2015), \textit{Plutella xylostella} (Lin et al., 2018a), and \textit{T. xiaojinensis} (Meng et al., 2015) (Table 1). Here we review lepidopteran PRRs that sense the presence of pathogens and parasites, mainly CTL, PGRP, βGRP, and GALE. Since PRRs from \textit{M. sexta} and \textit{B. mori} have been well reviewed (Xia et al., 2018), here we focus on lepidopteran PRRs besides those of these two species.
2. CTLs from lepidopteran insects in immune responses

CTLs are a large family of carbohydrate-binding proteins with characteristic carbohydrate-recognition domains (CRDs), and are also known as C-type lectin-like domain (CTLD) proteins. In most cases, the binding of CTLs is Ca\(^{2+}\) dependent (Dodd and Drickamer, 2001; Drickamer and Taylor, 2015). Several CTLs have been suggested to mediate important immune defense mechanisms, such as agglutination, nodulation, encapsulation, melanization and activation of phagocytosis (Ling and Yu, 2006; Sun et al., 2017; Wang and Wang, 2013; Wang et al., 2014; Xia et al., 2018; Zhang et al., 2016). Numerous genes encoding CTL proteins have been identified in many insect immune transcriptomes and genomes (Christophides et al., 2002; Evans et al., 2006; Gasmí et al., 2018; Gerardo et al., 2010; Meng et al., 2017; Rao et al., 2015a, 2015b; Shen et al., 2018; Waterhouse et al., 2007; Xia et al., 2015; Xiong et al., 2015; Zou et al., 2007). The number of CTL genes in lepidopteran insects varies from only seven in *P. xylostella* (Xia et al., 2015) to 34 in *M. sexta* (Rao et al., 2015a) (Table 2).

Genome-wide analyses have revealed that the CTLs in lepidopteran insects contain either a single-CRD or two tandem-CRDS. The dual-CRD CTLs are also known as immulectins, and were first reported in *M. sexta* in response to bacterial challenges (Yu et al., 1999). All of the lepidopteran insects have the dual-CRD CTLs, from only one in *P. xylostella* (Xia et al., 2015) to 23 in *T. xiaojinensis* (Meng et al., 2017) (Table 2). In contrast to dipteran, hymenopteran, coleopteran and hemipteran insects, none dual-CRD CTL was found, except *Tricholium castaneum* (Zou et al., 2007) that has one member.

Phylogenetic analysis of single-CRD CTLs from insects shows one clade including 12 members from *Spodoptera exigua* (Fig. 1). Since their amino acid sequences show high similarity to baclovirus lectins, this being named as *S. exigua* bracovirus-like lectins (SeBLLs). SeBLLs are proposed to be derived from horizontal gene transfer events from baclovirus and further domesticated by the *S. exigua*. Three distinct clades are present with the respective CTLs from the six lepidopteran species (Table 2) and other insects that are distant from other branches and are characterized as galactose binding CTLs with the QPD (Gln-Pro-Asp) signature sequence (Fig. 1). However, in contrast to the single-CRD CTLs, the dual-CRD CTLs from *M. sexta, H. armigera* and *T. xiaojinensis* are in the same clade, suggesting that these species have undergone significant evolutionary pressure leading to family expansion (Fig. 2).

Table 1

<table>
<thead>
<tr>
<th>Genotype</th>
<th>B. mori</th>
<th>M. sexta</th>
<th>H. armigera</th>
<th>P. xylostella</th>
<th>T. xiaojinensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGRP</td>
<td>12</td>
<td>14</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>βGRP/GNBP</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>18</td>
<td>4</td>
</tr>
<tr>
<td>GALE</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>CTL</td>
<td>23</td>
<td>34</td>
<td>26</td>
<td>7</td>
<td>32</td>
</tr>
<tr>
<td>FREP</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>TEP</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>SCR</td>
<td>18</td>
<td>10</td>
<td>15</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>67</td>
<td>64</td>
<td>59</td>
<td>56</td>
<td>64</td>
</tr>
</tbody>
</table>

Note: “–” indicates no information from current references.
Ovomermis sinensis is an entomopathogenic nematode that is parasitic during its developing stages in the hemocoel of the host. The number of nematodes emerged per larva significantly decreased following O. sinensis pre-coated with rHaCTL3 or H. armiger pre-injected with rHaCTL3. The endogenous HaCTL3 or the exogenous rHaCTL3 directly bind to the surface of O. sinensis. The encapsulation and melanization of H. armigera are promoted by rHaCTL3 and are suppressed through knockdown of HaCTL3 (Wang et al., 2017a).

HaCTL7 has a signal peptide that is synthesized in the fat bodies and then secreted into the hemolymph. The peptide contains two CRDs, and the C-terminal CRD2 of HaCTL7 has four conserved cysteine residues, not six as in other dual-CRD immunolectins. Both the recombinant HaCTL7 (rHaCTL7) and individual recombinant CRD2 (rHaCTL7-CRD2) have the agglutination ability against Gram-positive bacteria and negative bacteria in a Ca²⁺-dependent manner, whereas the individual recombinant CRD1 (rHaCTL7-CRD1) does not. Agglutination assays have shown that their agglutination capability to E. coli are inhibited by mannose, trehalose, PGN, and LPS. HaCTL7 binds to various bacteria due to its CRD2, while the binding activity to E. coli is inhibited only by PGN. Chromatography beads treated with rHaCTL7 or rHaCTL7-CRD2 can enhance cellular encapsulation in vitro, and the treated beads stimulated melanization in the larval hemocoel. Based on immunocytochemistry analysis, HaCTL7 is able to bind to granulocytes, plasmatocytes and oenocytoids, but not spherulocytes; rHaCTL7 and rHaCTL7-CRD2 bind to both granulocytes and

### Table 2
The domain structure of CTLs from lepidopteran insects.

<table>
<thead>
<tr>
<th>Reference</th>
<th>M. sexta</th>
<th>B. mori</th>
<th>H. armigera</th>
<th>P. xylostella</th>
<th>T. xiaojinensis</th>
<th>S. exigua</th>
<th>Ostrinia furnacalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number</td>
<td>34</td>
<td>23</td>
<td>26</td>
<td>7</td>
<td>32</td>
<td>25</td>
<td>14</td>
</tr>
<tr>
<td>Single-CRD</td>
<td>9</td>
<td>12</td>
<td>9</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Dual-CRD</td>
<td>19</td>
<td>6</td>
<td>15</td>
<td>1</td>
<td>23</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>CTL-X</td>
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<td>5</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Signal peptide</td>
<td>26</td>
<td>15</td>
<td>25</td>
<td>6</td>
<td>24</td>
<td>22</td>
<td>11</td>
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<td>Transmembrane region</td>
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<td>3</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: CTL-X indicates CTLs contain one CRD and other domains.
oenocytoids, and rHaCTL7-CRD1 can only bind to granulocytes. These results show that agglutinating, encapsulation, and melanization of HaCTL7 relies more on its C-terminal CRD2 with four cysteine structure, but not N-terminal CRD1. The latter may be involved in other immune processes, such as phagocytosis, due to its binding to granulocytes (Wang et al., 2014b).

HaCTL14 contains two tandem CRD, and presents a mannos-specific EPN (Glu-Pro-Asn) motif in the first CRD. The recombinant HaCTL14 (rHaCTL14) agglutinates the entomopathogen Beauveria bassiana in a Ca$^{2+}$-dependent manner. After being challenged by B. bassiana, HaCTL14 is only activated in the fifth instar but not in the second instar larvae, leading to the speculation that HaCTL14 may be the key factor modulating the age-dependent immunity of H. armigera against fungi. The phagocytic ability of B. bassiana in fifth instar larvae is higher than that in second instar H. armigera hemocytes, suggesting that the activity may be mediated by HaCTL14. Using immunoprecipitation and LC-MS/MS assay, 25 hemolymph proteins are identified with HaCTL14 following infection, including the serine proteinase (SP), serine proteinase inhibitor (serpin), prophenoloxidase (PPO), and vitellogenin (Vg). Double-stranded RNA (dsRNA)-mediated RNA interference (RNAi) in H. armigera was employed to assess the role of HaCTL14 in B. bassiana infection. Knockdown of HaCTL14 can inhibit the melanization pathway through down-regulation of SPs and up-regulation of serpins. HaVg interacts with HaCTL14 under the condition of fungal infection, implying its role in larval anti-fungal immunity (Cheng et al., 2018).

2.2. Other lepidopteran CTLs

2.2.1. S. exigua CTLs

S. exigua (Lepidoptera: Noctuidae), the beet armyworm, is a pest of many vegetable crops. Twenty-five CTLs were identified from immunotranscriptome of S. exigua (Pascual et al., 2012). These CTLs are divided into two groups based on the phylogenetic and sequence analysis. One group have 13 members that are homologous to CTLs from other lepidopteran insects, and they are named as S. exigua lepidopteran-like lectins (SeLLs). The other group including 12 members are SeBLLs (Fig. 1). Allof the SeBLLs have a single CRD that contains four conserved cysteine residues and one FXCE (Phe-X-Cys-Glu) motif (Gasmi et al., 2018). These SeBLLs homologue CTLs are found in other Spodoptera species, such as Spodoptera frugiperda, Spodoptera littoralis and Spodoptera litura. One hypothesis is that SeBLLs are obtained by horizontal gene transfer (HGT) from bracoviruses and further domesticated by the host (Gasmi et al., 2015).

Three recombinant lectins (rSeBL1, rSeBL2, and rSeBL3) agglutinate Gram-positive and negative bacteria in a Ca$^{2+}$-dependent manner in vitro. The agglutination capabilities to E. coli of rSeBL1 and rSeBL2 are inhibited by N-acetylglactosamine (GalNAc), and rSeBL3 is only inhibited by galactose. Among all the CTLs reported in insects, the SeBLLs are able to induce a broad range of bacterial species agglutination at the lowest concentration. However, SeBL1 and SeBL2 show high amino acid sequence similarities with only one residue variance, Tyr$^{100}$ (Y) in SeBL1 and Cys$^{100}$ (C) in SeBL2 (Gasmi et al., 2017).
The transcript level of SeBLL2 or SeBLL6 in the midguts is up-regulated after infection with *S. exigua* multiple nucleopolyhedrovirus (SeMNPV). When rSeBLL2 and SeMNPV fed to the *S. exigua* larvae, the rate of mortality is reduced by half compared to larvae fed with SeMNPV. Similarly, when *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) was preincubated with rSeBLL2, the viral infectivity to SF21 cells was suppressed in a dose-dependent manner (Gasmi et al., 2015). After infection with *Junonia coenia* densovirus (JcDV), a pathogenic virus for *Spodoptera* species, the expression of SeBLL2 and SeBLL6 in hemocytes was significantly reduced, while SeBLL4 in the midgut is up-regulated (Wang et al., 2013). The rSeBLL1, rSeBLL2, and rSeBLL3 had no effect on *S. exigua* tolerance to JcDV. However, when *S. frugiperda* larvae were orally infected with JcDV in the presence of rSeBLL3, the mortality rate was significantly reduced compared to larvae infected with JcDV only (Gasmi et al., 2018). All together, these results show that CTLs in *Spodoptera* species provide protection during anti-viral response.

2.2.2. Mythimna separate CTLs

*Mythimna separate* (Lepidoptera: Noctuidae), the rice armyworm, is a serious migratory pest of a variety of cereal and forage crops. A secretory dual-CRD lectin was identified as the gene involved in phagocytosis. However, the mRNA expression level of this gene was only significantly up-regulated by injection of the magnetic beads with diameters up to 90 μm. As this gene acts on encapsulation against larger invaders, it was named encapsulation promoting lectin (MseEPL). The recombinant MseEPL (rMseEPL) increased the rate of encapsulated beads (45 μm) and decreased the number of phagocytosed beads (1 μm) in an *in vitro* assay. MseEPL regulates cellular immunity by recognizing different sizes of immune targets. (Ishihara et al., 2017).

2.2.2.2. Pieris rapae CTLs

*Pieris rapae* (Lepidoptera: Pieridae), the cabbage white butterfly, is a worldwide pest of many vegetable crops. PrCTL was identified to be involved in distinct immune responses against Gram-positive bacteria, Gram-negative bacteria, and parasitoid wasp. *Pteromalus puparum*, is a pupal parasitoid of *P. rapae* that injects venom during oviposition to inhibit host cellular immune responses. When beads and the venom from *P. puparum* were injected together into *P. rapae*, the expression level of PrCTL was suppressed. Based on immunocytochemistry and immunoblotting analysis, PrCTL is mainly synthesized in hemocytes and then secreted into the plasma. After different kinds of immune challenge, PrCTL consistently showed higher expression in granulocytes than in plasmacytocytes. After knockdown of PrCTL through dsRNA-mediated RNAi, five immunity-related genes *Perca*, *Ply*, *PrPAP1*, *PrPAP3* and *PrSR* were down-regulated. The hemolymph antimicrobial activity, phenoloxidase (PO) activity, hemocytes phagocytosis and encapsulation ability were suppressed in the dsPrCTL-injected group compared with the control groups in an *in vitro* assay (Fang et al., 2011).

3. Downstream biological reactions of CTLs

3.1. Immune reactions

3.1.1. Antimicrobial immunity

Many studies have focused on the CTLs expression profiling of lepidopteran insects during bacterial, fungal, viral, and parasitic infection (Hou et al., 2014; Meng et al., 2017; Pascual et al., 2012; Shen et al., 2014; Vogel et al., 2011; Wang et al., 2010; Wu and Yi, 2018; Xia et al., 2015; Xing et al., 2017; Xiong et al., 2015; Zhang et al., 2018). Based on the transcriptomic and proteomic analysis, the expression levels of *HaCTL5* and *HaCTL7* were significantly reduced following nucleopolyhedrovirus infection (Xing et al., 2017). *Campothrix chlorideae* is an endoparasitoid wasp of many noctuid species; the wasp lays eggs into the hemocoele of its host. The C-type immunelectins of *H. armigera* (8 members, named as HaCTL1 to HaCTL8) showed dramatic changes in response to the *C. chlorideae* parasitization. The results suggest that *HaCTLs* (HaCTL1 to HaCTL8) might be involved in both inhibiting and promoting host immune defenses against parasitization (Wang et al., 2017b).

After infection with fungus *Ophiocordyceps sinensis*, larvae of *T. aoxajinensis* would survive 5–12 months and turn to mumification. After challenges by *Op. sinensis*, Cordyceps militaris or Enterobacter cloacae respectively, several *TxCTLs* were significantly induced. However, the expression of the *TxCTLs* was suppressed in the mumified larvae after one year being infected by *Op. sinensis*. This suggests that the *TxCTLs* may play an important role in the immune interactions between *T. aoxajinensis* and *Op. sinensis* (Meng et al., 2017).

3.1.2. Cellular immunity

Phagocytosis and encapsulation depend upon recognition of the non-self target followed by activation of down-stream signaling and effectors. In lepidopteran insects, granulocytes and plasmacytocytes are the main hemocyte types involved in phagocytosis and encapsulation (Lavine and Strand, 2002; Strand, 2008). Among the four functional validated dual-CRD CTLs of *H. armigera*, HaCTL1 and HaCTL14 participate in phagocytosis, while HaCTL3 and HaCTL7 play important roles in encapsulation (Chai et al., 2008; Cheng et al., 2018; Wang et al., 2014b, 2017a).

The rHaCTL3 promoted hemocytic encapsulation of protein-coated beads *in vitro*. After knockdown of HaCTL3 through dsRNA-mediated RNAi, the ratio of beads encapsulated was significantly reduced. HaCTL3 interacts with *H. armigera* β-integrin (Haβ-integrin) as shown by co-immunoprecipitation analysis. Haβ-integrin is a transmembrane receptor located on the surface of hemocytes. After knockdown of Haβ-integrin, the HaCTL3-coated beads encapsulation ability was impaired. According to these results, Haβ-integrin may be a receptor for HaCTL3 during encapsulation, and might be a mediator of cytoskeletal linkages with the extracellular matrix (Wang et al., 2017a).

The rMseEPL promotes hemocytic encapsulation, while suppressing phagocytosis. It has been speculated that rMseEPL may cause the shape of hemocytes to flatten. This process increases the adherent properties of hemocytes but inhibits the internalization of phagocytosis (Ishihara et al., 2017).

Upon reduction of PrCTL expression by RNAi, both the rate of phagocytosis of *E. coli* and encapsulation of beads decreased (Fang et al., 2011). The encapsulation carried out by host hemocytes is one of the major immune responses toward parasitoid wasps. As a recognition receptor, the rPrCTL can directly bind to the egg surface of the parasitoid wasp. Venom inhibits expression of PrCTL and also down-regulates the rate of encapsulation. These results indicate that PrCTL recognizes and binds to the immune invader, thus activating the immune system (Fang et al., 2011).

3.1.3. PPO activating system

CTLs can also enhance melanization, which is an important defense mechanism. The melanization pathway consists of a cascade of clip domain SPs (cSPs) that converts the inactive zymogen PPO to active PO, which is accurately regulated through their specific inhibitors, serpins. Active PO catalyzes the conversion of monophenols to o-diphenols, then oxidation to quinones, and the eventual formation of melanin is accurately regulated through their specific inhibitors, serpins. The plasma PO activity is suppressed in HaCTL3-depleted larvae (Wang et al., 2017a). The plasma PO activity is suppressed in HaCTL3-depleted larvae (Wang et al., 2017a). HaCTL4 interacts with some melanization-related proteins as shown by immunoprecipitation analysis. Compared to the EGP, dsRNA inoculated control, knockdown of HaCTL4 significantly influences these melanization-related proteins, reduces the gene expression levels of HaPSP6, HaSPH11, HaSPH50 and HaPPO1, and up-regulates the Haserpin-3. Meanwhile, the PO activity of *H.
armigera hemolymph is increased after B. bassiana infection, and decreased by dsHaCTL14 injection (Cheng et al., 2018). In P. rapae, after knockdown of PrCTL, the gene expression levels of PrPAP1 and PrPAP3 is down-regulated, and the PO activity is suppressed (Fang et al., 2011). Although many evidences indicated that CTL is probably involved in the melanization, but how CTL stimulates the downstream reactions is still unclear.

3.2. Physiological processes

In insects, steroid hormone 20-hydroxyecdysone (20E) plays important roles in multiple physiological and developmental processes, such as molting, metamorphosis, reproduction, and apoptosis (Kozlova and Thummel, 2000; Matsui et al., 2011). The level of 20E dramatically increases during the wandering stage relative to the feeding stage. This hormone also regulates the humoral immunity in the fat body of H. armigera (Wang et al., 2014a). Expression of HaCTL1 mRNA in larval hemocytes and fat bodies is induced by injection of 20E or RH-2485, a non-steroidal ecdysone agonist (Chai et al., 2008). The gene expression levels of HaCTL3 and HaCTL8 are dramatically induced post 20E injection, while HaCTL4, HaCTL5, and HaCTL6 is down-regulated (Wang et al., 2012). After knockdown of the 20E receptor (EcR) and ultraspiracle (USP), the expression of HaCTL3 is suppressed (Wang et al., 2017a). Diapause is a critical mechanism for H. armigera in harsh environmental conditions. The expression of HaCTL7 is significantly increased at the late stage of the sixth instar of diapause-destined larvae (Zhang et al., 2013). HaVg is another member captured by the HaCTL14 antibody from B. bassiana-challenged hemolymph that plays an essential role in insect development and egg maturation. HaVg shows high expression level in the fat body of fifth instar larvae, and significantly down-regulated under the condition of B. bassiana infection (Cheng et al., 2018).

4. Other PRRs from lepidopteran insects in immune responses

4.1. PGRPs

PGRPs are characterized by at least one PGRP domain homologous to bacteriophage and bacterial type 2 amidases (Kang et al., 1998). Based on the sequence length and the presence of the transmembrane domain, PGRPs could be divided into long (L) and short (S) forms. PGRPs, conserved from insects to humans, were firstly identified in the silkworm, B. mori (Yoshida et al., 1996). There are 12, 13, and 7 PGRP genes identified in the genomes of B. mori (Tanaka et al., 2008), Droso-phila melanogaster (Kurata, 2014), and Anopheles gambiae (Christophides et al., 2002), respectively. The immunotranscriptome of H. armigera has identified at least nine PGRPs (Xiong et al., 2015). PGRPs are a type of PRR that executes in initiating innate immune reactions. PGRPs are capable of recognizing peptidoglycans (PGNs) present in the cell wall of almost all bacterial species (Hetru and Hoffmann, 2009). PGNs are categorized into two types, namely the lysine type (Lys-type) PGNs in Gram-positive bacteria and the diaminopimelic acid type (Dap-type) PGNs found in Gram-negative bacteria and Bacillus. PGRPs differentiate these two types of PGNs and trigger different signaling pathways, such as the immune deficiency (IMD) pathway and the Toll pathway (Choe et al., 2002; Kaneko et al., 2004; Mellroth et al., 2005; Michel et al., 2001). PGRPs also participate in activation of PPO (Li et al., 2015; Sumathipala and Jiang, 2010),
facilitating hemocyte-mediated phagocytosis and encapsulation (Li et al., 2014; Ramet et al., 2002), and digestion of PGN to exhibit bactericidal activity (Mellroth et al., 2003).

Recent studies suggest that involvement of PGRPs in interactions with malarial parasites depend on the presence of gut microbiota (Gendrin et al., 2017). PGRP-LD functions in limiting Plasmodium infection by protecting the gut microbiota and promoting the peritrophic matrix structural integrity (Song et al., 2018). Silencing of PGRP-LE leads to a reduction of Wolbachia load, suggesting its role in facilitating colonization by these symbiotic bacteria (Pan et al., 2018). PGRPs also play critical roles in defending against viral infection by activating Toll or IMD pathways. Over-expression of Bombyx PGRP-S2 activates the IMD pathway and enhances antiviral resistance (Zhu et al., 2018). Apart from that, PGRPs mediate edcsyone to enhance innate immunity (Han et al., 2017; Rus et al., 2013). In addition to the involvement in immunity, PGRPs also participate in metabolic pathways and juvenile growth (Erickson et al., 2015) and in regulating homeostatic synaptic plasticity (Harris et al., 2015).

4.2. GNBPs

GNBPs also known as βGRPs, are biosensors that can bind to β-1,3-glucan in the cell wall of Gram-negative bacteria and initiate the immune responses in insects. The numbers of βGRPs shows large variation in Lepidoptera. In Manduca sexta and Helicoverpa armigera, the numbers of βGRPs are four and five, respectively, while in P. xylostella the number is 18, which illustrates the functional diversity of βGRPs (Xia et al., 2015; Xiong et al., 2015). The βGRP3 in Ostrinia furnacalis larvae could be activated by E. coli or Bacillus subtilis infection, and this increased the activity of PPO (Wu et al., 2018). In addition to activation of PPO, βGRP1 in P. xylostella also has the function of positive regulation in the expression of AMPs like cecropins (Huang et al., 2015). The expression levels of βGRP4a/b were significantly induced in resistant eighth instar larvae of Thitarodes pui and repressed in susceptible sixth instar after being challenged with conidia of B. bassiana implying their host defense functions against fungi (Sun et al., 2011).

4.3. GALEs

GALEs are an ancient family of β-galactoside binding proteins with CRDs. They are widely recognized for their participation in immune regulation and development (Kamhawi et al., 2004). Genome wide analyses revealed that the GALEs in dipteran insects contained 5, 8, and 12 transcripts in D. melanogaster, A. gambiae, and Aedes aegypti, respectively (Waterhouse et al., 2007). However, the number of GALEs shows small variation in lepidopteran insects, except for the three GALE genes in H. armigera, the other species contain four members (Table 1). The phylogenetic relationship shows that the GALEs in lepidopteran insects form four specific clades and displays a 1:1:1 orthologous relationship in H. armigera, M. sexta and B. mori (Fig. 3). The blue clade includes the dual-CRD GALEs from lepidopteran insects showing putative 1:1 orthologues (Fig. 3).

Galectins are the evolutionary conserved protein family distributed in vertebrates, invertebrates, and fungi. Originally, they were considered to only bind endogenous glycans. However, recent studies indicated that galectins also recognize glycans on the surface of microbes and parasitic worms and regulate immune homeostasis (Cerliani et al., 2011; Vasta, 2012). BmALECT4 is the only dual-CRD GALE in the silkworm, and is highly expressed in eggs. The recombinant BmALECT4 could agglutinate bacteria, but does not possess antibacterial activity (Rao et al., 2016). Although not many function studies were performed on lepidopteran GALEs, several GALEs from vertebrates or nematodes were applied for insecticidal activity. Mammalian GALE (GAL1) was identified to disturb the larval development of P. xylostella by interacting with chitosan/chitin, the essential module of peritrophic membrane (Chen et al., 2009). Nematode GALE (LEC-8) interacts with glycolipid in the midgut of H. armigera, thus enhancing the insect tolerance to Bt toxin (Ma et al., 2012).

5. Perspectives

Since different lepidopteran species survive under different biotic stresses, they have evolved diverse immune system to defend against various pathogens. Many entomopathogens such as fungi B. bassiana (Cheng et al., 2018; Xiong et al., 2015), baculovirus nucleopolyhedrovirus (Yuan et al., 2017), parasitoid wasp Microplitis mediator (Lin et al., 2018b, 2019b; Volovych et al., 2019), and merimidith nematode O. sinensis (Wang et al., 2019) are promising for pest control. Hence, clarification of the interaction between various pathogens and insect hosts would contribute to production of biological control agents. Importantly, the components present on the surface of pathogens recognized by PRRs should be specifically identified. Here, we discussed the function and phylogeny of CTLs, PGRPs, βGRPs, and GALEs. The function of FPRPs, TEPs, and SCRs are not studied very well in lepidopteran insects, except that SCR-C was found to be the receptor for Vip3Aa, a vegetative insecticidal protein in B. thuringiensis, in S. frugiperda cells (Jiang et al., 2018).

The interactions between PRRs and other proteins to exert their physiological functions, such as a SRC interacting with β-arrestin2 to induce the internalization of Virus (Yang et al., 2016), CTLs interacting with β-integrin to promote phagocytosis or encapsulation (Wang et al., 2014c, 2017a), and a CTL collaborating with a CD45 phosphatase homologue to facilitate virus infection (Cheng et al., 2010). Hence, in order to clarify the function of PRRs and the signaling pathway that PRRs are involved in, more PRR-interacting proteins need to be characterized under the physiological conditions. Microbiota may influence host insect development (Shin et al., 2011; Storelli et al., 2011) and are some are controlled by PRRs (Pan et al., 2018). It has been suggested that certain PRRs may be involved in modulating insect development through limiting or facilitating colonization by microbiota. Hence, identification of PRRs involved in modulating insect physiology and clarification of the underlying mechanism should become potential research directions.

Acknowledgements

This work was supported by the National Key Plan for Scientific Research and Development of China (No. 2017YFD0200400), National Science Foundation of China (No. 31872998), and Key Laboratory of Vector Biology and Pathogen Control of Zhejiang Province (HUZUL201901).

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