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Review

The immune response of silkworm, *Bombyx mori*

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The silkworm, *Bombyx mori* has been used to study numerous biological phenomena including innate immune response. *B. mori* could be infected by various pathogens such as bacteria, fungi and virus. After infection, these microorganisms induce different types of immune response, including humoral immune responses which secrete antimicrobial peptides (AMPs) into the hemolymph and cellular immune responses which engulfed invading microorganisms by plasmatocytes. Meanwhile, *B. mori* could be also efficiently killed by infection with human pathogens such as *Staphylococcus aureus, Streptococcus* pyogenes or Serratia marcescens when these bacterial are injected into the blood. It can now be used as an animal model for bacterial infection study. Here, we will review our current knowledge on molecular mechanisms of antimicrobial peptides production as well as recent progress made in the field of innate immunity in *B.mori*.

Key word: *Bombyx mori*, innate immune response, Toll/IMD pathway, phagocytosis.

INTRODUCTION

The innate immune system is the first-line defense mechanism against various bacteria and viruses infection. Defensive strategies in insect and in mammals are highly conserved at the molecular level, which will help to better understand the molecular mechanisms of innate immunity (Hoffmann et al., 1999; Kimbrell and Beutler, 2001; Muller et al., 2008). It was widely accepted that invertebrates fail to show a high degree of adaptive immune system (Flainik and Du Pasquier, 2004; Loker et al., 2004; Rowley and Powell, 2007). The innate immune responses which include production of antimicrobial substances, phagocytosis, encapsulation, clotting, nodule formation and melanization become the main defensive strategies for insects (Lemaitre and Hoffmann, 2007).

For the completion of the genome sequence of lepidopteran model insect, the silkworm *Bombyx mori,* it has now become the standard model system for pathological and genetic studies in insect (International Silkworm Genome, 2008; Mita et al., 2004; Xia et al., 2004, 2009). For defense against various infections, *B. mori* relies on three types of mechanisms (Ferrandon et al., 2007; Williams, 2007; Yamakawa and Tanaka, 1999). These include humoral immune responses which dependent on antimicrobial peptides (AMPs) secretion into the hemolymph from the fat body (a functional equivalent of the mammalian liver). The second is cellular immune responses, such as the phagocytosis of invading microorganisms by plasmatocytes. The third is an enzymatic cascade leading to melanization. In this review, we summarized recent progress in understanding innate immunity in *B. mori*.

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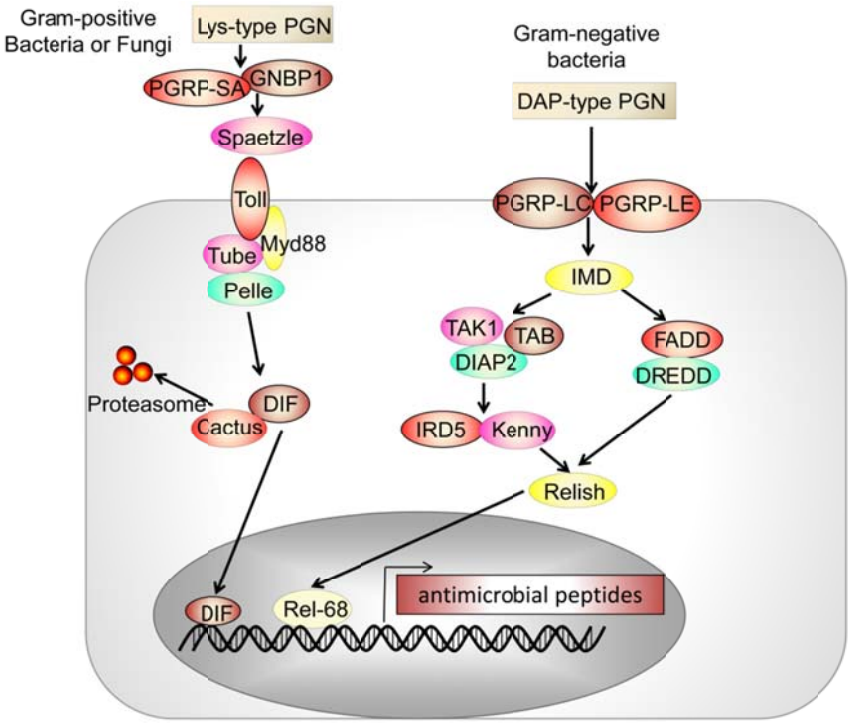

Figure 1. *B. mori* Toll and IMD pathway. The Toll pathway is activated by fungi and Gram-positive bacteria. The Lys-type PGN are recognized by a complex consisting of peptidoglycan recognition protein-SA (PGRP-SA) and Gram-negative binding protein 1 (GNBP1). Recognition of infection triggers a protease cascade that cleaves the precursor of Spatzle into an active form and activates the Toll receptor. Formation of this hetero-trimeric adapter complex leads to nuclear factor-κB (NF-κB)-dependent signaling and antimicrobial peptide (AMP) production. The immune deficiency (IMD) pathway is activated mainly by Gram-negative bacteria. The DAP-type PGN are recognized by a complex consisting of PGRP-LC and PGRP-LE. Recognition of infection induces the formation of a complex comprising of IMD. This complex induces the activation of two cascades and results in the activation of antimicrobial peptide (AMP) production.

HUMORAL IMMUNE RESPONSES

The hallmark of the *B. mori* immune response is the induction of antimicrobial peptides (AMPs) mainly synthesized by the fat body and secreted into the hemolymph (Cheng et al., 2006; Hu et al., 2013; Wu et al., 2010b). Since Steiner et al. (1981) purified and characterized the first AMPs from pupae of the cecropia moth, *Hyalophora cecropia*, more than 200 AMPs have been identified in insects (Bulet et al., 1999). In *B. mori*, 35 AMPs have been found in silkworm genome sequences (Cheng et al., 2006; Kaneko et al., 2008; Wen et al., 2009). These AMPs can be classified into six classes: cecropins, attacins, moricin, lebocin, defensins and gloverines (Bulet et al., 1999; Cheng et al., 2006; Govind, 2008; Imamura et al., 2006).

The expression of the AMPs is regulated by Toll and immune deficiency (IMD) pathways in the fat body (Figure 1). Fungi and Gram-positive bacteria induce the expression of AMP through the Toll signaling pathway, and Gram-negative bacteria induce the expression of AMP through the IMD signaling pathway. Toll and the IMD pathways are reminiscent of mammalian Toll-like receptor (TLR) and tumor necrosis factor-a (TNF-a) pathways (Govind, 2008; Hoffmann, 2003; Lemaitre and Hoffmann, 2007).

B. mori Toll pathway are composed of extracellular cytokine Spaetzle (or spätzle), transmembrane receptor Toll, MyD88 adaptors, tubes, Pelle kinase, Dorsal and DIF transac-tivators (Tanaka et al., 2008).

Spaetzle (which is similar to nerve growth factor and coagulogen (Mizuguchi et al., 1998)) was first found as a maternal effect gene that binds to the *Drosophila* Toll receptor. It activates the signal transduction pathways in both embryonic patterning and innate immunity (Morisato and Anderson, 1994). *B. mori* Spaetzle (BmSPZ1) was found to induce the expression of antimicrobial peptide genes when injected with renatured BmSpz1 into the silkworm larvae (Wang et al., 2007). In addition to BmSPZ1, two other Spaetzle homologs BmSPZ2 and BmSPZ3 were recently identified in the *B.mori* genome by

bioinformatic screening (Tanaka et al., 2008). Spaetzle is synthesized and secreted as an inactive precursor and cleaved by a serine protease cascade that are preferentially triggered by Gram-positive or by fungal infection (Valanne et al., 2011). After cleaving with its active C-106, the cleaved form of Spaetzle binds to the Toll receptor and activated Toll receptor. Unlike mammalian Toll-like receptors (TLRs), Toll does not function as a pattern recognition receptor that directly recognized the microbial ligands. Instead Lys-type peptidoglycan (PGN) is recognized either by the PGRP-SA–GNBP1 complex or by PGRP-SD (Lemaitre and Hoffmann, 2007; Valanne et al., 2011; Watanabe et al., 2006). To date, 11 Tolls and 2 Toll analogs genes have been identified in the *B. mori* genome (Cheng et al., 2008; Imamura and Yamakawa, 2002; Tanaka et al., 2008). Expression profiles of Bm Tolls showed that 10 of genes were induced or suppressed with different degrees by different invaders stimulation (Cheng et al., 2008). Bm Toll gene expression was strongly suppressed when lipopolysaccharide (LPS) was injected into silkworm hemocoel (Imamura and Yamakawa, 2002).

Escherichia coli, fungus and *Beauveria bassiana* infection also significantly increased BmToll9 expression in different parts of the gut, suggesting that BmToll9 is probably involved in the local gut immune response (Wu et al., 2010a). The expression of BmToll10-3 gene was significantly increased when infected with *B. mori* nucleopolyhedrovirus (BmNPV) (Sagisaka et al., 2010). The activated Toll receptor recruit a hetero-trimeric complexes composed of MyD88 (homologs of the human Myd88 protein), an adaptor protein Tube and the kinase Pelle (homologs of the human IL1 receptor associated kinase (IRAK)) (Leclerc and Reichhart, 2004; Valanne et al., 2011). *Bacillus bombysepticus* (Bb) infection induced the systemic immune response mainly by the Toll pathway in silkworm. Toll pathway genes including Spz1, Toll1, Toll6, MyD88, Tube and RelA were up-regulated after Bb infection. MyD88 was only expressed after Bb infection (Huang et al., 2009).

Formation of this hetero-trimeric adapter complex leads to rapid phosphorylation and degradation of Cactus (a homologue of the mammalian inhibitor of NF-KB (IKB)) by an uncharacterized mechanism, which is then degraded by the proteasome. As a consequence, the Rel transcription factors DIF are released and move from the cytoplasm to the nucleus (Kawaoka et al., 2008; Lemaitre and Hoffmann, 2007; Tanaka et al., 2005, 2009; Valanne et al., 2011). BmCactus, which constitutively expressed mainly in the fat body and hemocytes, can strongly inhibit activation of the CecB1 gene promoter by either BmRelA or BmRelB (Furukawa et al., 2009). Two BmRelish genes have been identified in the *B. mori*. *E. coli* infection induces the expression of BmRelish1 and BmRelish2 while deletion mutant of BmRelish of the BmRelish gene in transgenic silkworms resulted in failure of the activation of antimicrobial peptide genes (Tanaka et al., 2007).

The canonical components of *B. mori* IMD pathway are composed of IMD, TAK1 (TGF-β activated kinase 1), TAB, DIAP2, two IκB kinase (IKK) complex components, namely IKK-β (IRD5) and IKK-γ (Kenny), which are homologs of mammalian IKK signalosome. In addition, FADD functions as downstream of IMD, controlling the activity of DREDD (a caspase-8 homolog), which acts with the IKK complex to activate Relish (Brennan and Anderson, 2004; Kaneko and Silverman, 2005; Tanaka et al., 2008). Transcriptional profiling of midgut showed that IMD pathway, but not Toll pathway genes were up-regulated during the wandering stage, suggesting that IMD pathway probably regulates the production of antimicrobial peptides in the midgut during the wandering stage (Xu et al., 2012).

PGRP was first purified from hemolymph of the *B. mori* and was found to bind to peptidoglycan and triggered the prophenoloxidase cascade (Ochiai and Ashida, 1999b; Royet and Dziarski, 2007; Yoshida et al., 1996). Insect PGRP genes are divided into two subfamilies (short (S) and long (L) transcripts) based on their structure (Royet and Dziarski, 2007; Royet et al., 2011, 2005). The PGRP family comprises 12 members in *B. mori* with conserved PGRP domains (Figure 2). Six belong to the short (S) subfamily (Tanaka et al., 2008). Comparative proteomic approach identified that PGRP was up-regulated when reared on fresh mulberry leaves when compared with on artificial diet (Zhou et al., 2008). *B. mori* GNBP was found to constitutively express in fat body and rapidly induced following a cuticular or hemoceolien bacterial challenge (Lee et al., 1996). The solution structure of the N-terminal β-1, 3-glucan recognition domain of *B. mori* GNBP3 showed that GNBP3 is a β-1,3-glucan-recognition protein that specifically recognizes a triple-helical structure of β-1,3-glucan (Takahasi et al., 2009).

Cellular response

B. mori larvae contain several thousand hemocytes, which can be divided into the following three cell types on the basis of their structural and functional features: plasmatocytes, crystal cells and lamellocytes (Lemaitre and Hoffmann, 2007; Tan et al., 2013; Wago, 1982; Williams, 2007).

Plasmatocytes are professional phagocytes that function in the phagocytic removal of dead cells and microbial pathogens, which is most similar to the mammalian macrophage lineage and make up 95% of circulating hemocytes. The other 5% of circulating hemocytes consist of crystal cells, which secrete components of the phenol oxidase cascade for the melanization process, as well as for wound repair. A third cell type known as lamellocytes are rarely seen in healthy larvae and primarily function in encapsulation and neutralization of objects too large to be phagocytosed. Phagocytosis and encapsulation are two major mechanisms of the cellular response (Ling et al., 2005; Taniai et al., 1997).

B. mori are efficiently killed by infection with human

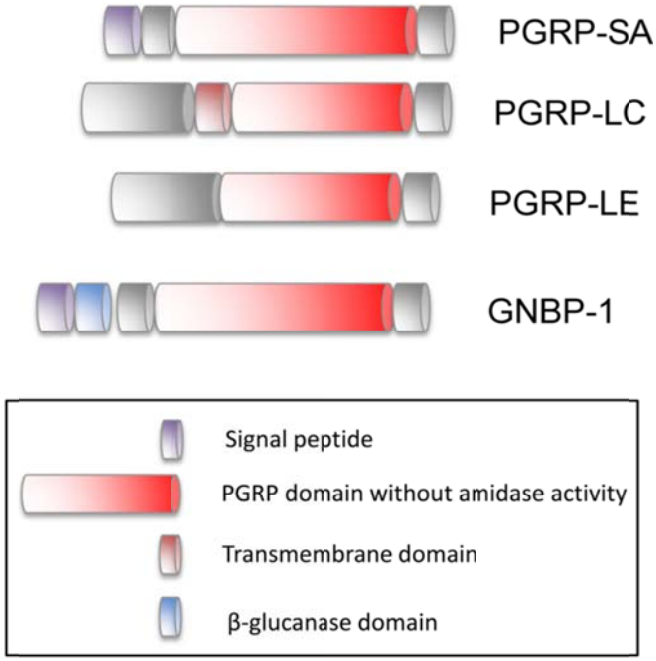

Figure 2. The structure of selected members of the PGRP and GNBP families.

pathogens such as *Staphylococcus aureus, Streptococcus pyogenes* or *Serratia marcescens* when these bacterial are injected into the blood (hemolymph) (Ishii et al., 2012). Silkworm larvae now have been used as an animal model of bacterial infection which is pathogenic to humans due to their low cost and ease of handling (Hamamoto et al., 2004; Ishii et al., 2012; Kaito et al., 2002, 2005; Kaito and Sekimizu, 2007). *B. mori* translationally controlled tumor Protein (BmTCTP) which expresses in intestinal epithet-lial cells and release into the gut lumen can promote the phagocytosis of invading substances by hemocytes (Wang et al., 2013). The phenoloxidase (PO) which exists in the hemocytes cascade regulates the melanization of invading pathogens in *B. mori* (Asano and Ashida, 2001; Diao et al., 2012; Ochiai and Ashida, 1999a; Wang et al., 2013). A high mass complex that contained PO was involved in hemolymph melanization in the *B. mori* (Clark and Strand, 2013). *B. mori* Reeler1 which was strongly induced by *E. coli* K12 and *B. subtilis* in silkworm larval hemocytess is involved in the *B. mori* melanization cascade (Bao et al., 2011).

CONCLUSION

As the completion of the genome sequence of *B. mori,* tremendous progress has been made in the past few years in *B. mori* research especially in *B. mori* innate immunity. However, the molecular mechanisms of *B. mori* response to the infection still need to be further clarified to help in understanding these conserved innate immune response both in insect and mammals.

Conflict of Interests

The authors have not declared any conflict of interests.

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