et al., 2016; Zhang et al., 2015). This implies a role for the intestine as the front line in defense against food-borne pathogens.

The fruit fly *Drosophila melanogaster* is an ideal model for the investigation of insect immune systems, and has been used extensively as such. The immune deficiency (IMD), Toll, and Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathways in this organism, as well as other defense responses, have been comprehensively deciphered (Bach et al., 2007; Brown et al., 2001; Dostert et al., 2005; Hoffmann, 2003; Hultmark, 2003; Lemaitre and Hoffmann, 2007; Wright et al., 2011). In contrast to our understanding of immune mechanisms in the fruit fly, those of the silkworm remain unclear. However, although the silkworm IMD, Toll, and JAK/STAT pathways are yet to be described, many laboratories have been working on the defensive responses of silkworms to infections by bacteria, viruses, fungi, and microsporidia, particularly since the sequencing of the silkworm genome (Xia et al., 2004). Based on the conservation of innate immune systems (Kang et al., 1998), studies of other insect species, especially lepidopterans, provide key clues that allow us to draw a picture of silkworm immune system. Here, we summarize studies of the silkworm immune system with a focus on responses to bacteria and fungi. Silkworm responses to viral and microsporidial infections are reviewed in other articles in this issue.

2. Immune responses to bacterial infection in the silkworm

Bacteria are important pathogens of insects, including silkworms, consuming host nutrients in order to rapidly proliferate. Bacterial shedding of peptidoglycans (PGs) or secretion of proteases may disrupt the host's cellular and biochemical processes (Karlsson et al., 2012; Kong et al., 2015). Over the course of their evolution, insects have developed innate but not adaptive immune systems to defend themselves against infections (Hoffmann, 2003; Hultmark, 2003; Tzou et al., 2002). These innate systems comprise the recognition of invading pathogens and subsequent production of effectors to eliminate them (Beutler, 2004; Brennan and Anderson, 2004; Hoffmann, 2003; Hultmark, 2003; Weber et al., 2003).

2.1. Bacterial pathogen-associated molecular patterns (PAMPs) and silkworm pattern-recognition receptors (PRRs)

Recognition of pathogens by insects relies on the interaction between PAMPs and PRRs. The principal bacterial PAMPs include PGs and lipopolysaccharide (LPS) (Akira et al., 2006; Karlsson et al., 2012; Medzhitov and Janeway, 2002; Yamakawa and Tanaka, 1999), although other elicitors produced by bacteria can also be recognized by insect PRRs (Charroux et al., 2009; Gottar et al., 2002; Hoffmann, 2003; Yamakawa and Tanaka, 1999). PGs are essential components of most bacterial cell walls, and are divided into two types based on structural differences: lysine (Lys) PGs from gram-positive bacteria, and diaminopimelic acid (DAP) PGs (Kurata, 2014). PGs have a stronger stimulatory effect than LPS on systemic humoral immunity in fruit flies (Akira et al., 2006; Kurata, 2014; Lemaitre and Hoffmann, 2007), and PGs from *Escherichia coli* and *Micrococcus luteus* are able to induce expression of certain antibacterial genes in silkworms, such as *cecropin B* and *lebocin 3* (Ha Lee et al., 2007; Tanaka et al., 2009). LPS (also known as endotoxin), the major and characteristic cell wall component of gram-negative bacteria, can trigger strong immune responses in most organisms (Raetz and Whitfield, 2002; Rietschel et al., 1994; Taniai et al., 1996). Production of the antibacterial protein Cecropin B is induced by LPS in the silkworm fat body and cell lines derived from this insect (Ha Lee et al., 2007; Taniai et al., 1996; Taniai and Tomita, 2000). Moreover, LPS promotes Cecropin A1 production in silkworm BmE cells (Hua et al., 2016; Morishima et al., 1997). These results clearly show that both PGs and LPS are immune stimulators in silkworms.

PGs are recognized by PG recognition proteins (PGRPs). Since the first PGRP was purified from silkworm hemolymph (Yoshida et al., 1996), more than 100 PGRPs have been identified in insects, including *D. melanogaster*, *Anopheles gambiae*, *Aedes aegypti*, and *B. mori*, and mammals (Christophides et al., 2002; Kang et al., 1998; Tanaka et al., 2008; Werner et al., 2000, 2003). Twelve silkworm genes have been identified that encode PGRPs (Tanaka et al., 2008), of which, the functions of BmPGRP-S1, -S2, -S4, -S5, and -L6 have been described (Chen et al., 2014, 2016; Tanaka and Sagisaka, 2016; Yang et al., 2015, 2017; Yoshida et al., 1996). BmPGRP-S1 binds to Lys-PGs from *M. luteus*, and BmPGRP-S4 recognizes both DAP-PGs and Lys-PGs from bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, and *Serratia marcescens*, but not *E. coli* (Yang et al., 2017). BmPGRP-S5 binds to DAP-PGs from *E. coli*, *Bacillus megaterium*, and *B. subtilis*, Lys-PG from *M. luteus*, and *S. aureus* (Chen et al., 2014, 2016). Finally, BmPGRP-L6 binds to Lys-PGs from *S. aureus* and DAP-PGs from *E. coli* and *B. subtilis* (Tanaka and Sagisaka, 2016; Yoshida et al., 1996). However, for most silkworm PGRPs, the events that take place following PG recognition and the regulation of these events remain obscure.

LPS is a component of gram-negative bacteria and can be recognized by secreted LPS-binding protein (LBP) in insects and mammals (Heumann et al., 2003; Kitchens and Thompson, 2005; Kopp et al., 2016; Ulevitch and Tobias, 1999). In humans, the LPS/LBP complex interacts with CD14, and LPS is delivered to Toll-like receptors to activate immune responses (Kitchens, 2000; Ranoa et al., 2013; Tapping and Tobias, 2000). LBP has been isolated from silkworm hemolymph by affinity precipitation using *E. coli*, and its involvement in the clearance of this bacterium from the hemolymph by nodulation has been demonstrated (Koizumi et al., 1999). However, the mechanism by which LBP recognizes LPS and activates AMP signaling and nodulation in the silkworms has not been determined.

2.2. Antibacterial defense in silkworms

The silkworm is not only a good invertebrate model to investigate the immune systems of lepidopteran insects (Tanaka et al., 2008), but also serves as an alternative to mammalian models for the evaluation of antibiotics and screening of bacterial virulence factors, including those of human pathogens such as *P. aeruginosa* and *S. aureus* (Kaito et al., 2002; Kurokawa et al., 2007; Panthee et al., 2017). To cope with infections, silkworm uses an array of strategies, such as generation of ROS, induction of AMPs, phenoloxidase (PO) mediated melanization, and cellular immune responses, to defend against harmful bacteria.

In insects, ROS can efficiently kill invading bacteria (Wink et al., 2011). Hydrogen peroxide (H_2O_2) and nitric oxide (NO) are the principal products of the ROS, the former being mainly produced by dual oxidase (Duox), and the latter being generated by nitric oxide synthase (NOS) (Ha et al., 2005a; Wink et al., 2011). *E. coli* induces *Duox* expression in the silkworm midgut, and larvae carrying a *Duox* deletion are more susceptible to bacterial infection (Hu et al., 2013). Concentrations of H_2O_2 and NO in the silkworm gut significantly increase upon *P. aeruginosa* and *Bacillus bombyseptieus* infection, and increased ROS levels can inhibit growth of these bacteria at the early stage of infection (Zhang and Lu, 2015; Zhang et al., 2015). NO functions both as an effector to kill pathogens and a messenger to induce immune responses (Nappi et al., 2000). Injection of LPS into silkworms induces *NOS1* expression, leading to production of NO, increased levels of which promote expression of *cecropin B* (Imamura et al., 2002). We have also shown that bacterial infection resulted in NOS1 upregulation in hemocytes, and

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increased NO in hemolymph after injection of bacteria or NO donors (sodium nitroprusside and diethylamine NONOate sodium) induceed AMP production in the fat body. Moreover, we established that the NO inhibitor L-nitroarginine methyl ester reduces silkworm AMP production during bacterial infection and results in decreased survival, whereas NO donors increase the survival of silkworms challenged with bacteria (unpublished data). These data indicate that ROS play an important role in inhibiting bacterial growth, maintaining homeostasis in the silkworm gut and hemolymph.

ROS act as a double-edged sword, given that low concentrations can kill pathogens, yet excessive levels are harmful to the proteins, nucleic acids, and lipids of host tissues (Hermes-Lima and Zenteno-Savin, 2002). Antioxidant enzymes such as catalase, superoxide dismutases, NADPH oxidase, and peroxiredoxins (Prxs) are responsible for eliminating excess ROS (Ha et al., 2005b; Shi et al., 2012b; Storr et al., 2013). *B. mori* catalase (BmCat) shares more than 50% sequence identity with catalases from other insects and mammals, including *D. melanogaster*, *Caenorhabditis elegans*, and humans. Recombinant BmCat functions as a ROS scavenger, degrading H_2O_2 (Yamamoto et al., 2005). Six Prxs have been shown to be involved in regulating ROS levels in the silkworm. Transcription of *BmPrx3* and *BmPrx5* is significantly upregulated in the midgut by exposure to *P. aeruginosa* and *S. aureus*, and the corresponding recombinant proteins can degrade H_2O_2 to protect DNA from oxidative damage (Kim et al., 2007; Lee et al., 2005; Shi et al., 2012a; Wang et al., 2008; Wang et al., 2016; Zhang and Lu, 2015). In addition, *P. aeruginosa* and *S. aureus* infection and H_2O_2 injection strongly induce expression of Oxidation resistance 1 (OXR1), which has an important antioxidant function through the JNK pathway (Su et al., 2017). These studies indicate that silkworm antioxidant enzymes are critical in the regulation of ROS levels following bacterial infection.

In contrast to ROS, AMPs are harmful to pathogens but not to the host. AMP production is mainly activated by NF-κB transcription factors through the Toll and IMD pathways in *D. melanogaster*. Gram-positive bacteria induce expression of AMPs, including Drosomycin, via the Toll pathway (Rutschmann et al., 2002), whereas gram-negative bacteria result in the production of others, including Diptericin, via the IMD pathway (Kaneko and Silverman, 2005). In silkworms, six families of AMPs (Attacin, Cecropin, Defensin, Gloverin, Lebocin, and Moricin) have been identified, the synthesis of members of which is triggered by bacteria, LPS, and PGs (Kaneko et al., 2007; Tanaka et al., 2008, 2009; Yamakawa and Tanaka, 1999). Compared to those of *D. melanogaster*, silkworm AMPs exhibit a broader spectrum of antimicrobial activity (Xia et al., 2013; Yang et al., 2011). Recombinant BmCecropinXJ exerts strong bactericidal effects against *S. aureus* (Xia et al., 2013), and we have confirmed that AMPs in the gut juice are able to inhibit *Yersinia pseudotuberculosis* and *B. bombyseptieus* growth, indicating the importance of these peptides in the silkworm midgut (unpublished data). *E. coli* and *B. subtilis* induce fat body-specific expression of Defensin B in the silkworm (Kaneko et al., 2008), and expression of Attacin, Cecropin, Defensin, Gloverin, Lebocin, and Moricin is induced in the fat body in response to *P. aeruginosa*. BmPGRP-S5 negatively regulates expression of these AMPs, likely through the IMD pathway (Chen et al., 2016). It has also been shown that BmCPT1 (Tweedle cuticular protein), BmPGRP-S5, and BmLBP work together to recognize *E. coli* and bring about AMP production (Liang et al., 2015). Isopropanol-killed *M. luteus* results in increased expression of Gloverin 3, Cecropin D, and Cecropin E in the fat body, and BmSerpin-5 downregulates the Toll pathway to reduce expression of these AMPs, probably by targeting BmHP6 and BmSP21 (Li et al., 2016a). Similarly, injection of recombinant BmSerpin-6 reduces *M. luteus*-induced expression of Gloverin 2 in the fat body and hemocytes (Li et al., 2017), and BmSerpin-15 downregulates Cecropin D, Gloverin 2, and Moricin expression stimulated by this bacterium in the fat body (Liu et al., 2015). The gram-positive bacterium *B. bombyseptieus* also activates AMP signaling, upregulating Attacin, Lebocin, Enbocin (which belongs to the cecropin family), Gloverin, and Moricin in the silkworm gut (Huang et al., 2009). In addition, PGs and LPS themselves have been shown to be strong elicitors of AMP signaling in the silkworm. In cells of the NISES-BoMo-Cam1 line, LPS from *E. coli*, *P. aeruginosa*, and *Salmonella enterica* Minnesota upregulates Cecropin B and Lebocin 3, and DAP-PG from *E. coli* appears to have a stronger stimulatory effect on AMP production than Lys-PG from *M. luteus* (Ha Lee et al., 2007). Bacterial and fungal cell wall components result in activation of the cytokine paralytic peptide (PP) from its precursor form in silkworms (Ishii et al., 2008), and activated PP strongly induces expression of Cecropin A and Moricin in the fat body (Ishii et al., 2010). These data suggest that AMPs are universal antibacterial effectors of the silkworm immune system. Some studies have indicated that Defensin B expression is mediated by both Toll and IMD pathways (Kaneko et al., 2008), and many AMPs are upregulated in response to both gram-positive and gram-negative bacteria (Chen et al., 2016; Huang et al., 2009; Wu et al., 2010b; Yang et al., 2011). Such observations imply that our understanding of the principal AMP production routes in silkworms, the Toll and IMD pathways, remains incomplete.

Signals derived from invading bacteria recognized by host PRRs may also trigger activation of the prophenoloxidase (PPO) cascade, leading to melanin production. Melanin is deposited on the surface of bacteria to inhibit their movement and growth, ultimately leading to their death. Furthermore, certain intermediate products of reactions catalyzed by phenoloxidase (PO) demonstrate broad-spectrum antibacterial activities (Nappi and Christensen, 2005; Nappi and Vass, 2001; Wang and Jiang, 2017; Zhao et al., 2007). Indeed, activation of the PPO cascade contributes to the survival of fruit flies infected with gram-positive bacteria and fungi (Binggeli et al., 2014). The silkworm genome contains two PPO genes encoding PPO1 (80 kDa) and PPO2 (78.7 kDa), which are processed into their respective active forms, PO1 (74 kDa) and PO2 (72.8 kDa), upon immune challenge (Clark and Strand, 2013; Kawabata et al., 1995; Yasuhara et al., 1995). In the silkworm hemolymph, PO and associated proteins form a high-mass complex (approximately 640 kDa) that is required for melanization (Clark and Strand, 2013). Both bacterial cells and PGs are potent elicitors of the PPO cascade, which, for example, is activated in the hemolymph by *M. luteus* and *Y. pseudotuberculosis* and PGs from *M. luteus*, *S. aureus*, *B. megaterium*, *B. subtilis*, and *E. coli* (Chen et al., 2014, 2016; Liu et al., 2015; Yoshida et al., 1996). The silkworm hindgut produces PPO, and subsequent activation of the PPO cascade leads to blackening of the feces and a reduction in the number of live bacteria at this site (Shao et al., 2012). PGRP-S1, PGRP-S4, and PGRP-S5 are involved in activation of the PPO cascade, for which their amidase activity is required (Chen et al., 2014, 2016; Yang et al., 2017; Yoshida et al., 1996). In addition, negative regulators have been identified that limit excess melanization in silkworms. In the hemolymph after injection of *M. luteus*, PO activity is significantly upregulated, the plasma becomes dark, and many clots form. Injection of recombinant BmSerpin-5, BmSerpin-6, and BmSerpin-15 significantly reduces the activity of PO, as these serpins inhibit specific serine proteases participating in the PPO cascade and block activation of PPO (Li et al., 2016a, 2017; Liu et al., 2015). Compared to the well-defined PPO cascades of *Manduca sexta*, *Tenebrio molitor*, and *D. melanogaster*, the components and regulation of that of the silkworm remain to be fully elucidated.

Several investigations also focused on cellular responses upon bacterial infection in the silkworm. Based on differences in

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morphology and function, silkworm hemocytes are divided into five types: prohemocytes, plasmatocytes, oenocytoids, granulocytes, and spherulocytes (Ling et al., 2003). Plasmatocytes and granulocytes are the main hemocytes responsible for cellular immunity in the silkworm, the former mediating phagocytosis of small particles, and the latter effecting encapsulation of larger materials (Wago, 1982, 1983). Our work indicates that elastase B secreted by *P. aeruginosa* can enhance silkworm hemocyte proliferation (unpublished data). However, the precise mechanisms underlying phagocytosis, encapsulation, and nodulation in the silkworm are unclear.

In addition to the above mentioned innate immune responses, silkworm utilizes other strategies to defend against bacterial infection. For example, it has recently been shown that silkworm transferrin and ferritin-1, production of which are induced by bacterial infection, have antimicrobial activity, likely through regulating the availability of iron to invading bacteria (Otho et al., 2016; Yun et al., 2009).

3. Silkworm immune responses triggered by fungi

Fungi are another important pathogens of insects, including silkworms. For instance, *Beauveria bassiana* is an entomopathogenic fungus and the cause of white muscardine, the chief fungal disease of silkworms (Lu et al., 2017b; Wang and Wang, 2017). *B. bassiana* spores spread among silkworm populations via the air, soil, mulberry leaves, tools used for rearing, and dead individuals. After adhering to the silkworm integument, the spores begin to germinate, before secreting enzymes to break and penetrate the cuticle to allow replication in the hemolymph, finally resulting in the death of the infected silkworm (Lu et al., 2017b; Wang and Wang, 2017). Using suppression subtractive hybridization and transcriptome analysis, many immunity-related genes have been found to be expressed differentially during *B. bassiana* infection, including those encoding Moricin, Cecropin B, ubiquitin, lysozyme precursor, and β-1,3-glucan recognition protein (βGRP)-3 precursor (Hou et al., 2011, 2014), suggesting that there exist undefined immune mechanisms in the silkworm response to fungal infection.

3.1. Fungal pathogen-associated molecular patterns (PAMPs) and silkworm pattern-recognition receptors (PRRs)

The fungal cell wall component β-1,3-glucan can be recognized by βGRPs, also known as gram-negative binding proteins (GNBPs), leading to activation of AMP production and the PPO cascade (Cerenius and Soderhall, 2004; Johansson and Soderhall, 1996; Kim et al., 2000; Ochiai and Ashida, 1988, 2000; Takahasi et al., 2009). In *D. melanogaster*, β-1,3-glucan is recognized by GNBP-3, which activates the Toll pathway. This pathway is also activated following detection of secreted fungal virulence factors by Persephone (PSH), and some results have indicated that these two routes act in a complementary fashion to achieve Toll pathway activation (Gottar et al., 2006; Lemaitre and Hoffmann, 2007). Expression of Cecropin A1, Drosomycin, and Metchnikowin is also induced to defend against fungal infection (Ekengren and Hultmark, 1999; Fehlbaum et al., 1994; Levashina et al., 1995). In *M. sexta*, βGRP-1 and βGRP-2 bind to β-1,3-glucan and are involved in activation of the PPO cascade (Jiang et al., 2004; Ma and Kanost, 2000). The N-terminal domain of βGRP-2 self-associates after binding laminarin, with which a soluble or insoluble complex is then formed. The insoluble complex exerts a stronger stimulatory effect on the PPO pathway than the soluble complex (Takahashi et al., 2014).

3.2. Antifungal defense in silkworms

Studies of insect defenses against fungal infection have focused on melanization and AMP production mediated by the Toll pathway. Silkworm βGRPs, four of which have been identified (Tanaka et al., 2008), also play important roles in the initial events in the activation of the PPO pathway. βGRP-1 contains a β-1,3-glucan-binding domain and a glucanase-like domain. Digestion of this protein with α-chymotrypsin results in two fragments, one of 20 kDa containing the first 102 residues of βGRP-1 that is able to bind to β-1,3-glucan, and another of 43 kDa containing a glucanase-

Table 1
Well-defined immune-related proteins in silkworm.

Name	Accession number	Function
PGRP-S1	AB016249	Bind to PGs; Involved in activation of the PPO cascade
PGRP-S2	KF906541	Participate in AMP production
PGRP-S4	XM_004928822.2	Bind to bacteria and peptidoglycans; Inhibit bacterial growth; Increase prophenoloxidase activity
PGRP-S5	NM_001043393	Bind to PGs; Inhibit bacterial growth; Involved in activation of the PPO cascade; Downregulate AMPs production.
PGRP-L6	XP_004929966	Bind to PGs; Involved in activation of Imd pathway
LBP	CAB38429.1	Bind to LPS and bacteria; Participate in bacterial clearance
βGRP-1	NP_001036840	Bind to β-1,3-glucan; Activate the PPO pathway
βGRP-3	NP_001036840	Bind to β-1,3-glucan; Activate the PPO pathway
Spätzle-1	NP_001108066	Required for the induced production of AMPs
PPO1	NP_001037335	Melanization
PPO2	NP_001037534	Melanization
PPAE	NP_001036832	Activate PPO
Serpin-5	AY566165.1	Downregulation of AMPs; Inhibit PO activity
Serpin-6	NP_001103823	Downregulation of AMPs; Inhibit PO activity
Serpin-15	NM_001146235	Downregulation of AMPs; Inhibit PO activity
NOS-1	XM_012690766.2	Produce nitric oxide and promote expression of cecropin B
Duox	XM_004932113	Produce hydrogen peroxide
Catalase	BAD38853.1	ROS scavenger
Prx-3	NP_001040464.1	Degrade H_2O_2; Protect DNA
Prx-5	NP_001040386.1	Degrade H_2O_2; Protect DNA
OXR-1	AK387280 NM_001145655 AK383272	Antioxidant function through JNK pathway
Transferrin	AF119098	Bind to iron; Antibacterial activity
Ferritin-1	DQ443196.2	Bind to iron; Block bacterial growth

AMPs: antimicrobial peptides; βGRP: β-1,3-glucan recognition protein; Duox: dual oxidase; GNBP: gram-negative binding protein; LBP: lipopolysaccharide-binding protein; NOS: nitric oxide synthase; OXR1: Oxidation resistance 1; PGRP: Peptidoglycan recognition protein; PG: peptidoglycan; PO: phenoloxidase; PPO: prophenoloxidase; Prx: peroxiredoxin.

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like domain that is able to activate the PPO pathway. This suggests that partial digestion of β-1,3-glucan by the βGRP-1 glucanase-like domain is required for PPO pathway activation, consistent with results showing that the amidase activity of PGRP-S5 is necessary for its involvement in activation of this same pathway (Chen et al., 2016; Ochiai and Ashida, 2000). In βGRP-depleted plasma, β-1,3-glucan fails to activate the PPO cascade, but does so after addition of recombinant βGRP-3 (Takahasi et al., 2009). These data suggest that silkworm βGRP-1 and βGRP-3 are involved in activation of the PPO cascade via recognition of β-1,3-glucan. In *M. sexta*, β-1,3-glucans induce self-association of βGRP-2, with which they form a complex that acts as a molecular platform, and the C-terminal of βGRP2 interacts with LA (low-density lipoprotein receptor class A) domains of HP14 to recruit HP14 zymogens (pro-hemolymph protease HP14, proHP14) to the molecular platform. This leads to conversion of proHP21 to its active form, further activating the PPO pathway (Dai et al., 2013; Ji et al., 2004; Takahashi et al., 2014, 2015; Wang and Jiang, 2007). There have been few reports concerning the structure of silkworm βGRPs and the molecular events involved in PPO pathway activation in this insect. BmHP6 and BmHP21 have been shown to form a complex with BmSerpin-5 during the PPO cascade, suggesting that these HPs are involved in the PPO pathway (Li et al., 2016a). However, many questions regarding this process in silkworms remain. For instance, what happens after β-1,3-glucan recognition by βGRPs? How does the β-1,3-glucan/βGRP complex interact with the PPO cascade? Is the PPO cascade in silkworms similar to that in *M. sexta*? Although reports have confirmed that in silkworms, certain PRRs (PGRP-S5, βGRP-1, and βGRP-3) and serine proteases (BmHP6 and BmHP21) are involved in activation of the PPO cascade, that β-1,3-glucan and PGs can activate proBAEEase (proBzArgOEtase) in the presence of Ca^{2+}, and that PPO-activating enzyme can directly cleave PPO (Katsumi et al., 1995; Satoh et al.,

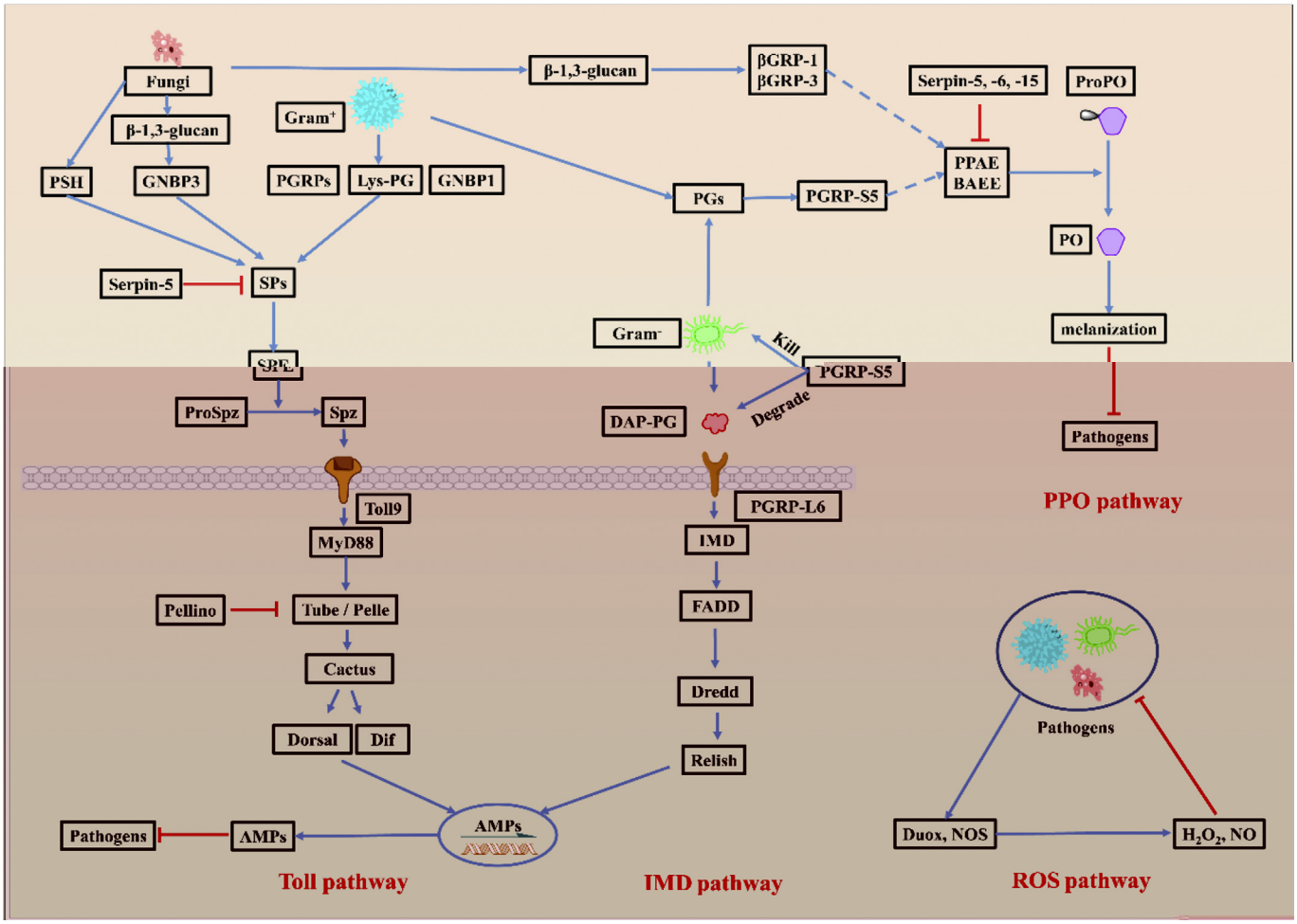

Fig. 1. Putative immune signaling pathways involved in the defenses against bacterial and fungal infections in *Bombyx mori*. This model was based on the Toll, IMD and melanization and ROS pathways of *Drosophila melanogaster* and *Manduca sexta*, and the findings in *Bombyx mori*. The β-1,3-glucan on the cell wall of fungi is able to be detected by GNBP-3 to active Toll pathway likely through Toll9 via a serine protease cascade and induce the expression of AMPs to kill the invading pathogens. Serpin-5 functions as a negative regulator of Toll pathway to reduce the expression of AMPs. The PGRPs are involved in the recognition of PGs on the cell wall of Gram-positive bacteria and Gram-negative bacteria to activate IMD pathway. Based on its amidase activity, PGRP-S5 is able to kill bacteria and degrade the PGs to downregulate the IMD pathway. PGRP-L6 is the only long type PGRPs of *Bombyx mori* without signal peptide and transmembrane domain, might function as an intracellular receptor like PGRP-LE of *Drosophila melanogaster* to positively regulate the IMD pathway. On the other hand, βGRP-1, βGRP-3 and PGRP-S5 are involved in the activation of PPO pathway via recognition of the PAMPs (Pathogen Associated Molecular Patterns) such as β-1,3-glucan on the cell wall of fungi and PGs of bacteria, and induce the production of melanization to kill the invading pathogens. The amidase activity of PGRP-S5 is required for the activation of PPO pathway which PGRP-S5 and PGs are involved in. Serpin-5, -6, -15 are able to limit the activation of PPO pathway. The illustration in the lower right corner is the generation of ROS. ROS pathway is able to be activated rapidly by invading pathogens such as fungi and bacteria, and upregulates Duox and NOS to produce hydrogen peroxide (H_2O_2) and nitric oxide (NO) respectively to inhibit pathogen growth. GNBP: Gram-Negative Binding protein, GNBPs also belong to βGRP family; PSH: Persephone; PGRP: Peptidoglycan recognition protein; PG: peptidoglycan; SP: serine protease; SPE: Spätzle-processing enzyme; proSpz: proSpätzle; Spz: Spätzle; IMD: IMmune Deficiency; AMPs: antimicrobial peptides; Lys-PG: Lysis-PG; DAP-PG: Diaminopimelic acid-PGs; proPO: prophenoloxidase; PO: phenoloxidase; Duox: dual oxidase; H_2O_2: produce hydrogen peroxide; NO: nitric oxide.

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1999), the structures and functions of each component of the silkworm PPO activation pathway and their interactions merit further study.

Induction of AMP expression is another silkworm defensive strategy against fungal infection. The Toll pathway is the principal mechanism by which fungi trigger AMP production, and the key molecules required for this pathway, such as BAEEase and Toll 9, have been found to be present in silkworms (Jang et al., 2006; Katsumi et al., 1995; Wu et al., 2010a). In silkworm, β-1,3-glucan and PGs are able to activate inactive proBAEEase. This enzyme is a homolog of the *D. melanogaster* Spätzle-processing enzyme, which cleaves proSpätzle to activate the Toll pathway (Jang et al., 2006); therefore, it seems likely that BAEEase performs this function in silkworms. The JNK/STAT pathway is also involved in immune responses to fungal infection. *B. mori* C-type lectin 5 (BmCTL5) may function as a receptor that activates this pathway to defend against fungal but not bacterial or viral infection. BmCTL5 RNAi downregulates expression of HOP and STAT, but upregulates that of SOCS2, SOCS6, and Ken in the JNK/STAT pathway. Moreover, silkworm survival and hemolymph antifungal activity significantly decrease when this pathway is inhibited (Geng et al., 2016). It has also been confirmed that Cecropin A, Defensin B, Gloverin 2, and Lebocin 5 exhibit antifungal effects against *B. bassiana*, and Gloverin 2 and Cecropin A show synergistic antifungal activity. Most of these proteins also have antibacterial properties (Kaneko et al., 2008; Lu et al., 2016, 2017a, 2017b).

A recent investigation showed that the cuticle protein BmSVWC maintains the integrity of the silkworm integument and is degraded during *B. bassiana* infection (Han et al., 2017), suggesting that it might be involved in antifungal defense. The cuticle-degrading enzyme CDEP-1 secreted by *B. bassiana* promotes conidial germination and penetration of the insect cuticle (Fang et al., 2002; Zhang et al., 2008). A number of studies have shown that the TIL-type protease inhibitors BmSPI38 and BmSPI39 (serine protease inhibitors/serpins) block melanization caused by CDEP-1, inhibit germination of *B. bassiana* conidia, and increase the survival of silkworm larvae infected with this fungus (Li et al., 2012; Li et al., 2015, 2016b). Thus, these proteins may play significant roles in defense against *B. bassiana* infection.

4. Conclusion and perspectives

From the above studies, we summarized to date well defined immune-related genes/proteins in the silkworm (Table 1) and constructed a rough picture of silkworm immune pathways and responses to bacterial and fungal infections (Fig. 1). Although we have identified the principal such pathways and responses, the connections and interactions between them and between their components are not yet clear and require further investigation. Such future studies will provide insights into the fine-tuning of silkworm immune responses and the differences between the silkworm immune system and those of other insects.

A domesticated insect, the silkworm has been reared on mulberry leaves for several thousand years indoors, and more recently, on an artificial diet in the laboratory for scientific study. This raises several questions. What is the role of mulberry leaves as the sole food of the silkworm in shaping silkworm immunity and antimicrobial defenses? How do silkworms fed on mulberry leaves differ from those given an artificial diet in terms of immune responses? Nutrients are critical in any physiological process, including immune responses, and also have an effect on the microorganisms (including invading pathogens and indigenous symbionts) living within the organism in question. The effects of nutrients on both the host and the microorganism can determine the outcome of their interaction (Ponton et al., 2013; Palmer-Young et al., 2017). It is known that short-term nutrient deprivation diminishes insect immune responses, such as PO activity (Akoda et al., 2009; De Block and Stoks, 2008; Siva-Jothy and Thompson, 2002). Moreover, dietary composition can have highly diverse effects on specific immune pathways and responses (Adamo et al., 2016; Brunner et al., 2014; Koella and Sorense, 2002; Srygley and Lorch, 2013; Unckless et al., 2015; Vogelweith et al., 2015; Dhinaut et al., 2017; Vogel et al., 2018). Starvation has been found to result in decreased growth and reduced baculovirus transmission and replication in silkworm larvae (Kang et al., 2011). In addition, our research has shown that the silkworm may regulate iron concentration through ferritin-1 to restrict the proliferation of bacteria in the hemolymph (Otho et al., 2016). Notably, use of proteomics has revealed differences in several immune-related proteins between silkworms reared on mulberry leaves and those administered an artificial diet (Zhou et al., 2016). What effects do dietary components have on silkworm immune responses and resistance to pathogen infection, and what are the mechanisms responsible? Is it possible to use dietary supplements to enhance silkworm antimicrobial resistance without adverse effects on growth and reproduction? It is time to initiate investigations to answer such questions.

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Appendix A. Supplementary data

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