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Interaction of entomopathogenic fungi with the host immune system

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ABSTRACT

Entomopathogenic fungi can invade wide range of insect hosts in the natural world and have been used as environmentally friendly alternatives to chemical insecticides for pest control. Studies of host-pathogen interactions provide valuable insights into the coevolutionay arms race between fungal pathogens and their hosts. Entomopathogenic fungi have evolved a series of sophisticated strategies to counter insect immune defenses. In response to fungal infection, insect hosts rely on behavior avoidance, physical barrier and innate immune defenses in the fight against invading pathogens. The insect cuticle acts as the first physical barrier against pathogens. It is an inhospitable physiological environment that contains chemicals (e.g., antimicrobial peptides and reactive oxygen species), which inhibit fungal growth. In addition, innate immune responses, including cellular immunity and humoral immunity, play critical roles in preventing fungal infection. In this review, we outline the current state of our knowledge of insect defenses to fungal infection and discuss the strategies by which entomopathogenic fungi counter the host immune system. Increased knowledge regarding the molecular interactions between entomopathogenic fungi and the insect host could provide new strategies for pest management.

1. Introduction

Entomopathogenic fungi cause many insect diseases and play important roles in regulating insect populations in nature (Hajek, 1997; Wang et al., 2011b). These fungi have many advantages as eco-friendly alternatives to chemical insecticide (Chen et al., 2017; Hajek, 1997). Those belonging to the genera *Beauveria* and *Metarhizium* have been widely used for the control of various agricultural insect pests and vectors of human diseases (Blanford et al., 2005; Farenhorst et al., 2009; Knols et al., 2010).

To combat infection, insects have evolved various defense systems. Cuticular integument provides the first and most effective physical barrier to prevent the entrance of pathogens into the body (Leger et al., 1991). Furthermore, insects employ both cellular and humoral immune defense mechanisms to fight fungal infections (Gottar et al., 2006). Cellular immunity is mainly mediated by hemocytes, which circulate in the hemolymph, and involves coagulation, phagocytosis, and encapsulation (Valanne et al., 2011). The humoral defense responses rely on specific signaling pathways. Among them, the Toll signaling pathway is significantly activated in response to fungal infection and eventually leads to production of anti-fungal peptides like drosomycin and metchnikowin in *Drosophila* (Lemaitre and Hoffmann, 2007). During long-term coevolutionary arms race, insect pathogenic fungi have developed multiple strategies that allow the fungus to overcome or evade the immune defense responses of the host.

Here we provide an overview of entomopathogenic fungi invasive and developmental processes in insect hosts and present a review of behavioral immunity in insects. We summarize studies that characterize signaling pathways or mechanisms of innate immunity of insects in response to infection. We also emphasize the current state of knowledge regarding fungal strategies that counter the host immune response. Finally, this review discusses the coevolutionary arms race between entomopathogens and insect host immune systems.

2. Invasion of entomopathogenic fungi and interactions on the surface

Entomopathogenic fungi typically infect their host insects by direct penetration of the external cuticle (Vega et al., 2012). The first step is adhesion of fungal conidia to the host surface. This occurs with the aid of mucilage and adhesive proteins (Wang and St Leger, 2007). Under appropriate conditions and given adequate

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nutrient availability, fungal spores can germinate on the insect cuticle and form a specialized structure called appressorium (Wang and Wang, 2017), then breach the insect cuticle via cuticle-degrading enzymes (Beys da Silva et al., 2010). The fungi then enter the insect hemocoel, where the fungal cells absorb nutrients, produce toxins, destroy host cells, and eventually kill the insect. Next, transmission is achieved by sporulation from the cadaver (Anderson et al., 2011; Mishra et al., 2015).

The insect cuticle is a complex multilayered structure composed of macromolecules such as tanned proteins and chitin that provides a series of challenges to the entry of pathogens (Leger et al., 1991). As the first physical barrier to pathogen infection, physiological environment of the cuticle is inhospitable for pathogens: It has low water activity, few nutrients, and contains antimicrobial compounds. Entomopathogenic fungi can actively penetrate the host cuticle by physical and enzymatic mechanisms.

The insect cuticle is hydrophobin, and some chemicals produced by host itself or the resident microbial community prevent fungal conidia from adhesion and germination (Greenfield et al., 2014; Ortiz-Urquiza and Keyhani, 2013; Toledo et al., 2011). For example, *Bacillus pumilus* on the surface of *Delphacodes kuscheli* and *Dalbulus maidis* cuticles inhibit *B. bassiana* conidial germination (Toledo et al., 2011). In addition, some social insects use microbicidal secretions to avoid fungal infection and disease spread. For example, ants secrete antimicrobials in response to fungal infection (Fernandez-Marin et al., 2006). *Tribolium castaneum* is resistant to *B. bassiana* infection by secreting defensive compounds called benzoquinones (Pedrini et al., 2015). The insect cuticle thus plays an important role in defense against fungi.

On the pathogen side of the arms race, fungi have evolved specific mechanisms that circumvent these defenses. Successful adhesion and fast germination of fungi spores on the insect cuticle surface are important attributes of virulent fungal strains. Fungal blastospores can produce mucilage that enables their adhesion on the insect cuticle, and it appears to provide a relatively stable underwater condition for spore germination. Entomopathogenic fungi are known to possess hydrophobin (*hyd*) or adhesion (*mad1*and *mad2*) genes, some of which are responsible for hydrophobicity, adhesion and virulence of strains (Holder et al., 2007; Sevim et al., 2012; Wang and St Leger, 2007; Zhang et al., 2011). In addition, other cell wall proteins play critical roles in adhesion and stress management. Examples are the non-hydrophobic cell wall proteins CWP10 and CP15, which are involved in the resistance to thermal and oxidative stresses (Li et al., 2010; Ying and Feng, 2011).

For entomopathogenic fungi, crossing the cuticle protein-chitin barrier is critical for infection. Entomopathogenic fungi produce many enzymes, including proteases, chitinases, lipases, esterase, phospholipase C, and catalase (Beys da Silva et al., 2010; Santi et al., 2010; Wang et al., 2009, 2011a; Wei et al., 2017), which are virulence determinants and necessary for penetration of the insect cuticle (Nunes et al., 2010; St Leger et al., 1998). Proteases, such as a virulence factor Pr1 (Shah et al., 2005), can enhance fungal spore germination and, together with other enzymes (e.g., Pr2 and chitinases) degrade the cuticle to enable the fungus to gain access to the hemocoel (Ortiz-Urquiza and Keyhani, 2013; Santi et al., 2010; Small and Bidochka, 2005). Comparative genomics studies show that entomopathogenic fungi have more genes encoding proteases and chitinases compared to plant pathogens (Gao et al., 2011; Hu et al., 2014; Xiao et al., 2012; Xiaoi et al., 2013; Zheng et al., 2011). This is likely an adaptation to the amount of chitin in the insect cuticle, and the expansion of proteases might reflect the insect host range of entomopathogenic fungi. In addition, internal lipids of the insect cuticle can also alternatively act as antifungal barriers. Thus, lipid assimilation can be considered as forming a coevolutionary web between insect hosts and entomogenous fungi (Keyhani, 2017).

Insect hosts exhibit a vast array of responses to counter entomopathogenic fungi during penetration (Fig. 1). In a bid to reduce hemolymph leakage during fungal penetration process, the insect hemostatic response is activated (Vilmos and Kurucz, 1998). Insect hemostasis involves clotting proteins such as lipophorins, vitellogenin-like proteins, and calcium-dependent transglutaminases containing a cysteine-rich domain homologous to the von Willebrand factor of mammals (Vilmos and Kurucz, 1998). In *Drosophila*, transglutaminase anchors the invading microbes to microclots to allow killing in the hemolymph by antimicrobial peptides (AMPs) and hemocytes (Wang et al., 2010). In addition, ecdysis can greatly improve insect larval survival when fungi have not reached the underling new cuticle; in this case, the pathogens are shed with the old cuticle (Vestergaard et al., 1995).

In addition to hemostasis and ecdysis, the cuticle also contributes to insect host immunity (Dubovskiy et al., 2013a). The epidermis initiates the integumentary defense by protease inhibitors and melanin synthesized by epidermal cells. An array of protease inhibitors are expressed by insects. One example is insect metalloprotease inhibitor (IMPI) that is involved in resistance to the entomopathogen-specific metalloproteases (Qazi and Khachatourians, 2007; Vilcinskas, 2010). During fungal penetration of the cuticle, the prophenoloxidase (proPO) cascade in epidermal cells is activated to synthesize melanin. Melanin and its precursors play important roles in protecting the insect cuticle against fungi invasion, not only limiting the growth of certain fungi but also preventing synthesis of cuticle-degrading enzymes (Söderhäll and Ajaxon, 1982; Yassine et al., 2012; Zhang et al., 2017). Moreover, it was recently found that prophenoloxidase activation is required for survival to infection by fungi and Gram-positive bacteria in *Drosophila* (Binggeli et al., 2014). AMPs are also detected in the integument (Brey et al., 1993). Hemocytes can penetrate the basement membrane and reach the fungi penetration sites to release AMPs (Gunnarsson, 1988). Although the extent to which AMPs in most insects contribute to defense against entomopathogenic fungi remains unclear, recent studies showed that *Bombyx mori* cecropin A and gloverin2 have high antifungal activity against entomopathogenic fungus *Beauveria bassiana* both *in vitro* and *in vivo* (Lu et al., 2016, 2017). The AMPs are likely involved in the early recognition and destruction of fungal structures in the external cuticle (Butt et al., 2016).

Once entomopathogenic fungi successfully gain entry into the insect hemolymph, they propagate as yeast-like blastospores or hyphal bodies. Then blastospores rapidly colonize the hemocoel, where they encounter host cellular and humoral defenses. The invading pathogen is recognized via pattern recognition receptors (PRRs) including peptidoglycan recognition proteins (PGRPs), Gram-negative-binding proteins (GNBPs), β-glucan-binding proteins (βGRPs), C-type lectins and others (Stokes et al., 2015). Carbohydrate heterogeneity at the fungal surface plays a critical role in nonself detection by insect hosts (Wanchoo et al., 2009). Compared with fungal conidia or hyphae, hyphal bodies appear to possess fewer carbohydrate epitopes, which allow them to avoid recognition by the host immune system in the hemocoel (Pendland and Boucias, 1993; Wanchoo et al., 2009). In addition, fungal protease PR1 could be detected and lead to cleavage of the insect host Persephone protease and then activates the serine protease cascade acting upstream of the Toll pathway (Ligoxygakis et al., 2002; Gottar et al., 2006).

3. Behavioral and social immunity to fungal infection

Entomopathogenic fungi have a dramatic impact on their insect hosts, and the survival of insects depends on their defense

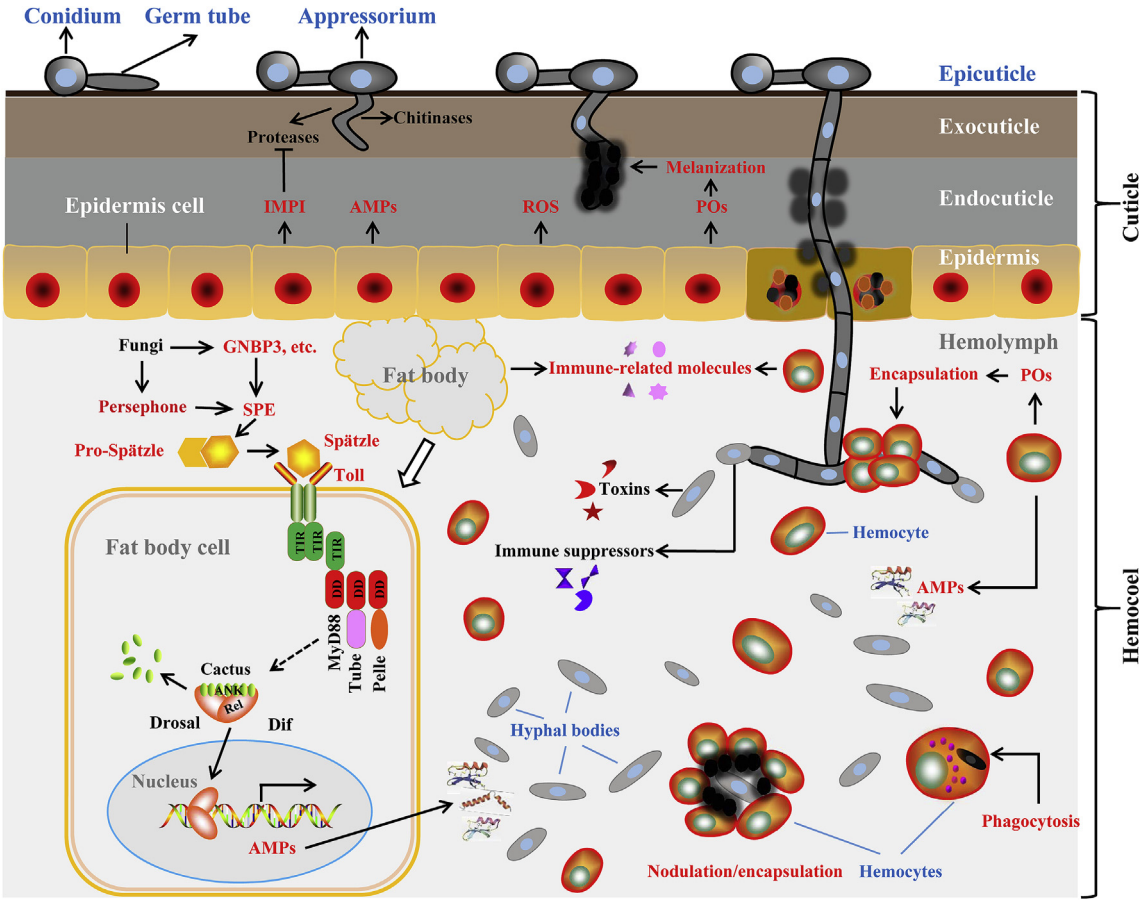

Fig. 1. Schematic of interactions between entomopathogenic fungi and the insect immune responses. Entomopathogenic fungi gain access to the insect by direct penetration of the host external cuticle, including spore attachment, germination, and formation of infection structure (appressorium), penetration of the cuticle, colonization in the hemocoel and eventual killing of the insect. Insects can detect fungal infections and activate innate immune responses, including humoral and cellular defenses. The defense strategy of the insect host against fungal pathogens includes multi-factorial reactions. The epidermis also contributes to host immunity. Insects have been selected to evolve protease inhibitors (e.g., IMPI) to the entomopathogenic fugni specific metalloproteases. Toll is the principal innate immune pathway in response to fungi. Gram-negative binding protein 3 (GNBP3), a member of the β-1, 3-glucan recognition protein family (βGRP3), binds to fungal cell components and initiates the Toll signaling pathway. Two CLIPs, Persephone and Späetzle-processing enzyme (SPE) cause the cleavage of a cysteine knot cytokine, Späetzle (Spz). The Toll receptor is activated when the proteolytically cleaved ligand Spz binds to the receptor. The signals are then passed into the intracellular signal transduction cascade consisting of MyD88, Tube and Pelle, eventually leading to the expression of AMPs by the potent NF-κB transcription factors Dorsal and Dif. AMPs are produced in fat bodies, epidermis cells and hemocytes. Fungi in the hemolymph could be recognized by hemocytes and induce cellular phagocytosis and encapsulation reactions of the hosts. The insect host also exploits immune-related molecules to fight against the invading fungi. To cause infection, the fungus has to avoid, subvert, or circumvent these defenses. After the fungus reaches the hemocoel, the invasive filamentous cells switch to a yeast-type proliferation strategy to form hyphal bodies (blastospores) for rapid proliferation and evasion of the host immune system. In addition, fungi secrete certain immune suppressors and toxins to suppress host immune responses. IMPI, insect metalloprotease inhibitor; AMPs, antimicrobial peptides; ROS, reactive oxygen species; PO, phenoloxidase; SPE, Spätzle processing enzyme; TIR, Toll-IL1 receptor domain; DD, death domain; ANK, Ankyrin repeats.

mechanisms. The most effective defense against fungal infection is probably behavioral avoidance of pathogens (de Roode and Lefevre, 2012; Shang et al., 2015). Although the ability of insects to detect and avoid entomopathogenic fungi depends on species (Baverstock et al., 2010), behaviors that enable insects to avoid infection or that lead to inhibition of fungal growth or to reduces disease symptoms are common (de Roode and Lefevre, 2012).

Some social insects (e.g., termites and ants) can detect the presence of virulent fungi on contaminated individuals from a distance using olfactory cues (Mburu et al., 2009; Ugelvig and Cremer, 2007; Yanagawa et al., 2008) The termite *Macrotermes michaelseni* can discriminate the higher virulent strains of *M. anisopliae* and *B. bassiana* from less virulent strains based on the volatile organic compounds emitted by these fungi (Mburu et al., 2011, 2013). Behavioral defenses against pathogens involve self-grooming (Tragust et al., 2013), grooming nest members (Ugelvig and Cremer, 2007), and intake or production of compounds with antipathogenic properties (Bulmer et al., 2009; Christe et al., 2003; Tragust et al., 2013). For example, *Formica selysi* workers increase their self-grooming in the presence of *Metarhizium brunneum*; this improved hygiene prevents pathogens from spreading (Tragust et al., 2013). Another example of hygienic behavior involves honeybee workers. The workers identify larvae infected with the fungus *Ascosphaera apis* by detecting the phenethyl acetate produced by pathogens and remove these larvae (Swanson et al., 2009). Some social insects spread antifungal secretions (e.g., formic acid, antimicrobial peptides, and proteinaceous salivary deposits) produced by salivary glands or a cuticular metathoracic gland to protect the insect themselves and their nestmates (Bulmer et al., 2009; Tragust et al., 2013). Two compounds secreted by termite salivary glands, termicin and GNBP2, have antifungal activity (Bulmer et al., 2009; Lamberty et al., 2001). Finally, some insects including locusts (*Schistocerca gregaria*) can raise their body temperature by basking in the sun to fight *Metarhizium* infection (Blanford and Thomas, 1999).

Density dependent prophylactic immunity has been observed in

various species. Individual hosts invest more in immunity when population densities are high (Wang et al., 2013a; Wilson-Rich et al., 2009). Like gregarious locusts, increased prophylactic immunity can reduce gregarious locusts susceptibility to *M. anisopliae* infection. This prophylactic immunity decreases population infection risk and likelihood of disease epidemics (Wang et al., 2013b).

Studies suggest that some insects are attracted to certain strains of entomopathogenic fungi. For example, female *Anopheles stephensi* mosquitoes are attracted to spores of *M. anisopliae* and *B. bassiana* (George et al., 2013). It is unclear whether the pathogens attract new hosts or whether the insect host is exposing itself to nonlethal doses of entomopathogenic fungi to "self-vaccinate". The phenomenon of immune memory in invertebrates has been a contentious issue (Rowley and Powell, 2007). Studies suggested that priming effects induced by a low dose of pathogens or environmental stress may only last 24–48 h (Browne et al., 2014).

4. Innate immune defenses against fungi

Unlike higher vertebrates, which possess the adaptive immune response, insects lack the ability to produce antibodies. Insects counter pathogen invasion with the aid of an innate immune system mainly composed of humoral and cellular mechanisms (Lemaitre and Hoffmann, 2007). Insects display various humoral defenses against fungal infection. Meanwhile, insect hemocytes function as the principle actors in the cellular immune responses to pathogen infections (Lavine and Strand, 2002).

4.1. Humoral immunity

AMPs play important roles in insect humoral immunity. Production of these peptides is activated primarily through two signaling pathways, the Toll and Imd signaling pathways, which are responsible for detection of different classes of microbes (Bulet and Stocklin, 2005; Valanne et al., 2011). In addition to AMPs, humoral immunity involves a wide range of defenses such as the melanization cascade, and the production of reactive oxygen and nitrogen radicals (Jiang et al., 2010).

AMPs are synthesized in the fat body, hemocytes, epidermis cells and epithelia, and subsequently secreted into the hemolymph to fight pathogens (Nehme et al., 2007). Insects produce specific AMPs in response to bacteria and fungi (Lemaitre and Hoffmann, 2007). Insect AMPs are small, typically cationic peptides that can form pores on the microbial membranes or repress the synthesis of cell wall proteins or nucleic acids (Brogden, 2005). For example, drosomycin binds to sphingolipids leading to pore formation in the fungal cell, whereas heliomicin interacts with fungal plasma membrane ceramides (e.g., glucosylceramides) (Fehlbaum et al., 1994; Gao and Zhu, 2008). Moreover, lantibiotic mersacidin could inhibit peptidoglycan synthesis by targeting lipid II (Brotz et al., 1998).

Invading microbes are recognized via PRRs of insect hosts leading to the amplification of signal of infection by serine proteases. Infection by fungi and Gram-positive bacteria activates the Toll signaling pathway leading to production of certain AMPs. Gram-negative bacteria trigger the Imd signaling pathway inducing expression of AMPs like the cecropins, diptericin, attacin, and drosocin (Lemaitre and Hoffmann, 2007). Eight insect antifungal peptides have been characterized, fewer than the number of antibacterial peptides (Faruck et al., 2016). In *Drosophila*, fungi infection results in the expression of AMPs drosomycin and metchnikowin (Kurata et al., 2006). Some other insect antifungal peptides are also well characterized like heliomicin from *Heliothis virescens* (Lamberty et al., 1999), termicin from termites (Da Silva et al., 2003), and gallerimycin from *Galleria mellonella* (Schuhmann et al., 2003). Moreover, other signaling cascades like the JAK/STAT and the JNK pathways are also activated upon fungal infection inducing expression of immune genes in the fat body of *Drosophila* (Lu and St leger, 2016). Recently it was demonstrated that AMPs produced by blowflies have synergistic interactions in response to pathogen infections (Chernysh et al., 2015; Poppel et al., 2015).

In addition to AMPs, insects produce other immune-related molecules like lysozyme, apolipophorin III, hemocyanin and transferrin. These molecules have multiple functions and tend to work synergistically to enhance efficacy. The immune effector apolipophorin III contributes multiple aspects of insect innate immunity including detection of fungi (via β-1,3-glucan), hemocyte-mediated encapsulation reactions, and nodule formation (Zdybicka-Barabas and Cytrynska, 2013). Co-incubation with apolipophorin III promotes the effects of *Galleria* lysozyme toward bacteria (Zdybicka-Barabas and Cytrynska, 2013). Similarly, the antifungal activity of lysozyme toward *C. albicans* is enhanced in the presence of anionic peptide 2 of *G. mellonella* (Sowa-Jasilek et al., 2014). The insect transferrin is detected in insect hemolymph; like mammalian transferrin it binds iron impeding pathogen survival (Geiser and Winzerling, 2012). In some insects like *Mastotermes darwiniensis* and *G. mellonella*, the expression of transferrin is significantly increased in response to infection with *B. bassiana* and *M. anisopliae*, suggesting it plays an important role in insect innate immunity (Dubovskiy et al., 2013b; Thompson et al., 2003). Recently, a modulator CLSP2 was showed to play a critical role in the defense against fungal infection in mosquitoes (Wang et al., 2015).

The Toll signaling pathway plays a critical role in insect hormonal immunity against fungi and Gram-positive bacteria, which was initially identified in *Drosophila*, and shares significant similarities with IL-1R-mediated activation of the NF-κB pathway in mammals (Belvin and Anderson, 1996; Valanne et al., 2011). In *Drosophila*, the Toll signaling pathway mediates embryonic development (Belvin and Anderson, 1996), immune responses (Hoffmann, 1995; Ip and Levine, 1994), morphogenetic movements (Gerttula et al., 1988; Hashimoto et al., 1991), and muscle development (Halfon et al., 1995; Nose et al., 1992).

Components of Toll signaling pathway have been identified in *Drosophila* and include the extracellular cytokine Spatzle, the transmembrane receptor Toll, the Tube and MyD88 adaptors, the Pelle kinase, Cactus, and the Dorsal and Dif transactivators (Belvin and Anderson, 1996; Tauszig et al., 2000). Deletion of some of these components leads to an immune-deficient phenotype characterized by the lack of expression of AMPs drosomycin and metchnikowin and makes the host more susceptible to fungal and Gram-positive bacterial infections (Lemaitre et al., 1996; Rutschmann et al., 2002). There are nine genes encoding Toll receptors in the *Drosophila* genome (Hetru and Hoffmann, 2009). Unlike vertebrate Toll-like receptors, Toll is activated by binding of the cleaved ligand Spatzle, trigging the expression of the NF-κB factors Dif and Dorsal and eventually leading to the production of AMPs like drosomycin (Hu et al., 2004; Weber et al., 2003). Cactus, the inhibitor of the Toll pathway, establishes a negative feedback on this cascade (Nicolas et al., 1998). It was showed that toll receptor Toll5A and cytokine Spz1C function in the toll signaling pathway following fungal challenge in the mosquito *Aedes aegypti* (Shin et al., 2006).

The Toll signaling pathway also plays a role in the cellular immune response, which involves phagocytosis of microbes and the encapsulation and killing of parasites (Hultmark, 2003). In addition, together with other pathways, the Toll signaling pathway controls hemocyte proliferation and hemocyte density (Sorrentino et al., 2004; Zettervall et al., 2004). Toll signaling is also required for melanization response in *Drosophila* larvae (Bettencourt et al.,

2004). Recent study revealed that thioester-containing proteins (TEPs) are involved in the defence against entomopathogenic fungi by activating Toll pathway in *Drosophila* (Dostalova et al., 2017).

4.2. Cellular immunity

Insect cellular immune responses rely on the circulating hemocytes, which are divided into different types based on morphological characteristics and functional features (Price and Ratcliffe, 1974). In *Drosophila*, there are at least three differentiated hemocyte types: plasmatocytes, crystal cells, and lamellocytes (Evans and Banerjee, 2003; Hartenstein, 2006; Lanot et al., 2001; Lemaitre et al., 1996). Insect hemocytes are involved in a series of cellular defenses including nodulation, phagocytosis, and encapsulation (Michael, 2008). Plasmatocytes represent 90–95% of all hemocytes in *Drosophila*. These cells recognize pathogens through phagocytic receptors like Eater and Dscam on their surfaces (Kocks et al., 2005; Watson et al., 2005). Moreover, a class of secreted thioester-containing proteins (TEPs) have important role in cellular defense. TEPs enhance phagocytosis by binding to pathogens and act through a mechanism similar to that of complement effector in vertebrates (Blandin et al., 2004; Stroschein-Stevenson et al., 2006). Interestingly, some studies indicate that the hemocytes play a part role in humoral immunity (Michael, 2008). Plasmatocytes trigger AMP production, and the expression of AMPs is abolished in *Drosophila* that lack hemocytes (Shia et al., 2009).

In addition to humoral and cellular immune responses, complex proteolytic cascades like the prophenoloxidase (PPO) pathway, which induces the melanization response, can be activated in response to fungal infection (Cerenius et al., 2010). During invasion, fungal cell wall pathogen-associated molecular patterns (PAMPs) can be recognized by host PRRs, inducing maturation of prophenoloxidase (PPO) to phenoloxidase (PO) through a series of enzymatic reactions, ultimately leading to the formation of toxic reactive quinones and melanin (Cerenius et al., 2008; Nappi and Christensen, 2005) These toxic substrates can aid in the killing of microbial pathogens and are effective against some fungal infections (Binggeli et al., 2014; Yassine et al., 2012). The PPO cascade is tightly regulated by serine proteases and serine protease inhibitors (Povelones et al., 2009). Many entomopathogenic fungi have evolved anti-PO activity via preventing their own proteases from activating the PPO cascade (Feng et al., 2015; St Leger et al., 1996).

5. Fungal strategies to counter host immune responses

Once inside the insect, entomopathogenic fungi face a series of potent immune responses from their hosts. Entomopathogenic fungi have evolved to circumvent these immune defenses through multiple strategies (Fig. 1). These involve masking the immunogenic carbohydrates of the fungal cell surface to avoid immune stimulation, secreting immune modulators to disrupt the host immune response and repress proteases that activate the PO cascade. The surface carbohydrate profile of entomopathogenic fungi depends on genera. It is the carbohydrates that are recognized by PRRs of host insects to trigger immune signaling cascades. *M. anisopliae* expresses MCL1, a collagen-like immune evasion protein acting as an anti-adhesive protective coat, to mask antigenic cell wall β-glucans and prevent hemocytes from recognizing its hyphal bodies (Wang and St Leger, 2006, 2007).

Entomopathogenic fungi secrete a wide range of bioactive compounds during invasion, including bassianin, bassiacridin, oosporeins, cyclosporine, and destruxins (Gibson et al., 2014; Molnar et al., 2010; Wang et al., 2010). Some of these metabolites are responsible for virulence and host specificity of fungi (Amiri-Besheli et al., 2000; Kershaw et al., 1999). Other bioactives could suppress the host immune response (Amiri-Besheli et al., 2000; Kershaw et al., 1999). For example, *Metarhizium* destruxins could inhibit expression of genes encoding AMPs and block phagocytosis by inhibiting V-ATPase (Chen et al., 2014). Oosporein produced by *B. bassiana* inhibits ProPO activity and downregulates expression of gallerimycin in the wax moth *Galleria* larvae (Feng et al., 2015). Moreover, some *Metarhizium* and *Beauveria* strains are resistant to the antifungal peptide drosomycin produced by activation of the Toll pathway (Lu et al., 2015; Tzou et al., 2002).

During the penetration phase, most entomopathogenic fungi produce proteases (e.g., Pr1) to degrade the cuticle and activate PPO pathway. Some entomopathogenic fungi can reduce insect PO activity by suppressing protease activity (Matskevich et al., 2010). Recently, it was reported that the entomopathogenic fungus *B. bassiana* can interact with the gut microbiota to accelerate mosquito death via down-regulation of antimicrobial peptides and dual oxidase in the mosquito midgut (Wei et al., 2017).

6. Coevolution of entomopathogenic fungi and the insect host immune system

During the complex interactions of entomopathogenic fungi and their insect hosts, there has been a dynamic coevolutionary arms race (Schmid-Hempel, 2003; Wertheim, 2015). Pathogens exert a strong selective pressure on the host genome, especially on immunity-related genes. For example, many studies have found that *Drosophila* immunity genes have significantly higher rate of adaptive substitution compared to other genes (Clark and Wang, 1997; Jiggins and Hurst, 2003; Lazzaro, 2008; Obbard et al., 2011). Different insects have their own distinct immune molecules (Juneja and Lazzaro, 2009) that play crucial roles in immune responses against invading pathogens. Accordingly, pathogens have evolved a series of strategies under long-term selection to evade or overcome the immune responses of their hosts. It was reported that *Beauveria* and *Metarhizium* have evolved resistance to drosomycin under strong selective pressure over modest evolutionary timescales (Lu et al., 2015; Tzou et al., 2002). The interactions between insect hosts and pathogens through these immune molecules promote continuous evolution of both organisms (Wertheim, 2015).

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