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REVIEW

The complexity of *Drosophila* innate immunity

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Abstract

Metazoans rely on efficient mechanisms to oppose infections caused by pathogens. The immediate and first-line defense mechanism(s) in metazoans, referred to as the *innate* immune system, is initiated upon recognition of microbial intruders by germline encoded receptors and is executed by a set of rapid effector mechanisms. Adaptive immunity is restricted to vertebrate species and it is controlled and assisted by the innate immune system.

Interestingly, most of the basic signaling cascades that regulate the primeval innate defense mechanism(s) have been well conserved during evolution, for instance between humans and the fruit fly, *Drosophila melanogaster*. Being devoid of adaptive signaling and effector systems, *Drosophila* has become an established model system for studying pristine innate immune cascades and reactions. In general, an immune response is evoked when microorganisms pass the fruit fly's physical barriers (*e.g.*, cuticle, epithelial lining of gut and trachea), and it is mainly executed in the hemolymph, the equivalent of the mammalian blood. Innate immunity in the fruit fly consists of a phenoloxidase (PO) response, a cellular response (hemocytes), an antiviral response, and the NF-κB dependent production of antimicrobial peptides referred to as the humoral response. The JAK/STAT and Jun kinase signaling cascades are also implicated in the defence against pathogens.

Key Words: *Drosophila*; innate immunity; signaling cascades

Introduction

Immune responses are typically distinguished in two main systems, the adaptive and the innate immune response. Adaptive immunity is specific, has memory and is generally considered to be restricted to vertebrates. It relies on the generation of immune receptors, like immunoglobulins and T-cell receptors, through somatic gene rearrangements in specified blood cells and on the clonal expansion of activated lymphocytes (B and T cells). Innate immunity, on the other hand, refers to the evolutionary ancient and presumably conserved first-line host defence against the early phases of microbial infection, and it is believed to be naive in recognizing broadly conserved microbial moieties. The model organism *Drosophila melanogaster* only seems to rely on this innate immune system for its defence against pathogens. *Drosophila* therefore has physical barriers (exoskeleton, chitinous epithelial lining of gut and trachea) to prevent microbial entrance into its body cavity. Additionally, specialized blood cells, called hemocytes, floating in the hemolymph participate in phagocytosis and encapsulation of foreign invaders and some of their components are needed for their subsequent melanization. The larval fat body, a functional analog of the mammalian liver and the main larval energy reservoir, is the main site of the fruit fly larva's humoral (or systemic) immune response, while in adults other tissues can take on major roles for immune surveillance (Hoffmann, 2003).

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Humoral response

The humoral response is the most intensively studied part of *Drosophila* innate immunity. It consists of recognition systems in the hemolymph which signal the presence of pathogens to the fat body, one of the main immune tissues. This leads to the activation of the Toll and/or the immune deficiency

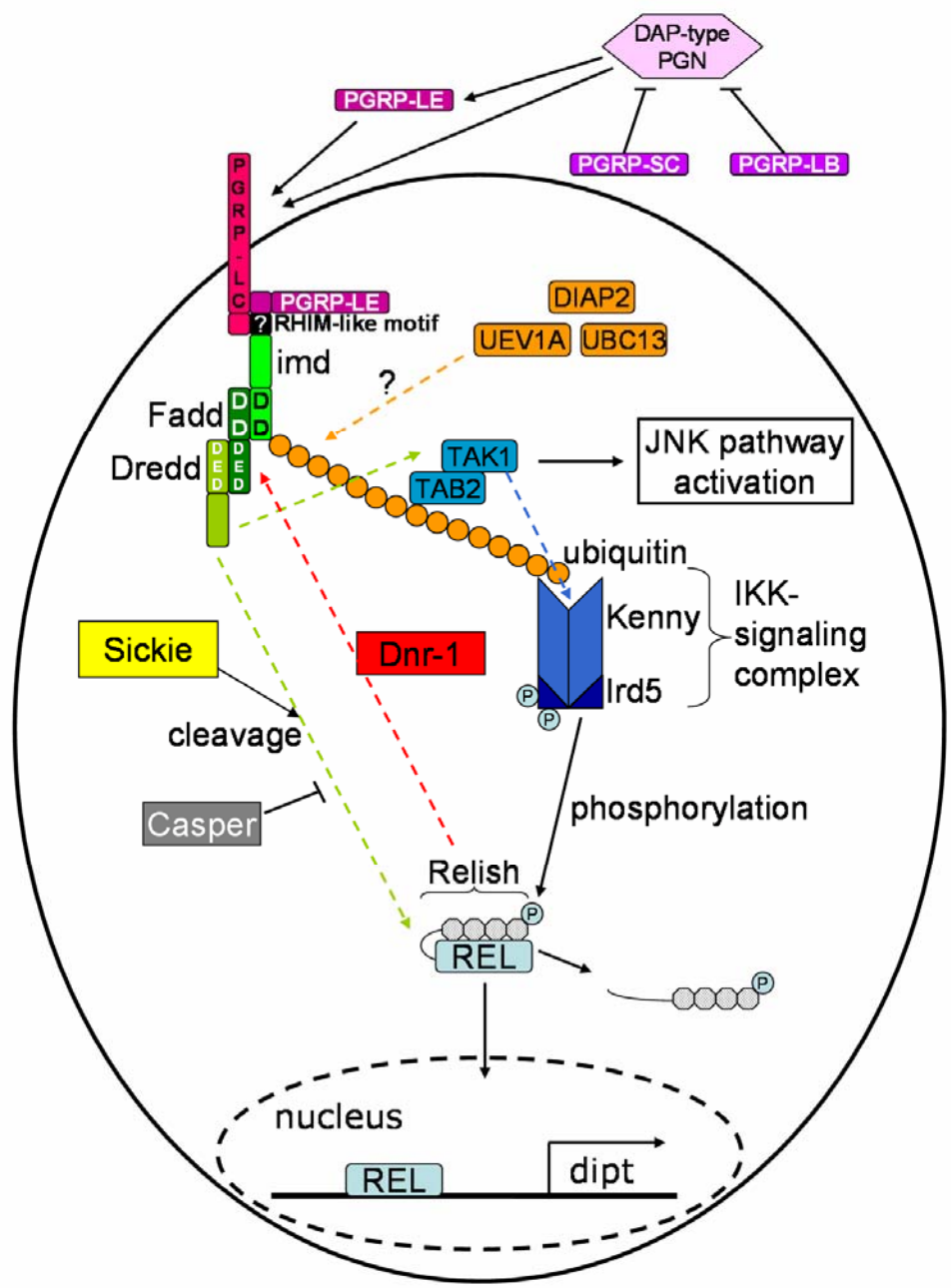

Fig. 1 The *Drosophila* imd pathway. Gram⁻ bacterial cell wall components (DAP type PGN) in the hemolymph are recognized by transmembrane or extracellular PGRP-LE and PGRP-LC. Imd pathway activation then is initiated by multimerization of transmembrane PGRP-LC and intracellular PGRP-LE both containing a RHIM-like [receptor interacting protein (RIP) homotypic interaction motif] motif. PGRP-SC and PGRP-LB mediate the intensity of this activation. The adaptor protein that links this RHIM-like motifs containing complex to imd is still unknown. Downstream of imd, several processes can be distinguished of which some probably depend on K63 linked ubiquitin chains for the assembly of active protein complexes. 1) Genetic evidence suggest the combined action of UBC-13 (ubiquitin conjugating enzyme), DIAP-2 (*Drosophila* inhibitor of apoptosis protein 2) and UEV1A (ubiquitin conjugating enzyme E2 variant 1) to be needed for the formation of these ubiquitin chains. 2) The complex formed between TAK1 and TAB2 mediates both JNK pathway and IKK signaling activation. The IKK complex composed of the regulatory subunit Kenny and the catalytic subunit IRD5 then phosphorylates Relish. 3) The unit consisting of imd, Fadd and Dredd, which interact with each other through their death domains (DD), respectively death effector domains (DED), is supposed to be involved in the activation of TAK1. Dredd further is presumed to cleave phosphorylated Relish resulting in the freeing of the REL transcription factor. Further processing steps of the phosphorylated inhibitory part of Relish are unknown. Dnr-1 (defence repressor 1) probably is part of a negative regulatory loop that keeps the imd-Fadd-Dredd complex inactive until imd pathway activation upon infection while Sickie positively regulates Dredd mediated cleavage of Relish (Foley and O'Farrell, 2004). The final result of all these processes is the translocation of the NF-κB transcription factor REL to the nucleus to start transcription of target genes. Caspar and the ubiquitin-proteasome pathway further negatively regulate imd pathway signaling.

(imd) pathway resulting in the synthesis of AMPs as well as other effector molecules, and their subsequent release into the hemolymph to fight infection (reviewed in Ferrandon *et al*., 2007).

Recognition of pathogens is mainly achieved by a diverse array of pattern recognition receptors (PRRs) belonging to the peptidoglycan recognition protein (PGRP) and Gram negative binding protein (GNBP) families which can discriminate between distinct classes of microorganisms (Lemaitre *et al.*, 1997). Detection of the diaminopimelic (DAP) type of peptidoglycan (PGN) constituting the inner membrane of G^- bacteria is mediated by PGRP-LC isoforms and PGRP-LE resulting in the activation of the imd pathway (Fig. 1) (Wu *et al.*, 2001; Choe *et al.*, 2002; Gottar *et al.*, 2002; Ramet *et al.*, 2002; Takehana *et al.*, 2004; Kaneko *et al.*, 2006). Lysine (Lys) type PGN of G^+ bacteria on the other hand is recognized by PGRP-SA, PGRP-SD and GNBP1 leading to Toll pathway activation (Fig. 2) (Michel *et al.*, 2001; Gobert *et al.*, 2003; Bischoff *et al.*, 2004). Sensing of fungal presence relies on the detection of glucan, a fungal cell wall constituent, by GNBP3 (Gottar *et al.*, 2006) and on the activation of the serine protease Persephone (Psh) in the hemolymph by virulence factors like fungal proteases (Ligoxygakis *et al.*, 2002). Both fungal infection detection cascades converge on Toll pathway activation (Fig. 2). The ability to form several PRR complexes furthermore expands the repertoire of microbial species of which the presence can be sensed (Bischoff *et al.*, 2004). Recent findings also suggest that these recognition systems presumably detect proliferation of bacteria rather than their presence (Ferrandon *et al.*, 2007). Furthermore, some microbial species can elicit both Toll and imd signaling through a yet unknown process (De Gregorio *et al.*, 2002b).

Upon detection of Gram negative bacterial infection, activation of the imd pathway (Fig. 1) is probably achieved through cooperation of transmembrane PGRP-LC and intracellular PGRP-LE (Takehana *et al.*, 2004; Chang *et al.*, 2005; Mellroth *et al.*, 2005; Kaneko *et al.*, 2006). PGRP-SC and PGRP-LB, which reside in the hemolymph, further modulate the intensity of the imd pathway stimulation (Mellroth *et al.*, 2003; Mellroth and Steiner, 2006; Zaidman-Remy *et al.*, 2006). This then results in the induction of several cascades downstream of the imd protein leading to activation of the Relish derived NF-κB transcription factor REL (Hedengren *et al.*, 1999), as well as to JUN N-terminal kinase (JNK) pathway activation (Boutros *et al.*, 2002; Park *et al.*, 2004). Both phosphorylation and cleavage of Relish are needed to liberate the transcription factor fragment REL from its inhibitory part. Phosphorylation is performed by the IκB kinase (IKK) signaling complex composed of Kenny and IRD5 (immune response deficient 5) whereas cleavage involves the coordinated action of at least three proteins, namely imd, Fadd (FAS-associated death domain) and Dredd (death-related ced-3/Nedd2-like protein) (Rutschmann *et al.*, 2000; Silverman *et al.*, 2000; Stoven *et al.*, 2000; Lu *et al.*, 2001; Leulier *et al.*, 2002;). IKK and JNK activation furthermore is mediated by TAK1 and TAB2 (TAK1-binding protein 2) (Vidal *et al.*, 2001; Boutros *et al.*, 2002; Silverman *et al.*, 2003; Gesellchen *et al.*, 2005; Geuking *et al.*, 2005; Kleino *et al.*, 2005). REL-induced gene activation then results in the synthesis and release of AMPs and other effector proteins. Among the *Drosophila* AMPs, diptericin (dipt), attacin (att), drosocin (dros) and cecropin (cec) were identified as being imd pathway induced (Hoffmann, 2003). Negative regulation of the imd pathway furthermore involves Caspar and the ubiquitin-proteasome pathway for controling Relish and Dredd activation levels (Khush *et al.*, 2002; Kim *et al.*, 2006). The physiological relevance of JNK signaling in the innate immune response remains elusive although it has been suggested to be involved in the control of the expression of some AMPs, and to regulate wound healing and melanization (Boutros *et al.*, 2002; Igaki *et al.*, 2002; Silverman *et al.*, 2003; Kim *et al.*, 2005) JNK signaling furthermore is shut off upon Relish activation. The JNK-dependent response thus is transient and it precedes the sustained induction of Relish-dependent innate immune loci (Park *et al.*, 2004).

Induction of the Toll pathway (Fig. 2) upon G^+ and fungal infection is preceded by the cleavage of Spätzle (Spz) by the Spätzle processing enzyme (SPE) (Jang *et al.*, 2006). Spz then binds to the membrane bound Toll receptor thereby initiating the assembly of the Toll induced signaling complex (TISC) which is composed of myeloid differentiation primary response gene 88 (MyD88), Tube and Pelle (Tauszig-Delamasure *et al.*, 2002; Weber *et al.*, 2003). TISC subsequently targets the Cactus-DIF (Dorsal related immunity factor) complex in an unknown way to induce the degradation of the NF-κB inhibitor Cactus resulting in the release of the NF-κB transcription factor DIF. Gene activation is then accomplished upon translocation of DIF to the nucleus (Belvin *et al.*, 1995; Fernandez *et al.*, 2001). Drosomycin (drom), defensin (def) and metchnikowin (metch) are the AMPs of which the synthesis is induced by Toll pathway activation (Hoffmann, 2003).

Both the induction of the Toll and the imd pathway thus leads to the activation of NF-κB transcription factors, DIF and REL, which recognize distinct κB binding sites.

Many more putative Toll and imd pathway components were discovered using several distinct assays, e.g., RNAi on cultured S2 cells (Avila *et al.*, 2002) and loss-off-function screens (Wu and Anderson, 1998; Wu *et al.*, 2001), but their sites of action in Toll and/or imd signaling still need to be explored.

Constitutive AMP activation furthermore has been demonstrated in several epithelia in close contact with the environment. Their expression however seems to be independent of both Toll and imd signaling. Upon infection, on the other hand, AMP expression in these epithelia solely relies on imd pathway activation (Ferrandon *et al.*, 1998; Tzou *et al.*, 2000).

Cellular response

The blood cells, or hemocytes, of *Drosophila* participate in the immune response through the production of AMPs, the phagocytosis of bacteria, the

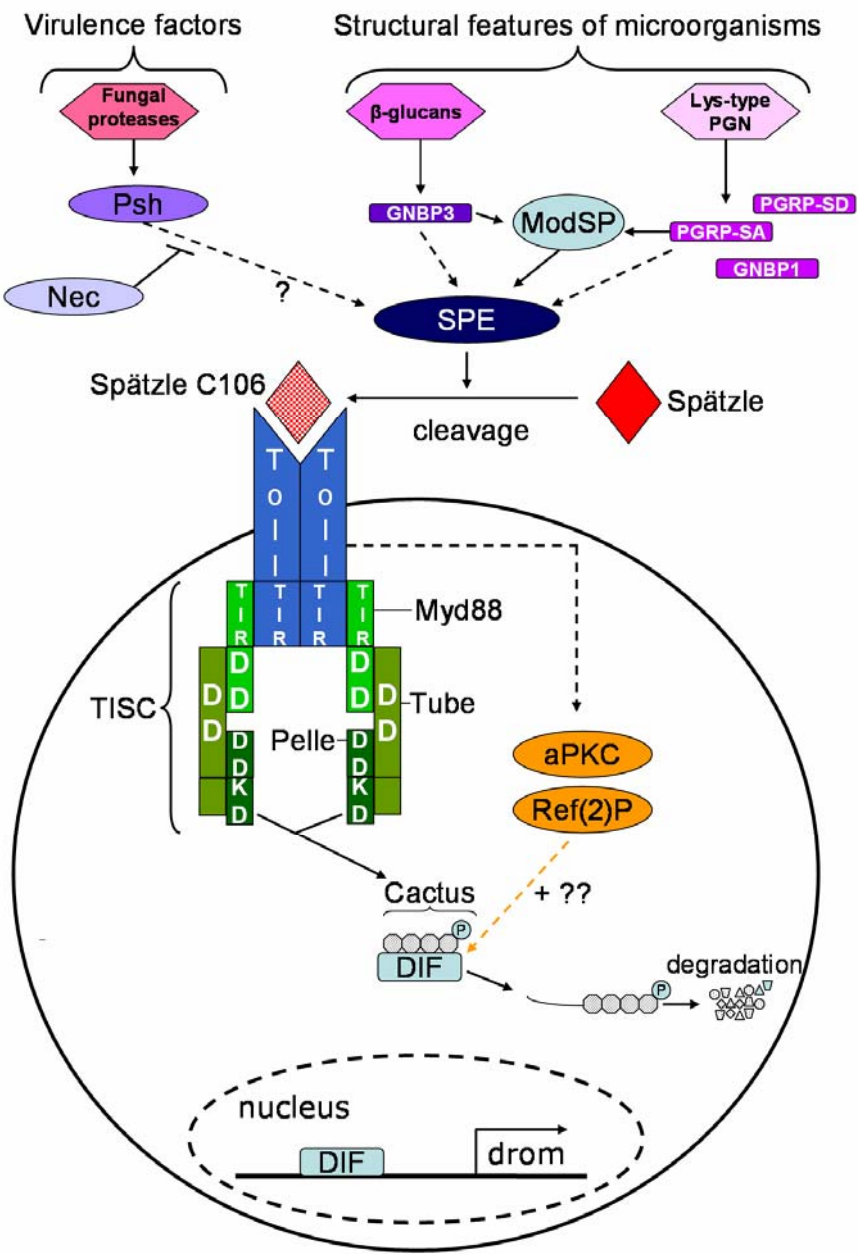

Fig. 2 The *Drosophila* Toll pathway. Structural components of the cell wall of G^+ bacteria and fungi are detected by soluble pattern recognition receptors in the hemolymph. PGRP-SA, PGRP-SD and GNBP1 recognize Lys type PGN probably upon formation of PRR complexes, and GNBP3 binds to fungal derived β-glucans. Virulence factors like fungal proteases [e.g., Pr1 (Gottar *et al.*, 2006)] also can initiate an immune response through activation of the CLIP serine protease Psh. The protease inhibitor Necrotic (not shown) furthermore seems to be involved in sensing fungal infection in the same proteolitic pathway as Psh (Levashina *et al.*, 1999). Recently a second proteolitic cascade acting downstream of circulating PRRs and including the activity of Grass (Gram positive specific serine protease) was uncovered (not shown) (El Chamy *et al.*, 2008). Both the Psh and the Grass dependent pathways are required for full activation of Toll upon fungal and G^+ infection. All these diverse pathogen recognition systems converge in an largely unknown way to the cleavage of Spätzle by SPE resulting in the release of its 106 amino acid C-terminal fragment (C106). Recently the modular serine protease (ModSP) was shown to integrate signals originating from GNBP3 and PGRP-SA and to connect them to SPE activity on Spz (Buchon *et al.*, 2009b) Spätzle C106 then binds to Toll thereby inducing conformational changes in the receptor resulting in its activation. Activated Toll induces the assembly of the TISC composed of three members bearing DDs. Tube links Pelle to MyD88 through DD interactions, and MyD88 probably interacts with Toll through its TIR (Toll/IL-1R) domain. TISC formation results in the activation of the kinase domain (KD) of Pelle, and in an unknown way in the phosphorylation of the NF-κB inhibitor Cactus thereby initiating its rapid polyubiquitylation and subsequent degradation. This allows the liberated NF-κB transcription factor DIF to translocate to the nucleus and to induce gene activation upon binding to NF-κB elements. A cell culture study furthermore suggest the existence of a second branch downstream of Toll activation consisting of atypical protein kinase C (aPKC) and Ref(2)P signaling components to aid in inducing DIF activation (Avila *et al.*, 2002).

encapsulation of larger foreign particles such as parasitic eggs as well as the signalling of pathogen presence to the fat body (Evans *et al.*, 2003). At the larval stage, three types of circulating hemocytes can be distinguished originating from the embryonic head mesoderm (Holz *et al.*, 2003), the larval lymph glands (Sorrentino *et al.*, 2002) as well as from sessile hemocytes attached to larval epithelial tissues (Markus *et al.*, 2009). Among these, the predominant type (90-95%) consists of small round plasmatocytes which essentially are phagocytic cells or macrophages. These engulf and degrade apoptotic and dead cells, and cellular detritus as well as microbial pathogens occurring in their hemolymph in a process known as phagocytosis. Phagocytosis is initiated by the recognition of these targets mainly by four classes of molecules: the complement-like opsonin family of TEPs (thioester-containing proteins), the class B scavenger receptors CD36, Peste and Croquemort, the EGF-like repeat containing receptors Eater, Nimrod C1 and Draper, and the Dscam (Down syndrome cell adhesion molecule) isoforms (reviewed in Stuart and Ezekowitz, 2008). Most of these components as well as a plethora of other intercellular phagocyte gene products were discovered with studies using a variant of *Drosophila* embryonic S2 (Schneider's line 2) cells combined with RNAi (Pearson *et al.*, 2003;Ramet *et al.*, 2001). Additional studies are required to explore their function(s) *in vivo*.

Plasmatocytes also secrete AMPs as well as other immune signaling components. Upd3 (unpaired 3), one of these molecules, has been shown to signal an infection induced by septic injury to the fat body thereby activating the JAK/STAT pathway in a cytokine-like manner (Agaisse *et al.*, 2003).

A smaller proportion of circulating hemocytes is formed by the crystal cells which are recognized by pronounced crystal-like inclusions in their cytoplasm. These contain the enzymes necessary for humoral melanization which accompanies many immune reactions (see below).

The third circulating hemocyte type, the lamellocyte, is normally not present in healthy larvae. Lamellocytes are large flat cells of which the amount substantially increases after specific stimuli, e.g., presence of parasitic wasp eggs, through a small burst of mitosis and subsequent differentiation of sessile cells in the lymph glands (Sorrentino *et al.*, 2002) and through differentiation of a subepidermal lamellocyte precursor population (Markus *et al.*, 2009). The balance between the multipotent prohemocytes and the differentiating blood cells upon infection is controlled by a small cluster of signalling cells in the lymph glands, termed the posterior signalling centre (PSC) (Krzemien *et al.*, 2007). Lamellocytes mediate the encapsulation of invaders, e.g., parasitoids, that are often too large to be phagocytized. Integrins seem to be involved in the strengthening of the capsule (Irving *et al.*, 2005). Encapsulation furthermore is often accompanied by a localized melanization reaction and an augmented nitric oxide (cytotoxic) production, resulting in the killing of the parasite within the black capsule (Carton and Nappi, 1997, Nappi *et al.*, 2000).

The hemocytes detected in *Drosophila* adults originate from the embryonic and larval lineages that persist during metamorphosis to populate this developmental stage. No adult hematopoietic organ has been described so far (Holz *et al.*, 2003).

Recently, it was found that priming *Drosophila* with sublethal doses of *Streptococcus pneumoniae* or the natural fly pathogen *Beauveria bassiana* has protective effects during subsequent challenges, and this persisted for the life of the fly. Phagocytes as well as Toll pathway activation, however independent of AMP synthesis, were required for this presumably species specific but not generally observed effect. These findings raise questions about the absence of memory in *Drosophila*'s innate immune responses (Pham *et al.*, 2007).

For a more in depth description of the cellular immune response, see for review Evans *et al.* (2003) and Stuart and Ezekowitz (2008).

The phenoloxidase response

The melanization reaction seems to be the most immediate immune response against invading pathogens in *Drosophila* (Cerenius and Söderhäll, 2004). It frequently assists the encapsulation reaction in the killing of microbial pathogens (Nappi and Christensen, 2005). Melanization is visible by the blackening of a wound site or of the surface of invaders, which results from the synthesis and deposition of melanin (Tang *et al.*, 2006). The melanization reaction starts off with the activation of prophenoloxidases (proPO) to active phenoloxidase (PO) enzyme in the hemolymph of arthropods, hence it is also referred to as PO response. This system had been extensively studied in large insects such as *Manduca sexta* (Cerenius and Söderhäll, 2004; Söderhäll and Cerenius, 1998). This has led to the present model (Fig. 3) in which the recognition of microorganisms triggers a proPO-activating enzyme (PPAE) proteolytic cascade dedicated to the activation of PO. The activated PO then catalyses the oxidation of tyrosine-derived phenols to quinones. Quinones then polymerize non-enzymatically to form insoluble melanin. Quinones as well as melanin and its biosynthetic byproducts, hydrogen peroxide and nitric oxide amongst others, are directly toxic to microorganisms (Nappi *et al.*, 2000, Nappi and Ottaviani, 2000). Melanin deposition is observed at all infection sites, where it possibly contributes to wound healing and to the control of microorganism growth as well as to their killing (Leclerc *et al.*, 2006, Nappi *et al.*, 1995, Carton *et al.*, 2009, Nappi and Christensen, 2005). Of note, nearly all arthropod proPOs are devoid of a secretion signal sequence. There presence in the hemolymph is therefore assumed to result from hemocyte rupture, as reported for some *Drosophila* proPOs (Bidla *et al.*, 2007).

In *Drosophila*, induction of the melanization response upon G^- infection involves PGRP-LE, which is also one of the key PRRs of the imd pathway (Fig. 1). The crystal cells furthermore are shown to express proPO A1 (or 54) as well as proPO45. The third *Drosophila* proPO is expressed exclusively in lamellocytes. The cells that mediate the encapsulation response thus also can provide

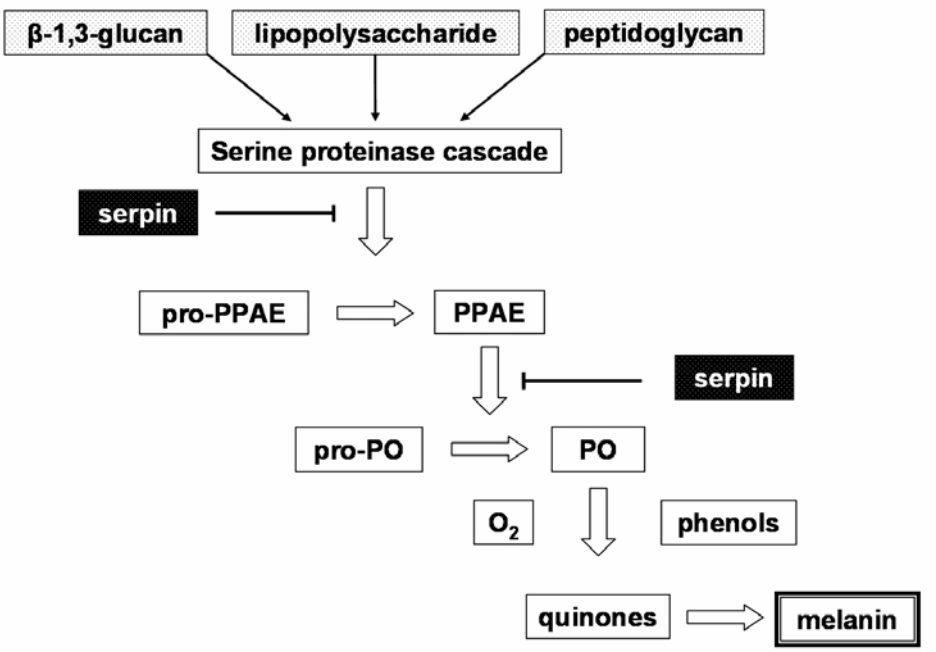

Fig. 3 Overview of the arthropod melanization cascade. The system is activated upon recognition of bacterial and fungal cell wall components by PRRs as well as by some endogenous factors produced upon tissue damage, e.g., during wounding. A cascades of serine proteases presumably will result in the cleavage of proprophenoloxidase activating enzyme (pro-PPAE) thereby activating it. Cleavage of proPO by active PPAE results in the activation of PO. Next, PO catalyzes the oxidation of phenols to quinones, which subsequently can polymerize to melanin. Control of PO activity is presumed to result from its synthesis as inactive precursor as well as from the presence of proteinase inhibitors which probably avoid excessive or premature activation (adapted from Cerenius & Söderhäll, 2004).

one of the key enzymes for melanization (Irving *et al.*, 2005). The activation of proPO in *Drosophila* is partially controlled by the serine protease inhibitor serpin 27A (Spn27A) (Nappi and Christensen, 2005). The target of Spn27A is thought to be PPAE since recombinant Spn27A is able to inhibit beetle PPAE (De Gregorio *et al.*, 2002). This however has not been demonstrated to occur *in vitro* or *in vivo* with *Drosophila* PPAE. Two immune inducible serine proteases, MP1 (CG1102) and MP2 (CG3066) (melanization protease 1 and 2), furthermore were identified which act in the PO cascade regulated by Spn27A. MP1 seems to be required for activation of the melanization process upon bacterial and fungal infection whereas MP2 is only involved in fighting fungal infection and thereby acting upstream of MP1. MP2 is furthermore able to induce Drosomycin expression independent of Toll pathway activation (Tang *et al.*, 2006). MP2 (or PPAE1) was furthermore reported to be a *Drosophila* PPAE as constitutive PPAE mutants showed constitutive PO activation (Leclerc *et al.*, 2006). Further elucidation of the *Drosophila* PO response thus is required to explore whether it is consistent with the proposed model (Fig. 3).

Recently, Colinet *et al.* (2009) identified the first serpin used as a virulence factor from the parasitoid wasp *Leptopilina boulardi* and they showed that it targets the *Drosophila* phenoloxidase cascades.

The JAK/STAT cascade in innate immunity

The evolutionary conserved Janus kinase (JAK)/signal transducer and activator of transcription (STAT) cascade plays a key role in a wide variety of biological processes (Arbouzova and Zeidler, 2006). In *Drosophila*, only one STAT protein, STAT92, seems to exist and Hopscotch (Hop) is the homolog of vertebrate tyrosine kinase JAK (Binari and Perrimon, 1994; Hou *et al.*, 1996; Yan *et al.*, 1996). Loss-of-function mutants of both STAT92E and Hop show a severe decrease in immune response activation upon infection, implicating the *Drosophila* JAK/STAT pathway in the innate immune defense (Sorrentino *et al.*, 2004). STAT92E furthermore is activated in the fat body upon immunization (Kwon *et al.*, 2000).

JAK/STAT pathway activation (Fig. 4) requires binding of an extracellular ligand, unpaired (upd), to a transmembrane receptor, Domeless/Master of Marelle (Dome/Mom). Upd, upd2 or upd3 constitute the *Drosophila* upd family of which only the latter is implicated in the fruit fly's immune response (Agaisse *et al.*, 2003). Ligand binding then results in the activation of receptor-associated JAKs which recruit STATs after their phosphorylation. Next, the STATs are phosphorylated and they will form the dimers that are responsible for gene activation upon translocation to the nucleus (see for review

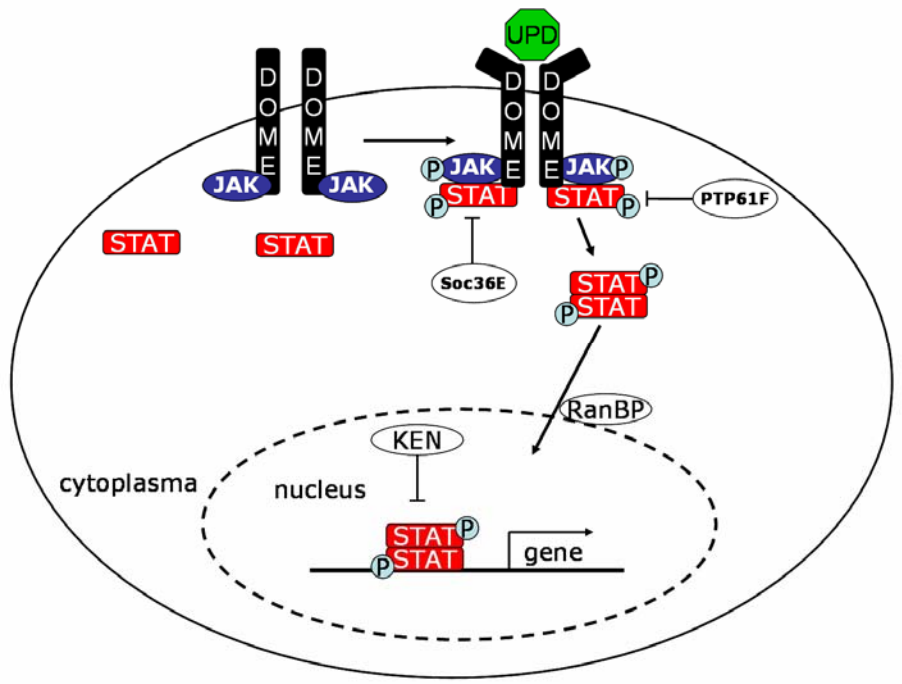

Fig. 4 The JAK/STAT pathway. Upd binding onto the Dome receptor dimer activates JAK kinases. JAKs phosphorylate (P) one another and subsequently attract STATs. After JAK dependent phosphorylation and subsequent dimerization, STAT dimers are translocated to the nucleus through nuclear pore complexes to act as activators of gene transcription.

Arbouzova and Zeidler, 2006). Upon infection, Turandot A (TotA), TotM and TotC, which normally accumulate in the hemolymph in response to various stress conditions including immune challenge, are expressed in the fat body thereby requiring JAK activity (Agaisse *et al.*, 2003). Gene activation of *vir-1* (*virus induced RNA 1*) furthermore is also attributed to JAK/STAT signaling since STAT92E binds to its promoter (Dostert *et al.*, 2005).

In *Drosophila*, several negative regulators of JAK/STAT signalling were identified (Fig. 4). Among these, SOC36E (suppressors of cytokine signaling 36E) and tyrosine phosphatase PTP61F (phospho-Tyr phosphatase 61F) both are a transcriptional target as well as a negative regulator, thereby forming a negative feedback loop to down-regulate pathway activity (Baeg *et al.*, 2005; Muller *et al.*, 2005). Furthermore, the *Drosophila* homologs of RanBP3 and RanBP10 control the nucleocytoplasmic transport of STAT92E (Baeg *et al.*, 2005). Ken and Barbie (KEN), a transcriptional repressor, selectively regulates STAT92E activity (Arbouzova *et al.*, 2006).

JAK/STAT pathway activity furthermore is also detected in hemocytes where it modulates hemocyte proliferation and differentiation, e.g., into lamellocytes in cooperation with the Ras and Toll pathway (Evans *et al.*, 2003).

Biological functions of JAK/STAT, besides its implication in the innate immune response, are summarized in Agaisse and Perrimon (2004) and in Arbouzova and Zeidler (2006).

The antiviral response: implication of RNA interference in the innate immune system

RNAi probably originated as an innate immune mechanism for fighting viral infections (Fig. 5). Viral dsRNA thereby is used to trigger host-mediated degradation of viral RNA. The identification of viral proteins, e.g., B2 of flock house virus and 1A from DCV (*Drosophila* C virus), that are able to inhibit the RNAi pathway (Li *et al.*, 2004) and the presence of viral small interfering RNAs in infected cells (Aliyari *et al.*, 2008) support this RNAi origin hypothesis. In *Drosophila*, three RNAi pathways are described of which at least two, an Argonaute 2 (AGO2) dependent and the Piwi pathway, seem to be implicated in the anti-viral defence although both are elicited upon different viral infestations (van Rij and Berezikov, 2009). Of note, Dicer 2, Argonaute 2 and R2D2 are encoded by the 3 % fastest evolving genes in *Drosophila*. This probably is driven by the likewise fast evolving viral inhibitor proteins (Obbard *et al.*, 2006).

In addition, studies using *Drosophila* X virus (DXV, a dsRNA virus) implied both Toll and imd pathway signaling in the detection of viral infection, although only a Toll induced NF-κB activation, dissimilar to the one described for AMP production (Fig. 2) seems to confer protective effects (Zambon *et al.*, 2005). DCV infection, adversely, results in JAK/STAT-induced immune signaling but not either Toll- nor imd-mediated immune responses (Dostert *et al.*, 2005). Viruses thus evoke different defense

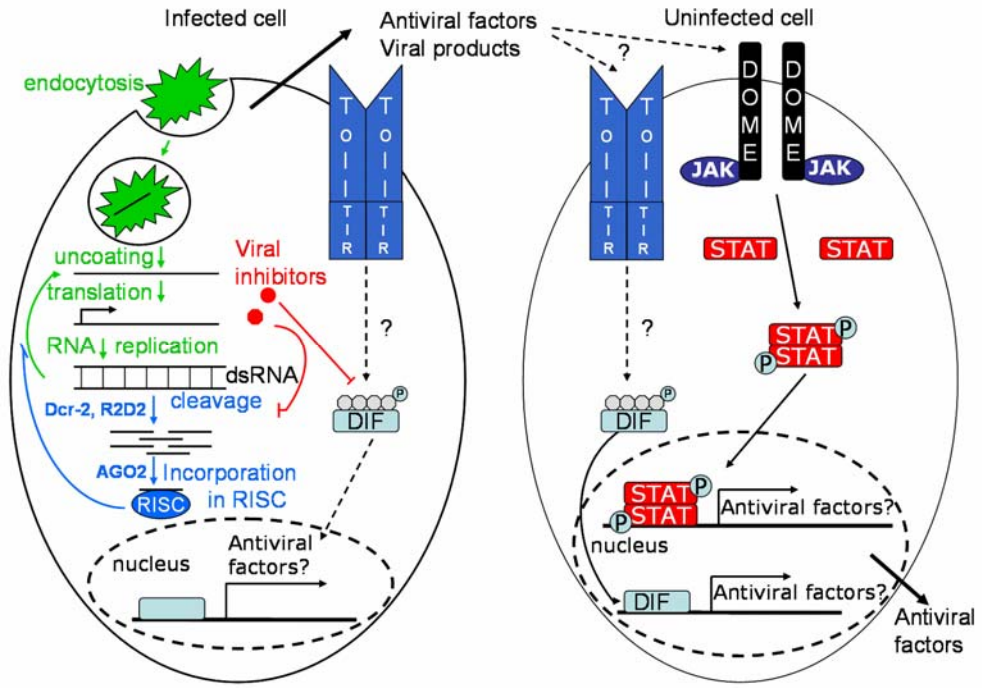

Fig. 5 Defense response against viral infection. Left: Viral infection (mainly through endocytosis) as well as viral replication in infected cells depend on endogenous cellular factors of the infected host (depicted in green). Many viruses depend on dsRNA production for replication. The hosts RNAi machinery, composed of Dicer-2 (Dcr-2) and its cofactor R2D2, mediate cleavage of the viral dsRNA into siRNAs. These are subsequently incorporated into an AGO2 containing RISC complex which is devoted to the targeted destruction of analogical sequence specific viral RNA (depicted in blue). Viruses object this RNAi mediated defence response by encoding RNAi inhibitor components (depicted in red). Some viral infections furthermore lead to the activation of a Toll induced, yet unknown signaling cascade that results in NF-κB induced (DIF) antiviral gene activation (depicted in blue). Recently, the amino terminal DExD/H box helicase domain of Dicer 2 was implicated in the inducible antiviral response thereby suggesting a connection between this and RNAi (Deddouche *et al.*, 2008). Right: Uninfected cells may be induced to produce and release antiviral factors by an unknown mechanism probably involving recognition of viral products, cell debris or other host antiviral factors. Both JAK/STAT and Toll pathway activation have been implicated in the production of the proteins of which some are assumed to have antiviral properties, e.g., Vago (Deddouche *et al.*, 2008) and vir-1 (Dostert *et al.*, 2005).

responses probably depending on differences in their pathogenesis and replication cycles.

The exploration of the antiviral response in *Drosophila* commenced about five years ago resulting in a yet limited understanding which is even more complicated by the different defense responses evoked by various viruses (see for reviews Kemp and Imler, 2009; Van Rij and Berezikov, 2009).

Immune defence systems in epithelial tissues

The innate immune responses described above were all explored in the two main immune tissues of *Drosophila*, the fat body and the hemocytes. They focus on the recognition and signaling of a pathogenic invader in the body cavity, and the subsequent production and release of immune effectors into its main battlefield, the hemolymph. This is referred to as the systemic immune response.

As is the case in mammals, also the epithelial linings of the digestive, respiratory and reproductive system that constitute the physical barrier for pathogen entrance into the body cavity do seem to rely on an effective immune defense system to try to prevent this invasion. Epithelial expression of antimicrobial peptides, for example was explored using reporter flies in which the promoter sequence of each of the seven AMPs (att, cec, def, droc, dipt, drom and metch) was fused to the green fluorescent protein coding sequence. This way, the expression pattern of the AMPs was explored both in larvae and in adults (Ferrandon *et al.*, 1998; Tzou *et al.*, 2000). This led to the finding that AMPs can be induced in surface epithelia in a tissue-specific manner and that IMD plays a critical role in the activation of this local response to infection (Tzou *et al.*, 2000). Malphigian tubule epithelia, furthermore, shown expression of PGRPs, e.g., PGRP-LE when induced by TCT (tracheal cytotoxin), disaccharide-tetrapeptide fragment of PGN (Kaneko *et al.*, 2006).

Furthermore, the *Drosophila* gut lumen is considered to be hostile to transient microbial

colonization due to physical (acidity) and physiological (peristalsis of the gut) properties and the presence of lysozymes. And the gut epithelium further was shown to express AMPs and to catalyze the generation of ROS that together most often provide an effective barrier against ingested microbes. Next, global gene expression analysis of *Drosophila* intestinal tissue, i.e., the gut minus the Malpighian tubules and gastric caeca, to oral infection with the G^- bacterium *Erwinia carotovora* recently revealed that immune responses in the gut are regulated by the imd and JAK-STAT pathways, but not the Toll pathway (Buchon *et al.*, 2009a). In addition, the Malpighian tubules also possess cell-autonomous, immune-sensing capabilities. All components of both the imd and the Toll pathway are expressed herein, and they are expected to mainly lead to the production of diptericin, cecropin and metchnikowin. The tubules furthermore produce nitric oxide (NO) which was also shown to be extremely important for AMP production as significant improvement in survival rates of the whole animal upon immune challenge was observed after forced NO production (Davies and Dow, 2009).

Similarities between mammalian and insect immune responses

Insects and vertebrates display considerable overlap in the signalling pathways that regulate innate immunity and in some of the effector mechanisms used against microbes. The mode of detection of microbial patterns through activation of pattern recognition receptors and NF-κB signalling cascades have been conserved throughout evolution. The *Drosophila* Toll pathway, for example, has some parallels to the mammalian signalling systems downstream of the interleukin 1 receptor (IL-1R) and the Toll-like receptors (TLRs). The main difference seems to be the fact that the *Drosophila* Toll receptor does not sense microbial inducers directly, as most mammalian TLRs do, but instead relies on an upstream recognition system. Furthermore, the mammalian system seems to be able to use the same NF-κB inducing cascade after recognition of both G^+ and G^- bacterial infection, while *Drosophila* uses two distinct systems, i.e., Toll and imd signaling. The *Drosophila* imd cascade furthermore is similar to the mammalian tumour necrosis factor receptor (TNFR) pathway (Hoffmann, 2003; Wang and Ligoxygakis, 2006).

A number of similarities between *Drosophila* hemocytes and mammalian blood cells such as AMP synthesis and cytokine production were also reported, as well as some specifics of invertebrate cellular immunity. The melanisation response, for example, has no clear counterpart in mammals but instead uses molecular building blocks (proteolytic cascades, integrins) that are conserved among phyla (Irving *et al.*, 2005). *Drosophila* blood cells consist of only a few terminally differentiated types whose functions resemble those of the cells of the vertebrate myeloid lineage which gives rise to macrophages among others (Evans *et al.*, 2003). A proteomic analysis of the phagosome content of *Drosophila* plasmatocytes, for example, has revealed that 70 % of its protein content (600 identified proteins) has a mammalian orthologue thereby validating fruit fly phagosomes as a model to study phagocytosis (Stuart and Ezekowitz, 2008). The encapsulation reaction furthermore has a similar function as the formation of granuloma in vertebrates (Markus *et al.*, 2009). In addition, the activation of TotA in the *Drosophila* fat body as a response to the release of upd3 by hemocytes, for example, is very similar to some aspects of the mammalian acute-phase response mediated by cytokines (Agaisse and Perrimon, 2004).

Next, the human genome encodes all major JAK/STAT pathway components. Molecular and functional data clearly indicate that a high level of conservation exists between the structural components of both the insect/*Drosophila* and the mammalian pathway (Arbouzova and Zeidler, 2006).

For fighting off viral infections, the mammalian system seems to depend largely on recognition of dsRNA, as does *Drosophila*, but in mammals this mainly leads to the production of interferons while in *Drosophila* RNA interference is the main immune effector (Cherry and Silverman, 2006). The study of the *Drosophila* antiviral immunity only just recently has gained serious interest. Further similarities and differences thus are expected to be uncovered in the near future.

Overall, discoveries made through research in the fruit fly, *Drosophila melanogaster* may be applicable to the study of innate immunity in humans.

The exploration of innate immunity in insects has also garnered increasing attention because of the role of many insects in transmission of human disease agents. Understanding how the insect immune system interacts with pathogens may contribute to the development of new strategies to block transmission of disease agents (Shi and Paskewitz, 2006).

Recently, questions were raised regarding the absence of memory and the absence of the diversity of immunoglobulin like recognition receptors in *Drosophila* innate immunity as seen in mammals. Induction of resistance to lethal doses of a pathogen by priming with sublethal doses was observed for *S. pneumoniae* and *B. bassiana* and required both the Toll pathway and phagocytosis (Pham *et al.*, 2007). The discovery that the neuronal immunoglobulin superfamily member DSCAM, which is encoded by a gene that can potentially generate 18,000 splice isoforms, is expressed by hemocytes and by cells in the fat body has also raised considerable interest as to the possibility of the generation of a large receptor repertoire in *D. melanogaster* (Watson *et al.*, 2005). Further research will probably shed light on these interesting findings.

Conclusion

In *Drosophila*, the epithelial defense system forms the first barrier to microorganismal infection. Upon entry into the body cavity, pathogens subsequently are mainly countered in the hemolymph with humoral, cellular and phenoloxidase defence responses. Both the JAK/STAT and the JNK pathways further seem to be implicated herein. *Drosophila* further seems to

rely on RNAi to combat viral infections. Appropriate experimental studies to identify the actual killing elements, however, are still awaiting. Interestingly, many *Drosophila* immune components have a counterpart in mammals making the study of fruit fly immunity of great importance for the unraveling of the conserved innate immune cascades.

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