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Review

Drosophila melanogaster model in innate immunity

Vincent Leclerc[1,*], Laure El Chamy[2], Monde Ntwasa[3] and Jean-Marc Reichhart[4]

[1]Université de Strasbourg and UPR9022 CNRS, IBMC, 15 rue René Descartes, 67084 Strasbourg Cedex, France. [2]Laboratoire de Génétique de la drosophile et virulence microbienne, EGFEM, Faculté des Sciences, Université Saint Joseph, Beirut, Lebanon. [3]School of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg, Private Bag 3, Wits. 2050, South Africa. [4]Université de Strasbourg and UPR9022 CNRS, IBMC, 15 rue René Descartes, 67084 Strasbourg Cedex, France.

ABSTRACT

Due to relative simplicity of the fly and availability of large amounts of genetic tools, *Drosophila melanogaster* has proven to be an excellent model to study the basic principles of innate immunity. This is illustrated by the discovery of the Toll-like receptor functions in pathogen sensing, recognised by the 2011 Nobel Prize in Physiology and Medicine awarded to Jules Hoffmann. *Drosophila* can also be used as an *in vivo*, genetically tractable model, to analyse various aspects of host-pathogen interactions including virulence factor mechanisms of action.

KEYWORDS: antimicrobial peptides, antiviral response, danger signal, *Drosophila*, gut infection, innate immunity, NFκB, PAMP, Toll

INTRODUCTION

The 2011 Nobel Prize in Physiology and Medicine was awarded to Ralph Steinman, Bruce Beutler and Jules Hoffmann for their discoveries in innate immunity. The laboratory of Jules Hoffmann used *Drosophila melanogaster* as a model system, demonstrating that research pursued on the tiny insect can provide important clues for our understanding of the human immune system. Recognition that the innate immune system has a paramount role in the detection of infections and the subsequent mounting of an adequate response directly stemmed from research on *Drosophila*. In this review we summarize our understanding of the fly immune system, explain how working on a simple animal model helped to decipher complex questions in the mammalian innate immune system and discuss what *Drosophila* is still able to bring to the field of immunity.

*Corresponding author: v.leclerc@unistra.fr

1. On the road to Toll

1.1. Discovery of antimicrobial peptides

The initial studies that led to the understanding of the *Drosophila* immune system actually utilized other insects such as larger flies or butterflies, which were easier to manipulate than *Drosophila*. The observation that insect larvae used to study the hormonal control of moulting and metamorphosis were rarely affected by infections prompted the researchers to look at mechanisms that could explain this protection. In the early 1980s, the group of Hans Boman in Stockholm discovered antimicrobial activities in the hemolymph (blood) of infected larvae. When purified, the active molecules appeared to be small peptides with antibacterial activities and were called anti-microbial peptides (AMPs) [1, 2]. They were later discovered in a wide range of organisms and more than eight hundred natural AMPs have since been isolated, including human cathelicidins, defensins and histatins. AMPs are mainly produced locally in epithelia or by phagocytes, but they are also secreted in the insect blood after infection, thus

facilitating their purification. They act through mechanisms either involving membrane disruption and pore formation or targeting essential bacterial processes and therefore induce rapid killing. Furthermore, it is currently clear that AMPs are also implicated in various immunomodulatory activities. AMPs are now considered as potential molecules for therapeutic use as an alternative to antibiotics [3, 4].

1.2. Inducibility and specificity of antimicrobial peptides

AMPs were also purified in infected *Drosophila* and the model was then chosen for further studies on the regulation of their expression. It was rapidly shown that different types of infection resulted in the expression of different AMPs: for example Cecropin and Diptericin were induced by and active against Gram-negative bacteria, whereas Drosomycin was induced after fungal and Gram-positive bacterial infections against which it was active. This specificity was not restricted to *Drosophila*, but the power of the model relied on the robust genetic tools, which had accumulated since the initial work of Thomas Hunt Morgan at the beginning of the 20th century and which provided powerful means to decipher the mechanisms of AMP expression. In the early 1990s, the group of Jean-Marc Reichhart and their colleagues in the Strasbourg CNRS laboratory headed by Jules Hoffmann, initiated the elucidation of the signalling pathways that control the expression of the genes encoding these AMPs in *Drosophila* (see [5, 6] for reviews).

1.3. Toll comes into the story

The first clue was the discovery that upstream regulatory sequences of AMP genes contain binding sites for the mammalian Nuclear Factor-κB (NF-κB). This factor was known for its involvement in the immune response in mammals, controlling the expression of many cytokines in response to immune challenge. This was a first link between immune responses in *Drosophila* and mammals. The second link came with the identification of the Toll pathway by Eric Wieschaus and Christiane Nusslein-Volhard (1995 Nobel Prize) who were looking for *Drosophila* mutants affecting early embryogenesis and found that the Toll signalling pathway was involved in the establishment of the embryonic dorso-ventral polarity. Activation of the Toll pathway culminated in the activation of a *Drosophila* NF-κB homolog, Dorsal (Figure 1). Furthermore the Toll transmembrane receptor cytoplasmic domain was similar to the cytoplasmic domain of the Interleukin-1 receptor (IL-1R). Altogether these observations suggested that the Toll pathway could have a second function in *Drosophila* by activating the expression of AMPs. Indeed it was shown that flies carrying a mutation in the Toll pathway are unable to synthesize Drosomycin and are highly susceptible to fungal and Gram-positive bacterial infections, demonstrating that the Toll pathway protects the flies against these microorganisms [7, 8].

1.4. Toll is not alone: the IMD pathway

1.4.1. The IMD pathway response to Gram-negative bacteria

Roughly at the same time, an independent mutation responsible for the susceptibility of *Drosophila* to Gram-negative bacteria and defining a second pathway was discovered and called Immune Deficiency (IMD) (Figure 2, see [9] for review). The IMD pathway culminates with the activation of another NF-κB factor, Relish, and controls the expression of a second set of AMPs active against Gram-negative bacteria [10]. The Toll and IMD pathways have great similarities with IL-1R and TNFα receptor pathways, respectively (see [11-13] for reviews).

1.4.2. Analysis of conserved signalling pathways

Identification of the molecular lesions pertaining to mutations affecting Toll and IMD pathways helped to understand how the immune signalling cascades function in both *Drosophila* and mammals. As an example, the recognition that a point mutation in a domain with no known structural feature of the IMD protein inactivated the pathway led to the discovery that IMD, a homolog of mammalian RIP1, is cleaved and ubiquitinated after activation, leading to the formation of a signalling platform [14]. Another example of the power of the *Drosophila* system was the identification of Akirin, a nuclear protein conserved in mammals and required in *Drosophila* for IMD signalling. Mammalian Akirin was shown to be required for activation of only a subset of NF-κB target genes, mostly pro-inflammatory cytokines.

Anti-inflammatory genes were unaffected by inactivation of Akirin. Thus, Akirin provides selectivity to NF-κB factors in both mice and flies by recruiting chromatin remodelling complexes [15, 16]. These data suggest that this newly identified protein could be targeted by future anti-inflammatory drugs with less side effects than those that completely inactivate NF-κB signalling [17].

1.5. From Toll to TLRs

1.5.1. Identification of Toll-like receptors

The discovery that the *Drosophila* Toll pathway was implicated in the defence against infections had three major impacts. First, it demonstrated that an immune response could be highly effective in the complete absence of antibodies and more generally of an adaptive response as it is the case for invertebrates. Second, it demonstrated that the innate immune response is not only based on phagocytosis, which was known, but also on a systemic antimicrobial response that is, to some extent, specific. The third impact was based on collaborations of the Strasbourg laboratory with mammalian immunologists and the recognition by Charles Janeway and Bruce Beutler (who later shared the 2011 Nobel Prize with Jules Hoffmann) that mammalian homologs of Toll, the Toll-Like Receptors (TLR), were responsible for the activation of the innate immune response in mammals [18-20].

1.5.2. The renewal of innate immunity

After these pioneering studies in flies and mice, the field of innate immunity, which had been neglected for a long time, showed a spectacular renewal, leading to the concept that this immune response is sufficient to fight infections in most animal species and represents the first and often completely efficient line of defence in vertebrates. The innate immune response acts largely before any adaptive response is set up and is required in order to activate the later more specific response (see [21-25] for reviews).

1.5.3. Why was the *Drosophila* model so helpful?

We should emphasize at this point that if the *Drosophila* model appears now to have been so successful in the field of innate immunity, as it is the case for other medically-related fields, it is due to several interesting aspects: first, *Drosophila* has been a genetic model for more than a century now and many tools have been developed that allow the study of gene functions *in vivo*; second, it is a simple model, with much less gene redundancy than in mammals and easy and cheap to work with; third, most signals, signalling pathways, transcription factors and so on are evolutionarily conserved and can be analysed in *Drosophila* by easy-to-screen phenotypes; fourth, the absence of an adaptive immunity allowed for the specific study of the innate immune system without interference. Despite all these facts, it is also obvious that a fly is not a mouse and even less a man, and that substantial differences exist between their respective innate immune systems.

1.6. The recognition of microorganisms

1.6.1. Peptidoglycan detection

The identification of the immune signalling pathways in the fly raised the problem of the identification of the receptors and the molecules that are recognized. Again, it was through an unbiased genetic approach that the first receptor was discovered and shown to be a Peptidoglycan Recognition Protein (PGRP) [26]. PGRPs belong to a family of proteins first identified in silkworms that can either bind to or hydrolyse bacterial peptidoglycan depending on the presence of a functional amidase domain [27]. It subsequently appeared that different PGRPs were involved in the activation of the Toll or IMD pathways and that they recognise peptidoglycan (PG), which is a conserved component of the bacterial cell wall. The precise detection is related to motifs that are different between Gram-negative (Diaminopimelic-type (DAP-type)) and Gram-positive (Lysine-type (Lys-type)) PGs. For the activation of the Toll pathway, a family of receptors belonging to the β-glucan Recognition protein/Gram-negative Binding Proteins (β-GRP/GNBP) family is also involved either in conjunction with PGRPs for detection of PG or alone for detection of fungal β-glucan cell wall components [28, 29].

1.6.2. PGRPs and peptidoglycan sensing in mammals

While GNBPs have only been found in insects, 4 PGRPs have been later identified in the human

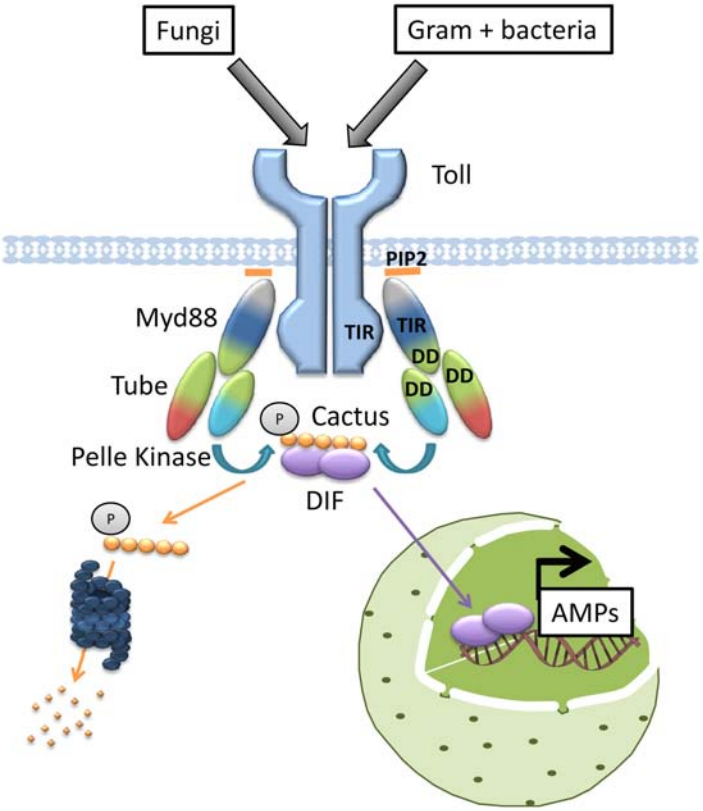

Figure 1

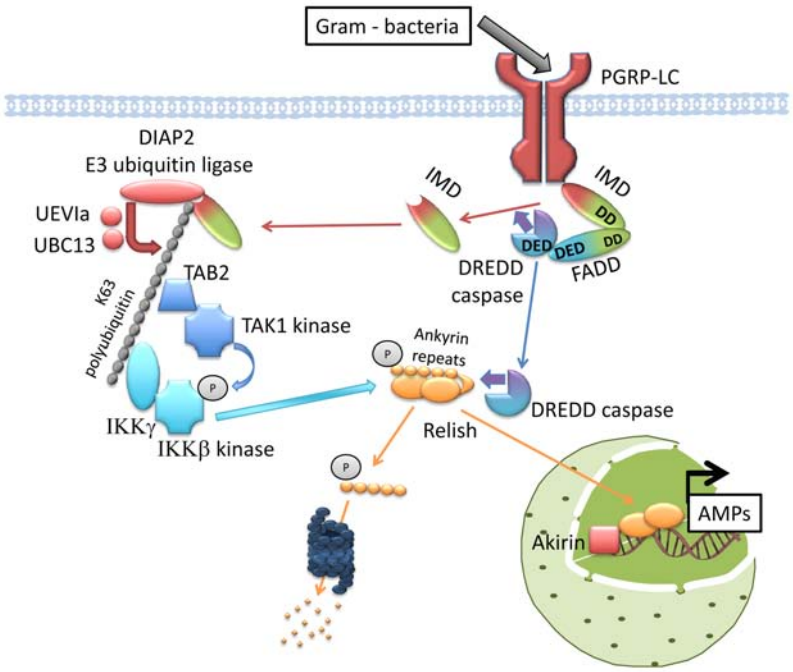

Figure 2

genome and were called PGLYRP1 to 4. However it soon became clear that they were not involved in the activation of the TLR pathways or of the innate immune response. Three of them function as anti-microbial peptides. The fourth, PGLYRP2, acts as an immune suppressor by degrading PG and therefore preventing its sensing by TLRs, as it is the case for several *Drosophila* PGRPs [30], in order to prevent an excessive or inappropriate immune response [31, 32]. In mammals, PG is sensed by NOD intracellular receptors and possibly by TLR2, which activate the innate immune response [33-35]. Mammalian TLRs are also able to recognize, directly or in collaboration with cofactors such as CD14, many different microbial ligands such as PG, Lipopolysaccharide (LPS), bacterial flagellin, nucleic acids etc. (see [24] for review).

1.6.3. Pathogen-Associated Molecular Pattern detection

Despite differences in how PG and other microbial determinants activate the immune response in flies or mammals, the process in both cases seemed to validate Charles Janeway's theory that recognition of microbes should be through invariant and conserved molecular patterns, usually cell wall components, that he referred to as "Pathogen-Associated Molecular Patterns" (PAMPs) [36]. These "patterns" cannot easily be modified by microorganisms and were therefore selected as infection signals by the innate immune system.

1.7. Extracellular signalling to Toll

1.7.1. A proteolytic cascade

As mentioned earlier, PGRPs or GNBPs are the sensors for PAMPs in *Drosophila*. The receptor for the IMD pathway is a trans-membrane PGRP whereas the receptors that activate the Toll pathway are secreted molecules that circulate in the hemolymph. The link between these receptors and Toll was suggested by analogy with the way the Toll pathway is activated in the embryo and later demonstrated by genetic studies in adult flies and biochemical approaches in larger insects. Toll is activated by binding to the cleaved form of a cytokine-like molecule called Spaetzle. This ligand is processed through proteolytic cascades, which are different in early development and in the immune response, but are both reminiscent of the complement cascade in mammals [37] (Figure 3).

1.7.2. Danger signal sensing

The surprising part of this story was the discovery that, during the immune response, Toll is activated not by one but by two different proteolytic cascades. The first comprises two serine proteases (Grass and ModSP) activated through a mechanism that is still unknown, by the binding of PAMPs to

Legend to Figure 1. The Toll pathway. The activation of Toll leads to the activation of a complex composed of Myd88, Tube and Pelle. Myd88 is anchored to phosphoinositol-phosphate (PIP2) at the plasma membrane. It binds to Toll through homophilic interaction by the TIR domain. Then Myd88 recruits Tube and Pelle via homophilic interactions by their Death Domains (DD). Pelle is a kinase that (probably indirectly) induces the phosphorylation of the ankyrin-repeats-containing Cactus inhibitor. Phosphorylated Cactus is targeted for degradation by the proteasome and Dorsal is then free to enter the nucleus and activate gene transcription.

Legend to Figure 2. The IMD pathway. The transmembrane receptor PGRP-LC recruits a complex of 3 proteins, IMD and FADD (FAS-associated death domain) that interact through a Death Domain (DD) and the caspase DREDD (death-related ced-3/Nedd2- like protein) that interacts with FADD through a Death Effector Domain (DED). This induces activation of DREDD and the cleavage of a small N-terminal domain of IMD revealing an interaction site to the E3 ubiquitin ligase DIAP2 (*Drosophila* Inhibitor of Apoptosis Protein 2). This leads to IMD K63-polyubiquitination, with the help of UEV1A (ubiquitin-containing enzyme E2 variant 1) and UBC13 (ubiquitin-conjugating enzyme 13), and the formation of a platform. Both TAK1/TAB2 (transforming growth factor-β (TGFβ)-activated kinase 1/TAK1-binding protein 2) and IKK (Inhibitor of NF-κB kinase) complexes interact with this polyubiquitin chain. TAK1 phosphorylates IKKβ, which then phosphorylates the ankyrin-repeats domain of Relish targeting it for degradation. However in the mean time, DREDD also cleaves Relish releasing its C-terminal NF-κB like domain. Relish C-terminal domain enters the nucleus where it somehow interacts with the nuclear factor Akirin and activates transcription of genes.

PGRPs and GNBPs. The second cascade, centered on a different protease called Persephone (Psh), senses microbial secreted proteases [38, 39]. Here extracellular microbial-derived proteases are interpreted as danger signals by the fly, validating Polly Matzinger's theory of danger signal sensing [40] and demonstrating that the ability to sense microbial activities, instead of only the microbial components themselves, is a property shared by the immune systems of flies and mammals.

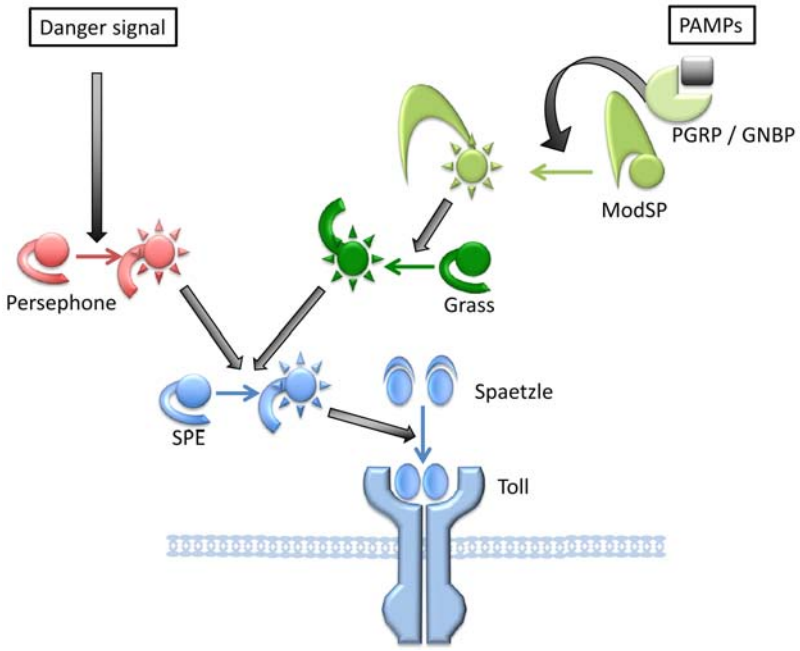

Figure 3

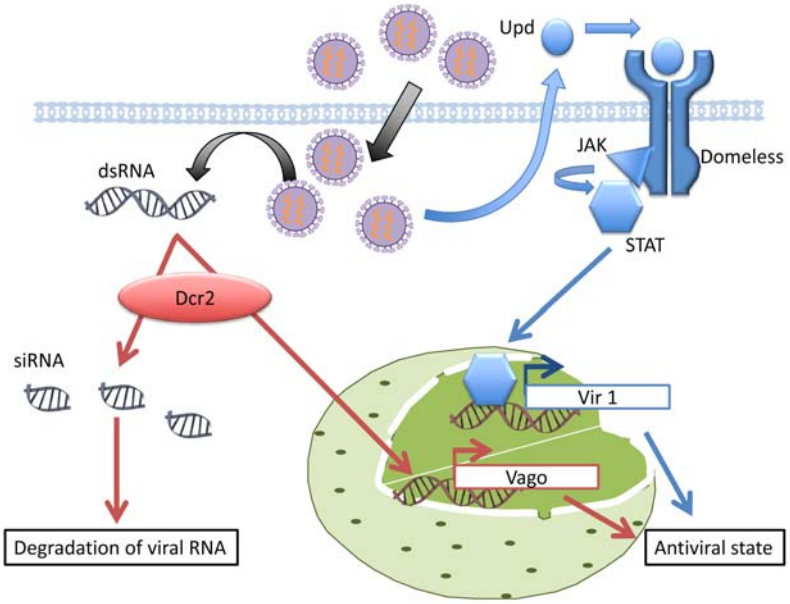

Figure 4

This property has two important benefits, as it not only allows detection of microorganisms that managed to hide their PAMPs, but also results in early detection of the infection before a significant amount of microbes reach the body cavity. We could indeed see that in the case of a fungal infection, where spore germination and growth requires pathogen-derived proteases to enter the fly host body cavity, danger signal sensing was effective the first day after challenge whereas sensing of PAMPs started only one day later. It was recently shown that, in *Drosophila*, the same pathway also detects endogenous danger signals such as cell death signals or cell-released components [41].

2. Beside Toll: what else can *Drosophila* teach us?

2.1. The *Drosophila* antiviral response

The Toll and IMD pathways mentioned so far are involved in the fight against bacterial and fungal infections. Various viruses infect *Drosophila*, which also emerges as a powerful tool for the investigation of the cellular processes required for viral replication. In a recent study using *Drosophila* C Virus, Majzoub *et al.* [42] have identified the ribosomal protein RACK1 as a cellular factor required for infection by internal ribosome entry site (IRES)-containing viruses. Interestingly, the authors showed that RACK1 is also required for the selective translation and infection of other insect and human viruses including Hepatitis C Virus. The study of the fly antiviral response took place recently but many aspects of this response have already been unveiled. (see [43, 44] for reviews) (Figure 4).

2.1.1. RNAi defence mechanism

Several single-stranded (ss) and double-stranded (ds) RNA viruses have been shown to infect *Drosophila*. Both RNA viruses produce dsRNAs that activate the host RNA interference (RNAi) pathway. The small interfering RNA (siRNA) pathway, which involves Argonaute2 and Dicer2, induces the processing of dsRNAs into small fragments (siRNAs) that are used as guides to degrade the whole viral RNA. The siRNA pathway is required to fight viral infections and flies mutant for this pathway are susceptible to viral infections, showing higher viral load [45]. Several *Drosophila* viruses also encode suppressors of RNAi that are required for an efficient infection, further demonstrating the importance of this antiviral defence mechanism [46-49].

2.1.2. Antiviral signalling pathways

In addition to the RNAi defence mechanism, *Drosophila* mounts a powerful transcriptional

Legend to Figure 3. Toll activation by proteolytic cascades. Toll is activated upon binding of the cleaved form of its Spaetzle ligand. Spaetzle is cleaved by the SPE protease (Spaetzle Processing Enzyme), which can be activated by two means. Danger signals, such as bacterial or fungal proteases activate Persephone protease that activates SPE. Pathogen Associated Molecular Patterns (PAMPs) are recognized by PGRPs and GNBPs circulating receptors that activate a first protease, Modular Serine Protease (ModSP), probably by binding to its long N-terminal domain. ModSP then activates Grass that cleaves SPE. All these proteases are synthesized as zymogens containing an N-terminal domain that has to be cleaved for activation, but remains bound to the catalytic domain by a disulfide bridge. The N-terminal domain contains a CLIP domain characterized by several intra-molecular disulfide bridges, with the exception of ModSP whose N-terminal part contains several protein-protein interaction domains.

Legend to Figure 4. *Drosophila* antiviral response. Upon viral infection, double-stranded RNA fragments, coming from the viral genome or its processing, are detected by the Dicer2 (Dcr2) complex. It induces the production of small interfering RNAs (siRNA) that will target viral RNA and induce its degradation. A second function of Dcr2 is to activate the transcription of some genes, including Vago, through an unidentified mechanism. A second response is the production of signals that in an autocrine or paracrine way will activate an antiviral response. Here is presented the JAK/STAT pathway, activated by the cytokine Umpaired (Upd) that binds to the Domeless receptor activating the JAK kinase and the STAT transcription factor. This induces the expression of various genes including Vir1. This pathway is activated in the case of DCV infection for example. Toll or Imd pathways are involved in some other cases. The mechanisms of sensing and activation of the pathways are unknown. The global consequence of gene transcriptions after viral induction is to activate an antiviral state that is still mostly undefined.

antiviral response illustrated by the modified expression of a large number of genes following viral infection. This response appears however to depend on the nature of the virus and no common scheme has been drawn for now. The JAK/STAT pathway is activated following DCV and Sindbis virus (SINV) infections and is required to control the viral load in the infected flies [50]. These data are reminiscent of the mammalian JAK/STAT pathway role in the interferon-based antiviral response. The already mentioned Toll and IMD pathways also play a role in the antiviral response [51, 52]. The Toll pathway is involved in the response to *Drosophila* X virus (DXV) and Dengue virus, and another member of the *Drosophila* Toll family, Toll7, activates autophagy in response to Vesicular Stomatitis Virus (VSV), a mechanism that reduces viral replication and pathogenesis [53]. The IMD pathway is involved in resistance to Cricket paralysis virus and SINV controlling the viral load independently of the AMP effectors. The Dicer2 pathway, which is part of the RNAi mechanism, also activates the transcription of numerous genes such as Vago, required to control the virus load in a cell-autonomous way following DCV and SINV infections [54]. The implication of the Dicer2 pathway in the *Drosophila* antiviral response illustrates the conservation of nucleic acids sensing mechanisms by DExD/H box helicases, Dicer-like enzymes and RIG-I-like receptors (RLR) in insects and mammals. Further work is clearly required to understand the function of these pathways, their mode of activation and their specificities in relation with the pathogenicity of different viruses.

2.1.3. Insect-borne viruses

Knowing the antiviral responses in *Drosophila* will help to understand the interactions between viruses and the close relatives of flies, mosquitoes. These insects are vectors for several virus-induced human diseases. Indeed several recent findings demonstrate that anti-viral induced responses are conserved between mosquitoes and flies (see for example [55]). The *Drosophila* model can be of great help for the studies of the strategies elaborated by both virus and mosquito to survive their host-pathogen interaction.

2.2. Host-pathogen interactions

2.2.1. Natural infection models, defence mechanisms and gut homeostasis

Most of the studies described so far were performed using injection of pathogens into the fly body cavity, which proved to be very efficient for deciphering signalling pathways. However direct injection bypasses the first step of infection, which is the crossing of epithelial barriers. The development of natural infection models, mostly oral administration of pathogens allows for the precise analysis of host-pathogen interactions during the course of an infection [11, 56]. Indeed these studies on the gut immune system have uncovered several important mechanisms, including the important role of phagocytosis to eliminate bacteria that succeed in entering the body cavity, that of reactive oxygen species (ROS) production by the dual-oxidase (DUOX) transmembrane protein and that of the localised AMPs expression by the IMD pathway [57-61] (Figure 5). In normal conditions, commensal bacteria only moderately activate the IMD or DUOX pathways, which are both triggered by the detection of microorganisms (Figure 6). However, commensal bacteria only induce these pathways to a basal level that is significantly shifted in the presence of pathogens. Recent studies suggest that the discrimination between commensal and pathogenic bacteria relies on the release of uracil that is responsible for DUOX activation [62]. The ensuing production of ROS damages the epithelial cells thus leading to their apoptosis. This pathological damage of the gut cells is followed by the stimulation of stem cell division and the induction of repair mechanisms [63]. Interestingly, this phenomenon is also activated in ageing flies due to a progressive change in gut microbiota [64]. The advantage of *Drosophila* as compared to mammals for the study of the influence of commensal microflora on gut homeostasis is the small number and the minimal diversity of bacteria found in the fly gut [65]. Therefore, the *Drosophila* model, given its simplicity and available genetic tools, can be used for analysing how the gut responds to pathogens, discriminates between commensal and pathogenic bacteria, activates repair mechanisms and modulates its response during ageing.

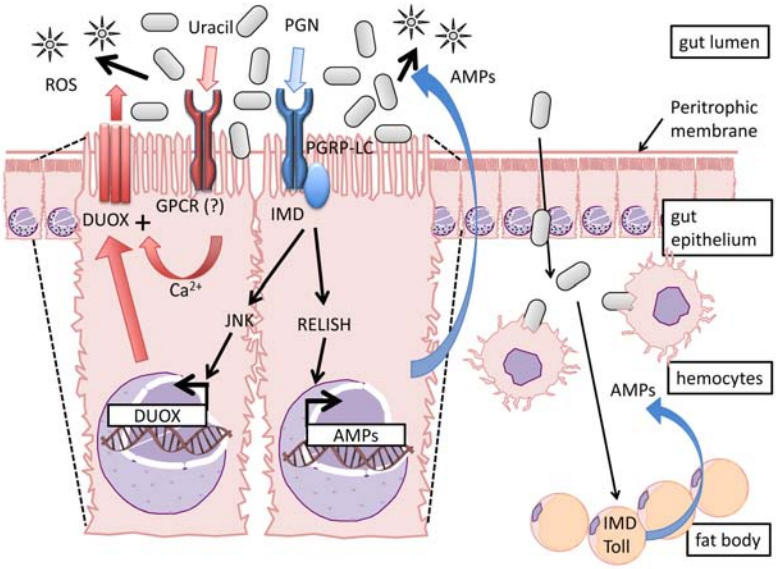

Figure 5. The gut immune defense mechanisms. High level of bacteria in the gut releases peptidoglycan (PGN) that activates the classical IMD pathway to express antimicrobial peptides (AMPs) as well as a Jun-kinase (JNK) pathway downstream of IMD to induce the expression of the dual-oxidase enzyme (DUOX). Uracil is sensed by a G-protein coupled receptor (GPCR) that activates DUOX activity through increase of intracellular Ca^{2+} concentration. Both increases in DUOX quantity and activity lead to a high level of reactive oxygen species (ROS) production. AMPs and ROS are effective killers of bacteria. However, if some bacteria succeed to cross the peritrophic membrane and the epithelial layer, they are detected by hemocytes and phagocytosed. If not sufficient to clear infection, the IMD or Toll pathways are then activated in fat body cells and AMPs released in the circulating hemolymph (blood of insects).

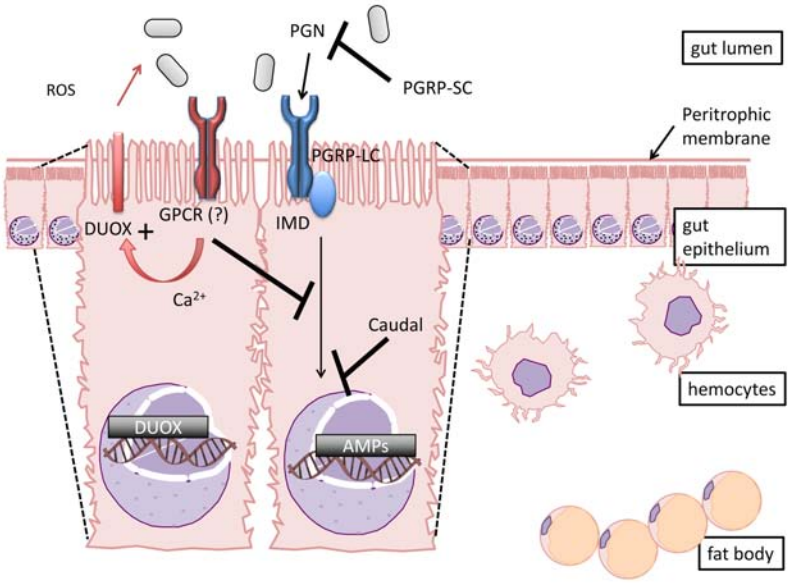

Figure 6. The gut tolerance to commensal flora. Commensal bacteria are kept away from the epithelia by the peritrophic membrane. A basal activation level of a G-protein coupled receptor (GPCR) only moderately activates the dual-oxidase DUOX enzyme, producing a low level of intestinal reactive oxygen species (ROS). Most of the few peptidoglycan molecules released are hydrolyzed by the amidase activity of PGRP-SCs. The basal level of IMD pathway activation is further blocked both by the transcription factor Caudal and an inhibiting signal emanating downstream of the GPCR.

Natural infection also involves the use of fly natural pathogens. This is important in order to analyse the mechanisms used by pathogens to evade or inhibit host defences and the mechanisms developed by the host to circumvent these evading strategies (see [66] for review). This area of research is still not well developed, as not a lot of natural pathogens have been isolated. This nevertheless includes transposable elements, viruses, bacteria, protozoan, fungi and parasitic animals such as wasps and nematodes. We know, for example, that genes encoding recognition proteins and Toll and IMD signalling pathway components are subjected to a high evolution rate, which is not the case for the effectors. This suggests that bacteria and fungi have developed mechanisms to avoid detection and inhibit immune pathways. Indeed, *Enterococcus faecalis* expresses an autolysin that trims the bacterial surface peptidoglycan to avoid recognition by PGRP receptors. In its absence, bacterial virulence is highly attenuated [67].

2.2.2. *In vivo* genome-wide genetic screens

The development of collections of *Drosophila* strains able to express RNAi constructs knocking down any transcript in any condition or tissue represents an exceptional tool. One of these collections has been used to screen for genes involved in the fly defence against gut infections by the opportunistic bacterium *Serratia marcescens* [58]. The approach uncovered several new aspects of host-pathogen interaction such as the role of intestinal epithelial homeostasis or metabolic regulation. Conversely, genetic analyses of pathogens can be conducted by screening collections of bacterial or fungal mutant strains for their ability to infect wild-type *Drosophila*. This approach has been used to identify virulence factors of the human pathogen *Pseudomonas aeruginosa* [68, 69] (Figure 7).

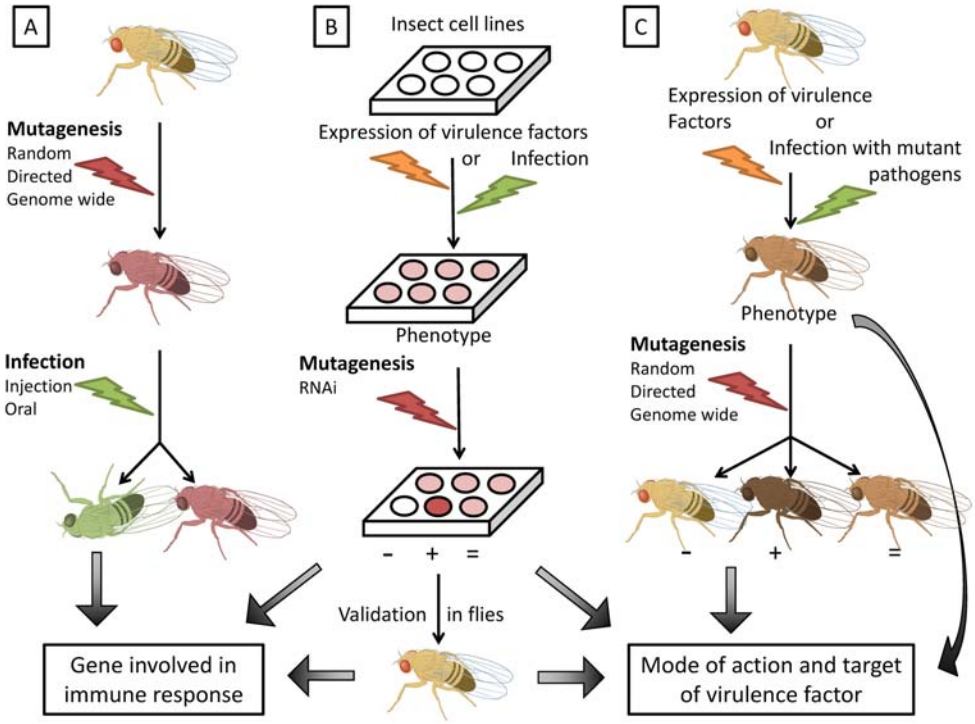

Figure 7. Host pathogen interaction studies. *Drosophila* can be used in different ways for identification of new genes involved in immune responses and the analysis of the functions and mechanisms of action of bacterial, viral or fungal virulence factors. A: classic forward genetic screen for genes involved in defense against various kinds of infection. B: use of insect cell cultures for genome-wide screens and subsequent validation in *Drosophila*. C: *Drosophila* as a test tube to identify the function of virulence factors or to analyze host-pathogen interaction via a double genetic approach. See text for details and examples.

2.2.3. *Drosophila* cell line RNAi screens

Genome-wide RNAi screens can also be conducted in *Drosophila* cell cultures [56, 70]. The few available cell lines are able to phagocytose microorganisms and have been used to identify several genes required for phagocytosis of bacteria and fungi. The interest of this model, when compared to mammalian cell lines, is the low level of gene redundancy and the possibility to validate the candidate genes retrieved from the screen in adult flies. A *Drosophila* cell line has even been used to purify and identify the proteins associated with the phagosome [71]. Host factors required for internalisation and replication of several viruses have also been identified in these cell lines.

2.2.4. Virulence factor studies

Many virulence factors produced by bacteria, fungi or viruses have been identified but their function is often difficult to decipher. The *Drosophila* model has been used successfully to analyse the mode of action of virulence factors from *P. aeruginosa* [72] and of toxins from *Helicobacter pylori* [73] or *Bacillus anthracis* [74]. One of the best systems for such studies is to express the virulence factor in the *Drosophila* eye and search for mutations that can suppress or enhance the obtained phenotype. The *Drosophila* eye is interesting for such approaches because it is dispensable for fly viability in laboratory conditions, its development requires many well understood conserved signalling pathways and any interference with its development leads in most cases to an alteration of its regular, easy to score, repetitive structure.

CONCLUSION

In this review we tried to give a short overview of the different ways the simplicity and the power of its genetics have led the *Drosophila* model to be used in innate immune research. The first aspect was the identification of basic and conserved innate immune mechanisms emphasizing the primary role of this defence mechanism in all animals. Several important outcomes for the field of mammalian immunity were the identification of Toll and TLRs, the validation of PAMP and danger signal theories, the identification of AMPs and PGRPs as antibacterial agents or the conserved function of Dicer/RLR proteins in activation of the antiviral response. Another aspect was the analysis of the insect defence mechanisms towards pathogens with important medical or economical impacts, such as viruses transmitted to humans by mosquitoes or microorganisms affecting honeybee viability. Finally, we tried to show that the fly could be used as a test tube to decipher the pathogenicity and the mode of action of virulence factors from human pathogens. Clearly, the *Drosophila* model has not said its last word in the field of immunology and host-pathogen interactions.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

GLOSSARY

Danger signal: Molecule or activity indicative of an abnormal physiological event sensed by the innate immune system. It can be microbial activities or endogenous molecules that are normally sequestered from their cognate receptors (elements of extracellular matrix or intracellular proteins) and are released upon damage of the self-tissues.

***Drosophila melanogaster*:** Dipteran insect chosen in the early 20th century by Thomas Hunt Morgan for genetics studies. In this species, adults are 2 mm long flies that naturally live on rotten fruits.

Hemolymph/hemocyte: The blood of insects and their blood cells. Insects have an open circulatory system.

Innate immunity: The first line of defence that provides immediate, but not long lasting protection against infection and does not involve memory in contrast to adaptive immunity.

Pathogen Associated Molecular Pattern (PAMP): A molecule conserved in a large group of microorganisms that can be detected by receptors of the innate immune system. It is often a component of the microbial cell wall.

PAMP Recognition Receptor (PRR): Receptors for PAMPs that activate the innate immune response. Some mammalian PRR are also involved in the detection of damage signals.

Peptidoglycan: A major component of bacterial cell wall. Different kinds of peptidoglycan can be recognized by different receptors.

RNA interference (RNAi): A cellular response to double stranded RNA that leads to the destruction or inactivation of all RNA molecules of the same sequence. The short interfering RNA (siRNA) response is mostly activated against exogenous RNAs and the microRNA (miRNA) response is involved in the regulation of endogenous expression of genes.

Reactive oxygen species (ROS): Highly reactive chemical molecules that contain oxygen, such as oxygen ions or peroxides. They may induce significant damage to cell structures.

TLR/NLR/RLR: Three families of PRR receptors involved in sensing of infections and the activation of the innate immune response. Toll-Like Receptors (TLR) are transmembrane molecules that sense various kinds of molecules (proteins, lipids, nucleotides etc.) either at the plasma membrane or in endosomes. Nucleotide binding and Oligomerization Domain (NOD)-like Receptors (NLR) are cytosolic receptors that sense various kinds of molecules including peptidoglycan motifs and danger signals. Retinoic acid inducible gene I (RIG I)-like receptors (RLR) are cytosolic receptors that sense viral RNAs.

Virulence factor: A molecule produced by a pathogen (bacteria, viruses, fungi etc.) that participates in its pathogenicity.

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