How *Drosophila* combats microbial infection: a model to study innate immunity and host–pathogen interactions
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During the past year, dramatic progress has been achieved in our understanding of *Drosophila* immune reactions. The completion of the *Drosophila* genome sequencing project, microarray analysis and the use of genetic screens have led to the identification of several new genes required to combat microbial infection, filling in some important gaps in the understanding of innate immunity. At the same time, this insect was used as a model for the study of host–pathogen interactions. The recent major advances on the mechanisms by which this insect defends itself against intrusion of pathogens are discussed in this review.

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Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>AMP</td>
<td>antimicrobial peptide</td>
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<tr>
<td>GNBP</td>
<td>Gram-negative-binding protein</td>
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<td>JAK</td>
<td>janus kinase</td>
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<tr>
<td>Imd</td>
<td>immune deficiency</td>
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<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
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<tr>
<td>PAMP</td>
<td>pathogen-associated molecular pattern</td>
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<tr>
<td>PGRP</td>
<td>peptidoglycan recognition protein</td>
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<tr>
<td>STAT</td>
<td>signal transducer and activator of transcription</td>
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<td>TEP</td>
<td>thiol ester protein</td>
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<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
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<td>TNF</td>
<td>tumor necrosis factor</td>
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Introduction

The fruit fly, *Drosophila*, spends its entire life cycle in decaying organic matter, such as injured or rotting fruit. In such an environment enriched in microorganisms, *Drosophila* often acts as a vector for microbial infection — adult flies transmit yeast and bacteria from one plant to another while the larvae deliver them deeper into the fruit (Figure 1). It is, therefore, not surprising that, during the four stages of the *Drosophila* life cycle, this insect uses efficient mechanisms to prevent microbial infection (reviewed in [1–3]).

First of all, the external cuticle offers an effective physical barrier against the penetration of microbes. In addition, both the gut and trachea, which are two main routes of infection, are lined with chitinous membranes. An environment hostile to microbial colonisation is maintained in the gut by its low pH and by secretion of antimicrobial factors such as lysozymes. When pathogens breach these physical and chemical barriers, they activate a wide range of inducible immune reactions (Figure 2). Firstly, breakage of the cuticle after injury or microbial infection induces rapid proteolytic cascades that lead to blood clotting and melanization (see later for a definition). Secondly, a cellular immune response that involves different types of haemocytes (blood cells), which participate in pathogen clearance by phagocytosis or by encapsulating larger parasites, is mounted. Finally, during systemic infection, a large set of inducible effector molecules, such as antimicrobial peptides (AMPs), stress response proteins and other factors required for opsonization and iron sequestration, are produced mainly by the fat body and secreted into the blood. As illustrated in Figure 2, the *Drosophila* immune responses are interconnected and synergistic in their effects. In this review, we discuss the recent major advances on the mechanisms by which *Drosophila* defends itself against intrusion by pathogens.

Haemocyte differentiation and function

Insects possess an open circulatory system that contains the haemolymph (the insect blood), which is pumped by a basic heart called the dorsal vessel. The haemolymph does not play a role in oxygen transport but is the major site of resistance during systemic infection. *Drosophila* larvae and adults contain a few thousand blood cells, which can be divided into the following four types on the basis of structural and functional features: secretary cells; plasmatocytes (the most numerous cells, essentially phagocytic); lamellocytes (required for encapsulation of parasites); and crystal cells (involved in the melanization process) [4,5,6••]. *Drosophila* haematopoiesis occurs in two major phases. The first population of blood cells appears during embryogenesis in the anterior mesoderm. These cells then rapidly colonise the whole embryo [7]. One of the functions of embryonic blood is to ingest apoptotic cells by phagocytosis. In addition, it has also been shown that blood cells have the capacity to engulf microbes injected into the embryo [8]. Toward the end of embryogenesis, the lymph glands (the larval haematopoietic organ) differentiate along the anterior portion of the dorsal vessel (Figure 3a). This organ, which disappears during metamorphosis, contains most haemocyte precursors, therefore serving as a haemocyte reservoir [6••]. In larvae, haemocytes can circulate freely in the haemolymph, but a large fraction of them, the sessile haemocytes, are attached to tissue [6••]. Four genes, *serpent*, *lozenge*, *U-shape* and *glial cells missing*, that regulate key steps of *Drosophila* hematopoietic lineage commitment have recently been identified (Figure 3b) [9,10••,11]. Given the similarities between Serpent, Lozenge and U-shape to mammalian haematopoietic factor GATA, Acute Myeloid Leukemia-1 (AML1) and Friend of GATA (FOG), respectively, these studies strongly suggest conservation of the molecular basis for blood cell lineage in mammalian and *Drosophila* haematopoiesis [9,10••,11]. The differentiation
and proliferation of larval haemocytes occur either in the lymph gland or in circulation. These processes are under the control of two conserved signalling pathways: the janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway, which is important for lamellocyte differentiation [12]; and the Töll pathway, which is required for proper haemocyte proliferation [13].

So far, we have little information on the mechanisms that underlie phagocytosis and encapsulation in *Drosophila*. A functional study using a dsRNA knockout in cultured cells of the mosquito *Anopheles gambiae*, however, indicates the role of a humoral protein called *Anopheles* thiol ester protein 1 (aTEP-1) in opsonization [14**]. This study suggests that aTEP-1 binds to the surface of both Gram-negative and Gram-positive bacteria and promotes phagocytosis by haemocytes. Interestingly, the *Drosophila* genome encodes four TEP-encoding genes that may play similar functions [15•]. Given the sequence similarities between TEP and vertebrate complement factors C3 and α-2-macroglobulin, these studies point to the ancient origin of complement-like protein in promoting phagocytosis [14**].

### Coagulation and melanization

As we mentioned above, breakage of the host cuticle in invertebrates immediately induces the clotting of blood and melanization at the injury site [16,17]. These two reactions are essential to limit the spread of microbes during systemic infection. Melanization is a common defence mechanism among invertebrates and, in addition to wound healing, it is associated with encapsulation [17]. It requires the activation of phenoloxidase, an enzyme that catalyses the conversion of dopamine to melanin, which is toxic for microorganisms. This reaction also leads to the production of cytotoxic reactive oxygen species (ROS) that may play a role in combating infection.

The coagulation and melanization reactions are poorly studied in *Drosophila*. However, studies performed in other arthropods indicate that these cascades are triggered after recognition of microbial elicitors, such as lipopolysaccharide (LPS) or β-1,3-glucan, via a serine protease proteolytic cascade [17,18]. Interestingly, the *Drosophila* genome contains a large number of genes that encode serine proteases, protease inhibitors and other metabolic enzymes that may be involved in coagulation and melanization [2]. The observation that some of these genes are upregulated after microbial infection supports this assumption of their roles during the immune response [19**].

### The fat body and the humoral response

One of the landmarks of the *Drosophila* immune response is the synthesis by the fat body of several antimicrobial peptides with distinct but overlapping specificity ([1,20]; Figure 3b). The fat body tissue originates from the mesoderm during embryogenesis and becomes immuno-competent only at the onset of the larval stage [21]. Unexpectedly, a recent report shows that, although embryos are well-protected by a thick chorion, they can still express the AMP gene *Cecropin A* upon injection of LPS or Gram-negative bacteria [22•]. The expression of this gene, however, does not occur in the fat body but, rather, in the yolk nuclei at the early embryonic stage and in the epidermis at later stages. During the larval stage, the
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size of the fat body cells increases dramatically by poly-
ploidization and their immune competence increases under
the control of ecdysone [23,24]. In the early adult stage,
the larval fat body desegregates and is replaced by a new
adult fat body with slightly different immune properties.

In some ways, the insect fat body plays a role similar to
mammalian liver during the acute-phase immune
response. The large size and the position of this tissue in
the insect body cavity make the fat body a powerful
machine that enables the secretion of peptides into the
haemolymph and ensures that they rapidly reach their
effective concentrations. The relevance of AMPs in the
immune response of Drosophila is supported by the high
susceptibility to infection of mutants that do not express
AMP-encoding genes [25,26]. AMP production is only a
subset of the humoral response; other induced humoral
factors are likely produced in this tissue as well. So far, we
still have little idea of how the fat body cells are informed
to induce AMPs and how these peptides are processed
and secreted.

Signalling cascades that regulate the humoral
response after systemic infection

Toll and Imd pathways

Several recent studies have greatly enhanced our under-
standing of the Drosophila humoral response by characterizing
new components that regulate expression of AMP-encoding
genes in the fat body (for a review, see [27,28]). The current
model is that each AMP-encoding gene is regulated by a
balanced activity of two distinct signal transduction
pathways: the Toll pathway, which is largely activated by
fungal infection and Gram-positive bacteria, and the
immune deficiency (Imd) pathway, which is mainly activated
by Gram-negative bacteria (Figure 4). Many components of
the Toll pathway were previously identified to be
mutations that affect embryonic dorsoventral patterning
[29]. By contrast, factors that function in the Imd pathway
to regulate antibacterial responses remained largely
unknown until the past year. Recently, several studies
have led to the genetic and molecular identification of six
components of the Imd pathway: Imd, dTAK1, Ird5, Kenny, the Dredd caspase and the rel factor Relish
[26,30,33,34•–37•,38••,39••,40]. The Imd and Toll
pathways do not appear to share any intermediate
components and mediate differential expression of
AMP-encoding genes via distinct NF-κB-like transcription
factors [41]. These pathways exhibit striking similarities
with the Toll-like receptor (TLR) and tumor necrosis
factor (TNF) cascades that regulate NF-κB activity in
vertebrates, suggesting common evolutionary roots for
the immune response pathways (discussed in [27,28]). However,
flies use two distinct pathways for controlling distinct
NF-κB proteins, whereas, in mammals, both the TLR and
TNF pathways converge to the activation of the IKK
complex, which regulates NF-κB factors. The use of two
pathways in fat body cells to regulate these AMP-encoding
genes may be an efficient mechanism to adapt the
antimicrobial response to different aggressors by producing
a specific subset of AMPs [34•,38••,41,42]. The mechanisms
by which the promoter of each AMP-encoding gene integrates
signals from the two pathways are not clear; other molecules, such as the GATA factor Serpent, may
play important roles [43]. Moreover, several studies
indicate some differences in the regulation of Drosophila
AMP-encoding gene expression, depending on the develop-
mental stage or the tissue, revealing the complexity of the
regulation of AMP-encoding genes [43,44,45•,46].

In addition to several developmental functions, the Toll
pathway regulates the antifungal response and is required
for proper haemocyte proliferation [13], as described
above. Therefore, Toll activation leads to a coordinated
immune response that comprises both cellular and humoral
response. In contrast, the Imd pathway is dispensable for proper development and cellular immune responses. Several studies suggest that, besides its role in antibacterial peptide gene regulations, the Imd cascade may be involved in apoptosis [39••,47,48].

**JAK/STAT**

Similar to the Toll cascade, the *Drosophila* JAK/STAT pathway is involved in multiple developmental events and regulates the cellular immune response [12]. This cascade is not essential for the activation of AMP-encoding genes in *Drosophila*, although studies in mosquitoes have shown that *A. gambiae* STAT (AgSTAT) is translocated in the nucleus of fat body cells of bacterially infected mosquitoes, suggesting that this cascade controls other genes encoding humoral factors [49]. More recently, the gene encoding the complement-like protein TEP1 has been identified as a target of the JAK/STAT pathway in *Drosophila* [15•]. The stimulus that activates the JAK/STAT pathway is not yet known, but there is evidence that this pathway can be activated by the Toll cascade [15•].

**Local versus systemic immune responses**

The use of green fluorescent protein (GFP) reporter transgenes has revealed that AMP-encoding genes, in addition to the fat body, can also be expressed in several surface epithelia that are potentially in contact with microorganisms ([44,45•]; Figure 3c). These include the epidermis, the reproductive system, the respiratory tract and the digestive tract that are in contact with the external environment [21,44,45•]. Preliminary studies indicate a predominant role of the Imd pathway in the control of expression of AMP-encoding genes in these tissues [21,45•], but the mechanisms that regulate the tissue specificity of this local immune response remain to be explored. Epithelial expression of AMPs appears to be a general feature of multicellular organisms, given that, in both vertebrates and plants, AMPs also play a critical role in the local response to infection.

**Recognition of pathogens**

In *Drosophila*, the mechanisms for detecting infectious microbes are largely unknown. The current view is that some receptors can recognise surface determinants (pathogen-associated molecular patterns, PAMPs) that are conserved among microbes but absent in the host. These PAMPs include LPS, peptidoglycan and mannans [50]. After recognition, the PAMP receptors may stimulate immune responses by activating extracellular proteolytic
cascades in the haemolymph and intracellular signalling pathways in immune-responsive tissues (Figure 2).

The Drosophila genome encodes a large number of proteins with putative recognition properties [2]. However, to date, there is no genetic demonstration of a role for these proteins in the activation of the immune response. Even though it is now clear that the Toll and Imd pathways are selectively activated in response to different microbes, the recognition proteins that target these pathways are still unknown [27]. In contrast to some of the mammalian TLRs, Drosophila Toll does not appear to function as a direct sensor of microbial compounds, but is activated via an unknown proteolytic cascade by a small cytokine-like molecule termed Spätzle [25,51]. The receptors that activate the Imd pathway in response to Gram-negative bacteria have not been identified. Given that TLR4 mediates recognition of Gram-negative bacteria in mice [52], it is tempting to speculate that one of the eight other Tolls encoded by the Drosophila genome may function in microbial recognition in the Imd pathway. However, expression of seven of the eight Toll homologues in cultured cells did not provide a clear demonstration of their function in the antibacterial defence [53].

Recently, two families of proteins have been implicated in pathogen recognition in Drosophila. Peptidoglycan recognition protein (PGRP) was identified as a Gram-positive-binding protein present in the moth Trichoplusia ni [54]. PGRP has also been implicated in the activation of the prophenoloxydase cascade in the silkworm Bombyx mori [55]. Interestingly, the Drosophila genome encodes 12 PGRP genes, and several of them are upregulated after septic injury [56]. In contrast to PGRP, Gram-negative-binding proteins (GNBPs) are only found in invertebrates and have been isolated as proteins that bind to LPS and β-1,3-glucan [57]. The Drosophila genome encodes three GNBPs and two immune-inducible-related proteins [19••,58]. Overexpression of dGNBP-1 enhances LPS induction of AMP-encoding genes in cultured cells [58]. Furthermore, the observation that TEP molecules can bind to the surface of bacteria also suggests a role for these...
...proteins in pathogen recognition [14**]. In addition to PAMP detection, the insect may selectively recognize more specific determinants of pathogens, as shown in plant defence. Possibly, some of the signals that trigger the *Drosophila* immune response may involve other indirect mechanisms that sense the presence of pathogens through the breakage of the integument.

**Monitoring the *Drosophila* immune response after different modes of infection**

So far, most of our knowledge on the *Drosophila* immune response has been built upon the analysis of the host's reaction after injection of non-pathogenic bacteria in *Drosophila* larvae or adults. Recently, the use of oligonucleotide microarrays encompassing the full genome has revealed that several hundred genes out of 13 600 *Drosophila* genes are modulated after septic injury, confirming that this stimulus induces a wide-ranging response [19**]. The possibility of infecting a large number of animals in a short time and the high reproducibility of the *Drosophila* immune response after microbial injection have allowed the successful identification of key regulators of expression of AMP-encoding genes via systematic genetic mutagenesis [33,38**,59–61]. The application of similar screens will probably help to identify new factors involved in little-studied processes such as melanization and phagocytosis. In addition to the introduction of microbes into the body cavity, microbial injection results in an injury that leads to wound-healing reactions and to a stress response [62,63**].

The limitation of the injection approach is that it bypasses the first step of infection, which includes the attachment and entry of the pathogen. Recently, alternative approaches have been developed that use natural *Drosophila* pathogens and a natural mode of infection. The use of a natural mode of infection has contributed to the discovery of the ability of *Drosophila* to activate an immune response adapted to the invading organism [42], and enables the study of all the phases of infection from the initial interaction between the pathogen and its host to the activation of the immune response.

*Beauveria bassiana* is an entomopathogenic fungus that infects many insect species by penetrating their cuticle. Genetic experiments indicate that natural infection of *Drosophila* by this fungus leads to the expression, via the selective activation of the Toll pathway, of genes encoding antifungal activity [42]. Microarray analysis also confirms that infection by this fungus leads to a more specific immune response, compared with infection by bacterial injection [19**]. Recently, some strains of the phytopathogenic Gram-negative bacterium, *Erwinia carotovora*, which causes soft rot in fruit and uses insects as its vector, have been isolated for their capacity to trigger a systemic and local immune response in *Drosophila* larvae after gut infection [64*]. In this case, the immune response is largely mediated by the Imd pathway [38**,45*]. Finally, parasitoid wasps that inject their eggs into young instar larvae are among the most formidable pests to *Drosophila* in nature. Resistance to parasitoid infection is correlated with the capacity of the host to encapsulate the wasp eggs. The interactions between *Drosophila* and several wasp species provide a model to analyse cellular reactions [65–68].

**Drosophila as a model host for studying human pathogens**

The idea of using simple, genetically tractable host organisms to study the virulence mechanisms of human pathogens has recently emerged. Studies from several groups have clearly established the nematode *Caenorhabditis elegans* as an attractive model host for the study of *Pseudomonas aeruginosa* and *Salmonella typhimurium* pathogenesis [69,70]. *P. aeruginosa* has previously been shown to be highly pathogenic to *Drosophila* [71], and it is now the focus of several groups that use *Drosophila* as an alternative model system to study this important pathogen. *Drosophila* can be used to screen for *P. aeruginosa* mutants with reduced virulence and to analyse the complex interactions between this bacterium and the innate host defence response [72,73].

Other pathogens that can be studied using *Drosophila* as a model host are human parasites transmitted by insects. Interestingly, *Plasmodium gallinaceum* ookinetes injected into *Drosophila* develop into infectious sporozoites, indicating that this parasitic step can be reproduced. The use of genetic screens in *Drosophila* may thus shed light on the host requirements for *Plasmodium* development and survival [74*].

**Conclusions**

Our knowledge of the *Drosophila* immune response has been greatly enhanced in the past year. Some aspects, such as the function of the Toll and Imd pathways in the induction of antimicrobial peptides, have been partially clarified. However, the molecular mechanisms of other critical cellular and humoral immune responses, including phagocytosis, encapsulation, melanization and coagulation, remain unknown. Two of the most important areas for research concern the mechanisms by which pathogens are recognised and how recognition leads to the activation of the immune response. The potential use of genetic and molecular analyses in *Drosophila*, combined with the fly's complete genome sequence and with new techniques such as microarray analysis, suggests that more aspects of the immune response will be unravelled. In addition, the development of alternative methods of infection that use natural *Drosophila* pathogens offers the possibility of studying the immune response under the conditions of a real infection. Finally, completion of the genome sequencing of other insect species, such as *B. mori* and *A. gambiae*, will enable interesting comparative studies and may improve our understanding of how insects resist microbial infections.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
** of outstanding interest

This study shows that the Drosophila NF-κB factor Relish by rapid endoproteolytic cleavage. *EMBO Rep* 2000, 1:347-352.

This study, along with [32**••**, 33**••**], demonstrates that Relish is processed upon infection by an endoproteolytic mechanism that is not proteosome-dependent but requires the caspase Ded1.


This genetic study demonstrates that the Drosophila MAPKKK dTAK1 regulates Drosophila antibacterial defense. Epistatic studies indicate that dTAK1 functions downstream of Imd and upstream of the IKK complex.


This study shows that the IMD gene encodes a protein with a death domain that shows some similarities with the mammalian receptor-interacting protein (RIP). Overexpression of *imd* leads to apoptosis.


This study, along with [44], reveals the complex pattern of expression of antimicrobial peptide genes on surface epithelia. Along with [21], this paper also indicates a predominant role of the IMD pathway in the control of expression of AMP-encoding genes in these tissues.


This is the first report to indicate that stress induces a humoral systemic response — the synthesis of a family of small peptides called Turdant.


This study reports the identification of bacterial strains of the *Erwinia* genus, which can activate a systemic antibacterial response after natural infection. *Erwinia* are phytopathogenic bacteria that use *Drosophila* as an insect vector. The *Drosophila*—*Erwinia* interaction provides a powerful tool to study *Drosophila* immune response after infection by Gram-negative bacteria.


This study reports that *Plasmodium gallinaceum* ookinetes injected into the fly can develop into infectious sporozoites. *Drosophila* can be used as an alternative model to study the host requirements for this important human parasite.