Immunity 25, 1–5, July 2006 ©2006 Elsevier Inc. DOI 10.1016/j.immuni.2006.07.002

Insect Immunity: The Post-Genomic Era

Meeting Report

Jenny Bangham,[1,*] Frank Jiggins,[1] and Bruno Lemaitre[2]
[1] Institute of Evolutionary Biology
The University of Edinburgh
Ashworth Laboratories
The King's Buildings
West Mains Road
Edinburgh EH9 3JT
United Kingdom
[2] Centre de Génétique Moléculaire
Centre National de la Récherche Scientifique
91198 Gif-sur-Yvette
France

Summary

Insects have a complex and effective immune system, many components of which are conserved in mammals. But only in the last decade have the molecular mechanisms that regulate the insect immune response—and their relevance to general biology and human immunology—become fully appreciated. A meeting supported by the Centre National de la Récherche Scientifique (France) was held to bring together the whole spectrum of researchers working on insect immunity. The meeting addressed diverse aspects of insect immunity and brought together geneticists working on *Drosophila melanogaster* with those working on other insects.

Introduction

The Jacques Monod Conference on "Innate immunity: the post-genomic era" was held in Roscoff, France (June 10th–14th), ten years after the identification of the first mutations to affect the *Drosophila melanogaster* immune response. Since then, the completion of several insect genome sequences and large-scale mutagenesis projects as well as the development of RNAi as an effective way to target genes in insects have furthered progress in the field.

The meeting was organized by Bruno Lemaitre (Gif-sur-Yvette, France) and Ulrich Theopold (Stockholm, Sweden) and touched upon all branches of the insect immune response—the recognition of foreign proteins, the signaling pathways that lead to the local and systemic production of antimicrobial peptides (AMPs), the wound response, the cellular responses, and some newly discovered antiviral defences, including RNAi and the Jak-STAT (The Janus kinase—signal transducer and activator of transcription) signaling pathway. Also discussed were the evasion and suppression mechanisms that pathogens use to avoid the host's immune system, and there was some tantalizing evidence of coevolution between parasites and their hosts.

Pattern-Recognition Receptors

Insects have an array of pattern-recognition receptors that bind to molecules (lipopolysaccharides, peptidoglycans, and glucans) associated with microbes and trigger signaling cascades to activate immune cells or the transcription of AMPs to isolate or kill invaders. There are two main families of pattern-recognition receptors, the peptidoglycan-recognition proteins (PGRPs) and the gram-negative binding proteins (GNPBs), which have homology to enzymes (amidase or glucanase). Perhaps one of the most surprising recent findings is the diversity of PGRP and GNBP functions, with roles not only in recognition but also in killing and immune regulation.

PGRPs were initially identified as extracellular sensors of bacterial infection, but recent results show that at least one PGRP functions intracellularly. Neal Silverman (Worcester, MA, USA) and Shoichiro Kurata (Sendai, Japan) discussed the roles of two isoforms of PGRP-LE (Kaneko et al., 2006). One is a short version that functions extracellularly as a coreceptor of PGRP-LC in the recognition of diaminopimelic acid-type peptidoglycan (Figure 1), whereas the longer version functions intracellularly and recognizes tracheal cytotoxin (TCT), a small peptidoglycan fragment released by bacteria. These researchers suggested that the long version of PGRP-LE might defend against intracellular bacteria. Interestingly, they also identified a domain in both PGRP-LE and PGRP-LC that is required for the activation of Imd signaling. This domain has weak homology to the RHIM motif that is responsible for the interactions of the mammalian TIR-adaptor proteins TRIF and RIP1 in Toll-like receptor 3 (TLR3) signaling.

Another subgroup of PGRPs, called catalytic PGRPs, have amidase activity that removes peptides from the glycan chains and thereby reduces peptidoglycan biological activity. Research shows that catalytic PGRPs negatively regulate the immune response. Julien Royet (Marseille, France) described the functions of two catalytic molecules, PGRP-SC1 and PGRP-SC2, that degrade peptidoglycan (Bischoff et al., 2006). Flies that lack these proteins have an overactive Imd pathway after infection with *Escherichia coli*, causing developmental defects and larval death. Bruno Lemaitre discussed a similar immune-regulatory role of another catalytic family member, PGRP-LB (Zaidman-Remy et al., 2006). Importantly, both PGRP-SC and PGRP-LB are primarily expressed in the gut, and their main function might be to prevent innocuous peptidoglycan in the diet from initiating an immune response. The importance of dampening the immune response is well documented in vertebrates, and these are the first data to indicate that it is also carefully regulated in invertebrates. Abdelaziz Heddi (Villeurbanne, France) reported that the bacteriome (the bacteria-bearing organ) of the weevil *Sitophilus zeamais*—an organism that harbors integrated intracellular bacterial symbionts—expresses a PGRP that is homologous to *D. melanogaster*'s PGRP-LB (Heddi et al., 2005). The expression of this gene might suppress the host's defense against endosymbiotic bacteria and thereby allow a long-term interaction.

Finally, Hakan Steiner (Stockholm, Sweden) described a secreted PGRP—PGRP-SB1—that acts as a scavenger by degrading peptidoglycan and that can also kill some

*Correspondence: jenny.bangham@ed.ac.uk

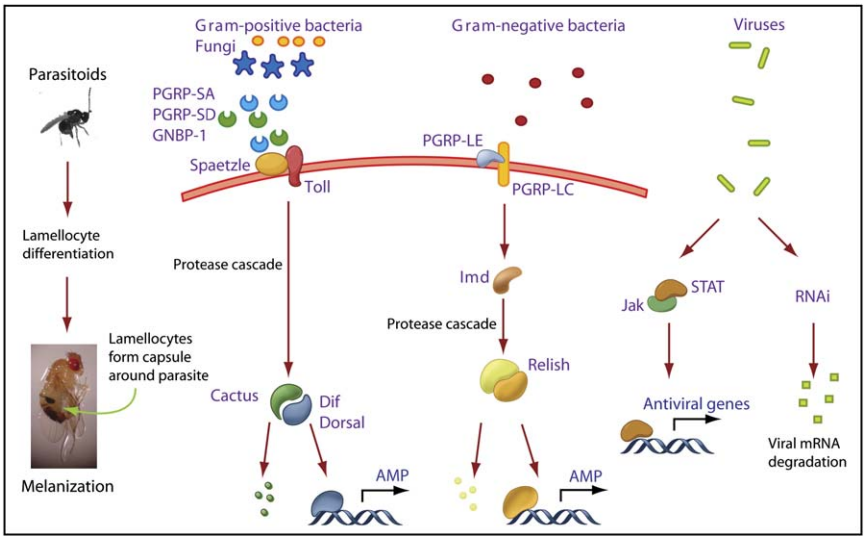

Figure 1. Simplified Description of Four of the Immune Responses of *Drosophila melanogaster*

From left to right. Parasitoids lay their eggs inside the larvae or pupae of other insects and, if successful, kill their hosts. In response to such parasitization, lamellocytes differentiate and form several layers around the parasitoid egg, which is melanized to form a hard black capsule. Gram-positive bacteria and fungi trigger the activation of the Toll pathway. Peptidoglycan recognition proteins (PGRPs) and gram-negative binding proteins (GNBPs) recognize the presence of Gram-positive bacteria and fungi and, through Spaetzle and Toll, activate a proteolytic cascade involving serine proteases and serine protease inhibitors. This results in the proteolytic degradation of inhibitor κB (IκB) protein Cactus and activation of the NF-κB proteins Dif and Dorsal, resulting in the transcription of antimicrobial peptides (AMPs). Gram-negative bacteria trigger the Imd pathway, which also results in a proteolytic cascade. This results in the cleavage of Relish—the C-terminal (IκB-like) part of which is removed and the N-terminal (NF-κB-like) part of which activates AMP transcription. Much less well understood are the antiviral responses of insects. Recent results indicate that viruses trigger the Jak-STAT pathway (involving a Jak kinase called Hopscotch) and the transcription of antiviral genes. RNAi-silencing machinery is also able to target animal viruses.

bacteria. This direct antibacterial activity constitutes a third function of PGRPs, in addition to pathogen recognition and immune regulation, and is reminiscent of the effector functions of some vertebrate PGRPs.

Peptidoglycans are long molecules that sometimes need to be processed to be recognized. The detection of gram-positive bacteria through their Lys-type peptidoglycan leads to the activation of the Toll pathway and requires the pattern-recognition receptors PGRP-SA and GNBP1. Petros Ligoxygakis (Oxford, UK) described how the activation of the Toll pathway by gram-positive infection requires the interaction between these two proteins (Filipe et al., 2005). GNBP1 is responsible for hydrolyzing the gram-positive peptidoglycan, and PGRP-SA binds to the peptidoglycan fragments, leading to activation of Toll.

GNBPs contain a glucanase-like domain and are important in detecting fungal infections. Dominique Ferrandon (Strasbourg, France) described how GNBP3 detects fungal β-1,3-glucan and leads to the activation of the Toll pathway and the production of antifungal peptides. Ferrandon proposed that, in addition to its function as a recognition protein, GNBP3 might also be an effector (agglutinating fungal cells) and thus illustrated how a single protein can have multiple immune functions.

Signaling Pathways

Both the Toll and the Imd pathways result in the transcription of AMPs (Figure 1). Some AMPs are specific to one pathway, and others are activated by both, but little is known about how this specificity is translated into a gene-expression profile. Both the Toll and the Imd pathways culminate in the activation of NF-κB family transcription factors—the Toll pathway activates Dorsal and Dif, and the Imd pathway activates Relish.

Although the Toll and Imd pathways are separate, knocking out both pathways can have a greater phenotypic effect than knocking out either Toll or Imd alone, so Tony Ip (Worcester, MA, USA) asked at which level the two pathways synergize. He found that crosstalk occurs at the level of Relish, Dif, and Dorsal and their interaction with the promoters of immune genes. Cooperation between these NF-κB factors (including the formation of heterodimers) results in the synergy between the Toll and Imd pathways.

In the same session, Steven Wasserman (San Diego, CA, USA) showed that the genes that are specifically upregulated by either the Toll or the Imd pathway have distinct NF-κB binding sites. The apparent simplicity of the Toll- and Imd-specific binding-site code contrasts with the complexity of NF-κB binding sites in vertebrates, where binding-site specificity is difficult to predict.

The similarities of the *D. melanogaster* Toll and Imd pathways to the vertebrate NF-κB pathway is often emphasized, but are these pathways involved in immunity in other insects? Using overexpression or in vivo RNAi in transgenic mosquito *Aedes aegypti*, Sang Woon Shin (Riverside, CA, USA) showed that a response to gram-negative bacteria requires REL2 (a Relish homolog), whereas anti-fungal immune signaling is mediated by REL1 (a Dorsal homolog) (Shin et al., 2005), the receptor AeToll5, and its cytokine ligand Spaztle1C; this is reminiscent of *D. melanogaster*. He also observed that, whereas the serine protease Easter and its inhibitor Spn27A regulate the Toll antifungal response in *A. aegypti*, they regulate Toll signaling in dorsoventral

patterning of the *D. melanogaster* embryo; this finding indicates a major evolutionary switch in extracellular Toll signaling.

Several important immune mechanisms, such as Toll and Imd-mediated defense and phenol oxidase (PO) activation, involve proteolytic cascades—these are mediated by serine proteases and controlled by the serine protease inhibitors (serpins). Studies of serine proteases and serpins are made difficult by the large number of serine proteases encoded in the genome (more than 200 in *D. melanogaster*), but genome-sequence information and RNAi have recently boosted this field. Kristin Michel (London, UK) aims to analyze all functional serpins in the *Anopheles gambiae* genome. She showed that knocking down the genes SRPN2 and SRPN6—the two serpins that she and her colleagues have described so far—compromises the ability of mosquitoes to clear Plasmodium parasites through melanisation or lysis (Abraham et al., 2005; Michel et al., 2005). Mike Kanost (Manhattan, Kansas, USA) discussed proteases and serpins that function in the PO cascade in the enormous caterpillars of the Tobacco Hawkmoth *Manduca sexta*. He described a branch of the proteolytic cascade in which the haemolymph protease HP14 (activated in response to gram-positive bacteria or fungi) activates HP21, and, in turn, proPO-activating protease (proPAP). Serpin 3 inhibits PAP, whereas Serpin 4 and Serpin 5 form covalent complexes with HP1, HP6, and HP21 in response to bacterial infection (Tong et al., 2005).

In *D. melanogaster*, Serpin27A regulates the processing of pro-PO in the melanization response. To identify proteases involved in this pathway, Carl Hashimoto (New Haven, CT, USA) and colleagues took advantage of the constitutive melanization that results from loss of Spn27A and screened for suppressors of this phenotype. They identified two such proteases, MP1 and MP2, and were surprised to find that these have infection-specific roles—MP1 activates melanization in response to bacteria and fungi, but MP2 is involved in an antifungal response.

Finally, several talks discussed the immune response from a physiological perspective. In *D. melanogaster*, the fat body not only is the site of expression of antimicrobial peptides but also modulates host metabolisms, including nutritional balance. Marc Dionne (Stanford, CA, USA) found that *D. melanogaster* that have been infected with *Mycobacterium marinum* progressively lose metabolic stores and become hypoglycaemic, in a situation reminiscent of tuberculosis in humans, and Kerstin Isermann (Kiel, Germany) found that starvation stimulates AMP gene expression. Both talks suggest complex crosstalk between immune and metabolic pathways.

The Cellular Response

In *D. melanogaster*, there are three classes of hemocytes with specialized immune functions. Crystal cells are involved in melanization, which occurs at wound sites or around microbes; plasmatocytes are professional phagocytes that digest microorganisms and apoptotic cells; and the lamellocytes are responsible for the encapsulation of parasites.

Several talks discussed the production and differentiation of hemocytes in the lymph gland during *D. melanogaster* larval development. Utpal Banerjee (Los Angeles, CA, USA) described lymph-gland development and hemocyte differentiation. This process requires interactions between three distinct lymph-gland subregions—the cortical zone (containing differentiated hemocytes), the medullary zone (containing hemocyte precursors), and the posterior signaling center (PSC), which acts as an organizer (Jung et al., 2005). Michele Crozatier (Toulouse, France) described how the PSC, which requires the transcription factor Collier (Crozatier et al., 2004) and the Jak-STAT signaling pathways, is required for immune-specific differentiation of hemocytes in the lymph gland (Crozatier et al., 2004). The structure of the PSC is reminiscent of vertebrate hematopoeisis, in which stromal cells act as a niche for the differentiation of blood cells. Will Wood (Lisbon, Portugal) has been studying how hemocytes find their way to a wound site in the *D. melanogaster* embryo. He showed that phosphoinositol 3 kinase (PI3K) is required for haemocyte chemotaxis toward wounds, a mechanism different from the migrations of hemocytes during development (Wood et al., 2006).

How do phagocytes recognize their targets? Recent studies indicate that insect phagocytosis might involve a unique class of pattern-recognition receptor. Christine Kocks (Boston, MA, USA) and colleagues have identified a transmembrane receptor with EGF-like repeats. Called Eater, this receptor binds to and helps internalize a broad range of bacteria (Kocks et al., 2005). Eater-deficient flies have defective phagocytosis and reduced survival after bacterial infection. In the beetle *Holotrichia diomphalia*, Bok Luel Lee (Busan, Korea) isolated a 40 kDa LPS recognition protein (LRP) with six EGF repeats. LRP is a secreted protein in the hemolymph and aggregates gram-negative bacteria by associating with LPS. This work indicates that insects might use EGF-like repeat-containing proteins to phagocytose or aggregate bacteria via LPS.

Finally, Ulrich Theopold analyzed the rapid release of PO by crystal cells after injury. He shows that none of the classical immune pathway is involved in this process but that the rupture of crystal cells, and the consequential melanization, is blocked when the function of the GTPase Rho A is altered, pointing to a key role for cystoskeleton reorganization in this process.

Evasion Strategies by Parasites

Encapsulation is the primary defense mechanism that insects use against parasitoids. Parasitoids are insects—normally wasps or flies—that lay their eggs inside the larvae or pupae of other insects and that, if successful, kill their hosts. When *D. melanogaster* is parasitized, lamellocytes differentiate and form several layers around the parasitoid egg, which is then melanized to form a hard black capsule (Figure 1).

However, parasitoids have adopted a range of counterstrategies against encapsulation. Perhaps the most remarkable of these is the use of polydnaviruses, which the parasitoid injects into its host during egg laying and which suppress the host's immune response. Jean Michel Drezen (Tours, France) and Michael Strand (Athens, GA, USA) have studied the symbiotic relationship between polydnaviruses and their wasp hosts by using the completed genome sequence of two symbiotic polydnaviruses, *Cotesia congregata* bracovirus (CcBV,

567 kb, containing 156 genes) (Espagne et al., 2004) and *Microplitis demolitor* bracovirus (MdBV, 189kb, containing 65 genes).

Members of Drezen's group discussed the cysteine protease inhibitors encoded by Bracoviruses and their cysteine protease targets encoded by their hosts. Elisabeth Huguet (Tours, France) showed that the CcBV-encoded protein cystatin 1 inhibits a range of cysteine proteases and speculated that the target cysteine protease in *Manduca sexta* is involved in antiparasite defense (Espagne et al., 2005).

Strand and colleagues have identified two IκB proteins encoded by MdBV. Called H4 and H5, these proteins bind to the NF-kB factors Dif and Relish but, unlike host-encoded IκBs, do not possess the target sites of degradation (Thoetkiattikul et al., 2005). In this way, H4 and H5 suppress the expression of Attacin (target of Relish) and Drosomycin (regulated by Toll). In addition, the Strand lab has also identified MdBV-encoded surface proteins that are expressed in infected hemocytes and that disrupt encapsulation by interfering with surface molecules that regulate adhesion and phagocytosis (Beck and Strand, 2005).

Marylène Poirié (Nice, France) focused on a *D. melanogaster* parasitoid called *Leptopilina boulardi*, which injects particles resembling viruses (VLPs), but containing no DNA, into its host. Poirié has identified a VLP virulence factor called P4, which is a Rho-GAP protein that alters the morphology of the lamellocytes produced in response to parasitization (Labrosse et al., 2005) and suppresses the encapsulation response of the host.

The encapsulation response is not the only part of the insect immune system to be sabotaged by pathogens. Ferrandon found that the fungus *Beauveria bassiana* avoids detection by GNBP3 and actively suppresses the activation of PO. Curiously, however, even GNBP3 mutant flies manage to upregulate their Toll pathway when they become infected by *B. bassiana*. This pathway is thought to be triggered by the fungal protease PR1, which cleaves the host's serine protease Persephone and leads to Toll activation. Ferrandon suggested that the pattern-recognition receptors such as PGRPs and GNBPs form a basal detection system of the innate immune system and that, because some pathogens have evolved to evade these pattern-recognition receptors, insects have evolved ways of also detecting virulence factors such as PR1.

Most studies of the antimicrobial response in *D. melanogaster* have used assays that involve septic wounding, but oral infection is potentially more crucial, and several research groups are focusing on the ways that insects fight infection in the gut. Won-Jae Lee (Seoul, Korea) showed that reactive oxygen species (ROS) produced by a dual oxidase is an efficient mechanism used by *D. melanogaster* to eliminate most bacteria entering the gut (Ha et al., 2005) and that Imd-dependent gut AMPs provide a second barrier against bacteria that resist the ROS. Nadine Nehme (Strasbourg, France) and Peter Liehl (Gif-sur-Yvette, France) showed that a local immune response, mediated by the Imd pathway, has a predominant role against oral infection by the gram-negative entomopathogenic bacteria *Serratia marcescens* and *Pseudomonas entomophila*. Curiously, *P. entomophila* remains in the gut and triggers a systemic immune response by the fat body, whereas *S. marcescens* crosses the intestinal barrier to reach the hemolymph without eliciting such a systemic response. This indicates that the presence of bacteria is not sufficient to trigger an immune response and that these bacteria have sophisticated evasion strategies. Liehl also reported that *P. entomophila* expresses a zinc metalloprotease virulence factor, AprA, that degrades AMPs produced by the gut epithelia and thereby promotes bacterial persistence (Liehl et al., 2006). Similarly, Richard Ffrench-Constant (Bath, UK) showed that the entomopathogenic bacteria *Photorhabdus luminiscens* use an extracellular protease, prtA, to suppress the melanization reaction cascade in the hemocoel of *Manducta sexta* by degrading a serine protease homolog, SPH3. Such proteases could represent a common strategy used by entomopathogenic bacteria to resist the insect host defense.

Antiviral Defense

Little is known about antiviral responses; indeed, it is not yet clear whether there is a dedicated antiviral pathway in insects (Figure 1). Jean-Luc Imler (Strasbourg, France) described how microarray analysis of DCV infection revealed genes controlled by a Jak kinase and STAT transcription factor (Dostert et al., 2005). The Jak-STAT pathway has a role in interferon signaling in mammals and could represent an ancient conserved mechanism for dealing with viral infections. However, the stimuli that trigger the Jak-STAT pathway and the nature of induced antiviral molecules remain to be determined.

A role for RNAi in antiviral defense in animals was first described in 2002, when *D. melanogaster* S2 cells were infected with flock house virus (FHV). Imler's group demonstrated that for FHV to infect and kill adult flies, it must express a protein called B2, which suppresses RNAi. Conversely, flies with a loss-of-function mutation in the gene encoding Dicer-2 (*Dcr-2*), which is an essential component of the RNAi system, are more susceptible to infection by members of three families of RNA viruses: FHV, DCV, and Sindbis virus (Galiana-Arnoux et al., 2006).

Population Genetics and Evolution

Comparisons of the immune gene "repertoire" of different insects could tell us a lot about the variation and conservation of insect host defense mechanisms. Georges Christophides (London, UK) described how genome sequencing projects on other insects could be used. For example, the variation in the number of PGRPs and their genomic organization could provide key information about bacterial detection in other species, based on the data gained in *D. melanogaster*.

We expect there to be strong selection on parasites to outwit the host's immune strategies and, conversely, for the host to kill invaders. Such an "arms race" between host and parasite is predicted to result in the rapid evolution of the genes involved in the interaction. Seven distinct *Drosophila* species have now been sequenced, allowing researchers to probe the extent and type of genetic polymorphism in *Drosophila* populations. Frank Jiggins (Edinburgh, UK) used this information to show that three *D. melanogaster* genes involved in the RNAi response against viruses are in the top 3% of the most

rapidly evolving genes in the *D. melanogaster* genome. As well as illustrating the evolutionary consequences of host-parasite interactions, this provides a further support to the importance of RNAi in antiviral defense in insects (Obbard et al., 2006).

There is enormous variation in the level of immune competence among individuals in wild populations. Which genes are responsible for this natural phenotypic variation? Brian Lazzaro (Ithaca, NY, USA) has found that it is the signaling molecules, such as cactus, Dif, and Imd, that are responsible for most of the variation in antibacterial immunocompetence, whereas little variation is found at the level of recognition molecules (PGRPs) or effectors (AMP) (Lazzaro et al., 2004).

Meeting Outcomes

Insect immune systems are complex, and the last few years have been characterized by the need to analyze the immune response physiologically and to use real pathogens that coevolve with insects. Studying insect immunity provides a unique opportunity to dissect the molecular mechanisms that underlie the basic modules of the immune system, to analyze the contribution of each defense mechanism throughout natural infections, and to analyze variation in immune competence among populations and species (such analysis will lead to a better understanding of adaptation). This leaves us with a vast plan of research, and combining different areas of expertise (in genetics, entomology and evolution) will reveal some coherent features among insect-parasites interactions and impact the field of immunology in general.

Acknowledgments

Thanks to all of the speakers for permission to discuss unpublished work and to Anastasia Fytrou for providing photographs for the figure. Thanks also to Ulrich Theopold for comments on the manuscript.

References

Abraham, E.G., Pinto, S.B., Ghosh, A., Vanlandingham, D.L., Budd, A., Higgs, S., Kafatos, F.C., Jacobs-Lorena, M., and Michel, K. (2005). An immune-responsive serpin, SRPN6, mediates mosquito defense against malaria parasites. Proc. Natl. Acad. Sci. USA *102*, 16327–16332.

Beck, M., and Strand, M.R. (2005). Glc1.8 from Microplitis demolitor bracovirus induces a loss of adhesion and phagocytosis by insect High Five and S2 cells. J. Virol. *79*, 1861–1870.

Bischoff, V., Vignal, C., Duvic, B., Boneca, I.G., Hoffmann, J.A., and Royet, J. (2006). Downregulation of the Drosophila immune response by peptidoglycan-recognition proteins SC1 and SC2. PLoS Pathogens *2*, e14.

Crozatier, M., Ubeda, J.M., Vincent, A., and Meister, M. (2004). Cellular immune response to parasitization in Drosophila requires the EBF orthologue collier. PLoS Biol. *2*, e196.

Dostert, C., Jouanguy, E., Irving, P., Troxler, L., Galiana-Arnoux, D., Hetru, C., Hoffmann, J., and Imler, J. (2005). The Jak-STAT signaling pathway is required but not sufficient for the antiviral response of Drosophila. Nat. Immunol. *6*, 946–953.

Espagne, E., Douris, V., Lalmanach, G., Provost, B., Cattolico, L., Lesobre, J., Kurata, S., Latrou, K., Drezen, J.M., and Huguet, E. (2005). A virus essential for insect host-parasite interactions encodes cystatins. J. Virol. *79*, 9765–9776.

Espagne, E., Dupuy, C., Huguet, E., Cattolico, L., Provost, B., Martins, N., Poirie, M., Periquet, G., and Drezen, J.M. (2004). Genome sequence of a polydnavirus: Insights into symbiotic virus evolution. Science *306*, 286–289.

Filipe, S.R., Tomasz, A., and Ligoxygakis, P. (2005). Requirements of peptidoglycan structure that allow detection by the Drosophila Toll pathway. EMBO Rep. *6*, 327–333.

Galiana-Arnoux, D., Dostert, C., Schneemann, A., Hoffmann, J.A., and Imler, J.-L. (2006). Essential function in vivo for Dicer-2 in host defense against RNA viruses in Drosophila. Nat. Immunol. *7*, 590–597.

Ha, E.-M., Oh, C.-T., Bae, Y.S., and Lee, W.-J. (2005). A direct role for dual oxidase in Drosophila gut immunity. Science *310*, 847–850.

Heddi, A., Vallier, A., Anselme, C., Xin, H., Rahbe, Y., and Wackers, F. (2005). Molecular and cellular profiles of insect bacteriocytes: Mutualism and harm at the initial evolutionary step of symbiogenesis. Cell. Microbiol. *7*, 293–305.

Jung, S.H., Evans, C.J., Uemura, C., and Banerjee, U. (2005). The Drosophila lymph gland as a developmental model of hematopoiesis. Development *132*, 2521–2533.

Kaneko, T., Yano, T., Aggarwal, K., Lim, J.H., Ueda, K., Oshima, Y., Peach, C., Erturk-Hasdemir, D., Goldman, W.E., Oh, B.H., et al. (2006). PGRP-LC and PGRP-LE have essential yet distinct functions in the Drosophila immune response to monomeric DAP-type peptidoglycan. Nat. Immunol. *7*, 715–723.

Kocks, C., Cho, J.H., Nehme, N., Ulvila, J., Pearson, A.M., Meister, M., Strom, C., Conto, S.L., Hetru, C., Stuart, L.M., et al. (2005). Eater, a transmembrane protein mediating phagocytosis of bacterial pathogens in Drosophila. Cell. Microbiol. *123*, 335–346.

Labrosse, C., Eslin, P., Doury, G., Drezen, J.M., and Poirie, M. (2005). Haemocyte changes in D. Melanogaster in response to long gland components of the parasitoid wasp Leptopilina boulardi: A Rho-GAP protein as an important factor. J. Insect Physiol. *51*, 161–170.

Lazzaro, B.P., Sceurman, B.K., and Clark, A.G. (2004). Genetic basis of natural variation in D. melanogaster antibacterial immunity. Science *303*, 1873–1876.

Liehl, P., Blight, M., Vodovar, N., and Lemaitre, F.B.B. (2006). Prevalence of local immune response against oral infection in a Drosophila/Pseudomonas infection model. PloS Pathogens. *2*, e56.

Michel, K., Budd, A., Pinto, S., Gibson, T.J., and Kafatos, F.C. (2005). Anopheles gambiae SRPN2 facilitates midgut invasion by the malaria parasite Plasmodium berghei. EMBO Rep. *6*, 891–897.

Obbard, D.J., Jiggins, F.M., Halligan, D.L., and Little, T.J. (2006). Natural selection drives extremely rapid evolution in antiviral RNAi genes. Curr. Biol. *16*, 580–585.

Shin, S.W., Kokoza, V., Bian, G., Cheon, H.M., Kim, Y.J., and Raikhel, A.S. (2005). REL1, a homologue of Drosophila dorsal, regulates toll antifungal immune pathway in the female mosquito Aedes aegypti. J. Biol. Chem. *280*, 16499–16507.

Thoetkiattikul, H., Beck, M.H., and Strand, M.R. (2005). Inhibitor kappaB-like proteins from a polydnavirus inhibit NF-kappaB activation and suppress the insect immune response. Proc. Natl. Acad. Sci. USA *102*, 11426–11431.

Tong, Y., Jiang, H., and Kanost, M.R. (2005). Identification of plasma proteases inhibited by Manduca sexta serpin-4 and serpin-5 and their association with components of the prophenol oxidase activation pathway. J. Biol. Chem. *280*, 14932–14942.

Wood, W., Faria, C., and Jacinto, A. (2006). Distinct mechanisms regulate hemocyte chemotaxis during development and wound healing in Drosophila melanogaster. J. Cell Biol. *173*, 405–416.

Zaidman-Remy, A., Herve, M., Poidevin, M., Pili-Floury, S., Kim, M.S., Blanot, D., Oh, B.H., Ueda, R., Mengin-Lecreulx, D., and Lemaitre, B. (2006). The Drosophila amidase PGRP-LB modulates the immune response to bacterial infection. Immunity *24*, 363–366.