# Genome-wide analysis of the *Drosophila* immune response by using oligonucleotide microarrays

**Ennio De Gregorio*, Paul T. Spellman†, Gerald M. Rubin†, and Bruno Lemaitre*‡**

*Centre de Génétique Moléculaire, Centre National de la Recherche Scientifique, 91198 Gif-sur-Yvette, France; and †Department of Molecular and Cell Biology and the Howard Hughes Medical Institute, University of California, Berkeley, CA 94720-3200

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**To identify new *Drosophila* genes involved in the immune response, we monitored the gene expression profile of adult flies in response to microbial infection by using high-density oligonucleotide microarrays encompassing nearly the full *Drosophila* genome. Of 13,197 genes tested, we have characterized 230 induced and 170 repressed by microbial infection, most of which had not previously been associated with the immune response. Many of these genes can be assigned to specific aspects of the immune response, including recognition, phagocytosis, coagulation, melanization, activation of NF-κB transcription factors, synthesis of antimicrobial peptides, production of reactive oxygen species, and regulation of iron metabolism. Additionally, we found a large number of genes with unknown function that may be involved in control and execution of the immune response. Determining the function of these genes represents an important challenge for improving our knowledge of innate immunity. Complete results may be found at http://www.fruitfly.org/expression/immunity/.**

innate immunity | fungal infection | septic injury

*Drosophila*, like other insects, is able to mount a rapid and efficient immune reaction in response to microbial infection despite the lack of an adaptive immune system (1, 2). Genetic and molecular studies, conducted mainly in *Drosophila*, have underlined the striking similarities between the mechanisms that regulate insect host defense and the mammalian innate immune response. These similarities make the fruit fly a potent model for deciphering animal innate immunity.

Microbial infection of *Drosophila* activates multiple cellular and humoral responses (1, 2). Septic injury, for example, rapidly triggers proteolytic cascades that lead to localized blood coagulation and melanization. This stimulus also activates the synthesis by the fat body, an analogue of the mammalian liver, of several antimicrobial peptides (AMPs), which are secreted into the hemolymph and directly kill invading pathogens. Finally, circulating blood cells perform an analogous function to mammalian macrophages, engulfing microbes, whereas larger pathogens are encapsulated by a specialized blood cell, the lamellocyte.

The mechanisms of *Drosophila* for recognizing infectious microbes are largely unknown. The current view is that some receptors can recognize surface determinants conserved among microbes but absent in the host such as lipopolysaccharides, peptidoglycans, and mannans. After recognition, these receptors stimulate immune response(s) by activating proteases present in the hemolymph and by using intracellular signaling pathways in immune-responsive tissues (1, 2).

Recent studies have demonstrated the critical role of evolutionary conserved signaling cassettes, such as the TLR-NF-κβ and the JAK-STAT pathways, in the regulation of both the humoral and cellular immune responses in *Drosophila* (3, 4). The nuclear factor-κB-like transcription factors are major regulators of AMP gene expression and their activity is modulated by two distinct signaling pathways, Toll and Immune deficiency (Imd), which are similar to the TLR-IL-1 receptor cascade in mammals (4). The Imd signaling pathway mediates defense against Gram-negative bacterial infection, whereas the Toll pathway is essential for the antifungal response. Although septic injury triggers the activation of both antibacterial and antifungal responses, leading to the expression of all AMPs, studies using natural modes of infection have demonstrated the ability of *Drosophila* to differentiate between pathogens and mount "specific" immune responses (5).

Knowledge of the *Drosophila* immune response is based on work conducted on a limited number of factors. These studies have shown that most genes participating in the immune response are transcriptionally modulated after infection. In an effort to identify new *Drosophila* genes involved in the immune response, we have monitored the genome-wide expression profile of adult flies in response to microbial infection by using high-density oligonucleotide arrays.

## Materials and Methods

**Infection Experiments.** For septic injury and natural infection experiments, we used *Drosophila* Oregon$^R$ adult males, aged 3–4 days at 25°C. Septic injury was performed by pricking the thorax of the flies with a needle previously dipped into a concentrated bacterial culture of *Escherichia coli* and *Micrococcus luteus* (5). Then flies were incubated at 25°C and collected at specific times after infection (1.5, 3, 6, 12, and 48 h). Natural infection was initiated by shaking anesthetized flies in a Petri dish containing a sporulating culture of the entomopathogenic fungus *Beauvaria bassiana* and incubated at 25°C (5) for specific times (12, 24, 48, and 96 h).

**Analysis of mRNA Expression by Using Oligonucleotide Arrays.** Total RNA was extracted from ≈500 flies for each time point by using Trizol reagent (GIBCO/BRL), and poly(A)-RNA was isolated from ≈1 mg of total RNA by using the MICROFASTTRACK 2.0 mRNA isolation system (Invitrogen). Gene expression analysis was performed by using the Affymetrix (Santa Clara, CA) *Drosophila* GeneChip, using the laboratory methods in the Affymetrix GeneChip expression manual. Briefly, double-stranded cDNA was synthesized by using 2 μg of poly(A)-RNA. Biotin-labeled cRNA was synthesized by using the BioArray high-yield RNA transcript-labeling kit (Enzo Biochem), and 15 μg of fragmented RNA were hybridized to each array. The arrays were washed with the EukGW2 protocol on the GeneChip Fluidics Station 400 series and scanned by using the GeneArray scanner. Gene expression analysis was performed by using multiple arrays and multiple independent mRNA samples for each time point (see Table 2, which is published as supporting information on the PNAS web site, www.pnas.org).

Abbreviations: AMP, antimicrobial peptide; Imd, Immune deficiency; DIRG, *Drosophila* immune-regulated gene; PO, phenoloxidase.

‡To whom reprint requests should be addressed. E-mail: lemaitre@cgm.cnrs-gif.fr.

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**Data Analysis.** Genes are represented on the DrosGenome1 chip by one or more transcripts, which in turn are represented by a probe set. Each probe set has 14 pairs of perfect match (PM) and mismatch (MM) oligonucleotides. Data were collected at the level of the transcript, but for ease in the text, the data are referred to by gene. Intensity data for each feature on the array was calculated from the images generated by the GeneChip scanner, using the GeneChip Microarray Suite. These intensity data were loaded into a MySQL database where information on each of the features was also stored. The difference between the PM and MM oligonucleotides (probe pair) was calculated, and the mean PM–MM intensity for each array was set to a constant value by linearly scaling array values. The mean intensity of individual probe pairs was calculated across all 34 arrays, and the $\log_2$ ratio of each value to this mean was stored. Next, all log ratios for each probe pair set (transcript) were averaged, creating one measurement for each transcript on each array. The final dataset was generated by averaging data for each transcript on replicate arrays and subtracting the average log ratio of the uninfected sample from each measurement.

A set of selection criteria for identifying new immune-responsive transcripts was created, using 33 genes previously known to be immune-responsive as a reference. Transcripts were ranked by the absolute value of their second and third largest log ratio, and the geometric mean of the rank was calculated (this will select genes that are both repressed and induced, but select against genes that are only highly induced or repressed at a single time point). The transcripts were sorted by their scores, and the position of known genes in this list was examined. Only 1 of the 33 known immune-responsive genes appears in this list of 13,197 genes below position 400 (*Tep1* at position 1874), whereas 21 known immune-responsive genes occur before position 100, 5 between 101 and 200, 2 between 201 and 300, and 4 between 301 and 400 (the full ranking for all genes is available at http://www.fruitfly.org/expression/immunity/). The correlation between rank and previous identification, and the fact that genes previously reported to be immune-responsive were very rare below position 400, made us conclude that it was reasonable to truncate the list of genes for further study by limiting the list to 400 transcripts.

## Results and Discussion

We used high-density oligonucleotide microarrays capable of assaying nearly every *Drosophila* gene to monitor the gene-expression response to microbial infection. Adult male flies were subjected to septic injury with a mixture of Gram-negative (*E. coli*) and Gram-positive (*M. luteus*) bacteria, or to natural infection with the entomopathogenic fungus *B. bassiana* (see *Materials and Methods*). Septic injury triggers a rapid and widespread response in *Drosophila* adults (5). This response might involve, together with defense genes required to combat infection, genes activated by physical injury. By contrast, natural infection by *B. bassiana* induces a slow but more specific antifungal response that starts 12–24 h after infection (5). Changes in relative transcript levels were measured 1.5, 3, 6, 12, 24, and 48 h after septic injury and 12, 24, 48, and 96 h after natural infection.

Among the large number of genes displaying changes in mRNA expression, we have selected a set of 400 *Drosophila* immune-regulated genes (DIRGs) out of 13,197 genes tested with patterns of gene expression similar to previously known immune induced genes (see *Materials and Methods*). The threshold was defined to minimize the inclusion of gene-expression patterns that seemed to be unrelated to immune response while including 32 of the 33 immune-response genes identified in previous studies; 368 of the selected genes had not previously been associated with the immune response.

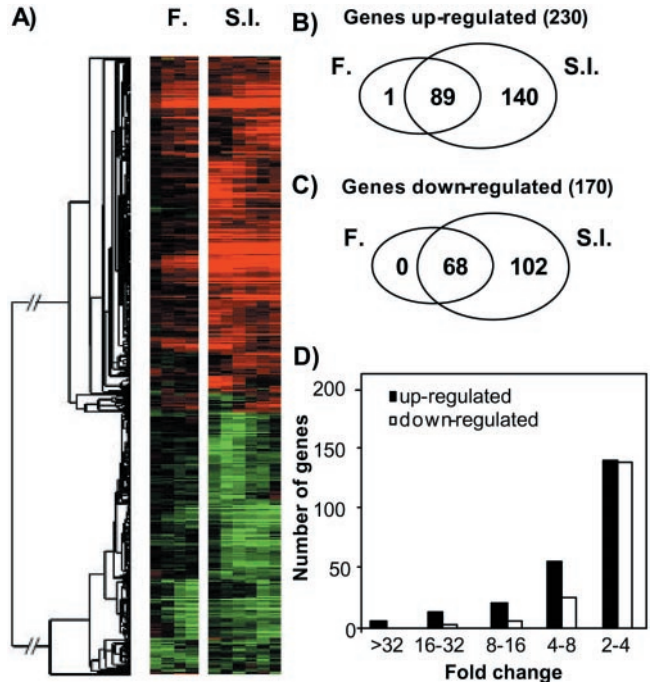


**Fig. 1.** General statistics on the *Drosophila* immune-regulated genes. (*A*) Cluster image of the 400 DIRGs. The expression profiles after fungal natural infection (F.) and septic injury (S.I.) are shown. Columns correspond to different time points and rows to different genes. Red indicated increased mRNA levels, whereas green indicated decreased levels compared with uninfected flies. The brightest reds and greens are 6-fold induced and repressed, respectively. The graphs in *B* and *C* show the number of the genes induced (*B*) and repressed (*C*) responding to fungal natural infection (F.), septic injury (S.I.), or both. (*D*) Distribution of induced (white bars) and repressed (black bars) genes based on their fold change.

**General Statistics.** The expression patterns of all 400 DIRGs were hierarchically clustered (6). Fig. 1*A* shows the complete dendrogram, which contains two main branches composed of 230 induced and 170 repressed genes. Within these two branches, we identified other clusters based on the differential response between septic injury and fungal infection and subsequently on the kinetics of the immune response. Septic injury causes gene-expression changes in nearly all of the 400 DIRGs, whereas fungal infection regulates a lower number of DIRGs (89 induced, 68 repressed; Fig. 1 *B* and *C*). Moreover, the change in gene expression is generally less marked after natural infection by *B. bassiana* compared with septic injury. These findings are in agreement with the previous observation showing that fungal infection is more specific than septic injury (5). We identified one gene (*CG14599*) that responds only to *B. bassiana* infection and could be a defense gene responding specifically to this entomopathogenic fungus. On the other hand, genes regulated by septic injury, but not by fungal natural infection (140 induced, 102 repressed), could be specific genes that fight bacterial infection or genes involved in the response to physical injury (Fig. 1 *B* and *C*). It should be noted that our study has a slight bias toward identifying more genes responsive to septic injury than to fungal infection because of the greater number of experimental observations devoted to septic injury; however, this effect should be quite small. The terminal branches of the cluster separated the DIRGs according to the kinetics of their expression profiles (acute, intermediate, and late)

The complete set of DIRGs (as well as the complete underlying data) is available at http://www.fruitfly.org/expression/immunity/. A large number of the DIRGs encode proteins

**Table 1. Expression profiles of selected DIRGs**

| | | Septic injury | | | | | | Fungal infection | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1.5 | 3 | 6 | 12 | 24 | 48 | 12 | 24 | 48 | 96 |
| **Recognition and phagocytosis (GO:0006909)** | | | | | | | | | | | |
| PGRP (GO:0016019) | | | | | | | | | | | |
| CG11709 | PGRP-SA | 8.5 | 9.5 | 8.1 | 5.6 | 4.9 | 5.5 | 1.2 | 1.3 | 1.5 | 1.9 |
| CG14745 | PGRP-SC2 | 1.2 | 1.9 | 2.7 | 2.8 | 2.6 | 2.3 | 1.0 | 0.8 | 0.9 | 1.0 |
| CG14704 | PGRP-LB | 2.9 | 3.7 | 2.9 | 2.5 | 2.1 | 2.4 | 1.0 | 1.0 | 0.9 | 0.7 |
| CG9681 | PGRP-SB1 | 10.2 | 16.0 | 21.7 | 17.7 | 15.2 | 15.2 | 1.0 | 1.1 | 1.2 | 1.7 |
| CG7496 | PGRP-SD | 3.6 | 7.4 | 18.9 | 15.8 | 9.5 | 7.8 | 1.4 | 1.4 | 1.5 | 1.7 |
| CG4432 | PGRP-LC | 3.1 | 2.8 | 2.4 | 3.1 | 1.6 | 3.4 | 1.3 | 1.2 | 1.0 | 1.2 |
| CG4437 | PRGP-like | 3.1 | 2.2 | 2.2 | 2.1 | 1.6 | 2.2 | 1.3 | 0.9 | 0.9 | 1.1 |
| GNBP | | | | | | | | | | | |
| CG13422 | GNBP-like | 6.9 | 7.1 | 11.4 | 13.3 | 9.7 | 11.2 | 2.1 | 3.5 | 5.0 | 5.1 |
| CG12780 | GNBP-like | 3.4 | 3.4 | 3.2 | 2.2 | 1.8 | 2.6 | 1.1 | 1.7 | 1.8 | 1.6 |
| Putative chitin-binding lectins (GO:0005530) | | | | | | | | | | | |
| CG4559 | Idgf3 | 2.2 | 2.3 | 2.8 | 2.1 | 1.8 | 2.1 | 0.9 | 1.2 | 1.1 | 1.1 |
| CG4472 | Idgf1 | 4.1 | 2.9 | 2.0 | 1.4 | 1.0 | 2.6 | 0.9 | 1.2 | 0.8 | 0.9 |
| Complement-like | | | | | | | | | | | |
| CG7052 | Tep2 | 1.7 | 1.5 | 7.9 | 3.9 | 7.2 | 2.6 | 1.1 | 1.2 | 1.2 | 3.1 |
| CG10363 | Tep4 | 3.2 | 3.1 | 3.4 | 2.2 | 2.0 | 2.7 | 0.8 | 1.3 | 1.7 | 2.5 |
| Complement-binding | | | | | | | | | | | |
| CG4823 | α2M-receptor-like | 1.3 | 1.1 | 2.5 | 2.3 | 4.5 | 3.8 | 0.9 | 1.5 | 1.4 | 2.1 |
| **Trypsin-like serine proteases (GO:0008236 )** | | | | | | | | | | | |
| Up-regulated | | | | | | | | | | | |
| CG2056 | | 1.6 | 2.2 | 3.0 | 6.1 | 6.7 | 6.2 | 1.3 | 0.8 | 1.3 | 0.8 |
| CG11842 | | 1.1 | 1.2 | 3.3 | 6.4 | 5.1 | 4.3 | 1.4 | 1.8 | 2.1 | 1.7 |
| CG9645 | | 2.3 | 1.5 | 1.7 | 2.0 | 2.9 | 3.1 | 1.0 | 1.6 | 2.2 | 2.5 |
| CG9649 | | 2.0 | 0.9 | 1.2 | 1.6 | 2.1 | 4.6 | 1.2 | 1.8 | 1.8 | 2.5 |
| CG2045 | | 1.7 | 1.9 | 1.4 | 2.7 | 2.6 | 2.5 | 1.1 | 2.3 | 2.6 | 2.5 |
| CG5909 | | 5.2 | 4.6 | 1.8 | 1.8 | 1.2 | 1.1 | 1.1 | 1.5 | 2.1 | 2.8 |
| CG15046 | | 5.6 | 3.9 | 2.2 | 1.3 | 2.0 | 1.5 | 0.9 | 1.1 | 1.0 | 1.2 |
| CG8215 | | 1.3 | 2.5 | 2.1 | 1.9 | 3.3 | 7.0 | 1.2 | 1.0 | 1.6 | 6.0 |
| CG6639 | | 2.8 | 1.4 | 2.0 | 8.6 | 23.4 | 49.8 | 2.0 | 2.1 | 6.8 | 18.7 |
| CG6361 | | 1.6 | 2.4 | 3.2 | 2.4 | 2.4 | 2.3 | 1.3 | 1.2 | 1.2 | 1.1 |
| CG6467 | | 2.1 | 2.3 | 2.4 | 1.5 | 1.7 | 1.9 | 1.1 | 1.2 | 2.3 | 2.5 |
| CG9631 | | 2.7 | 3.3 | 2.4 | 2.0 | 2.1 | 2.6 | 1.1 | 1.2 | 1.7 | 1.6 |
| CG11841 | | 1.2 | 0.8 | 1.6 | 2.7 | 2.5 | 2.8 | 1.3 | 2.6 | 3.0 | 3.9 |
| CG18563 | | 1.1 | 1.3 | 2.4 | 4.1 | 6.2 | 9.9 | 1.1 | 1.0 | 1.8 | 3.1 |
| CG9733 | proPO-AE | 8.1 | 5.2 | 2.0 | 1.7 | 2.0 | 1.3 | 0.9 | 1.2 | 1.2 | 1.0 |
| CG1102 | proPO-AE | 3.9 | 3.1 | 1.5 | 2.9 | 2.7 | 3.9 | 1.2 | 1.6 | 1.4 | 1.5 |
| CG3066 | proPO-AE | 5.5 | 5.3 | 3.0 | 3.5 | 3.0 | 3.4 | 1.1 | 1.3 | 1.0 | 1.2 |
| CG3505 | proPO-AE | 5.3 | 3.7 | 5.0 | 5.8 | 4.7 | 5.9 | 0.8 | 1.7 | 2.1 | 2.5 |
| CG16705 | proPO-AE | 2.2 | 2.4 | 3.5 | 4.8 | 4.8 | 4.7 | 1.1 | 2.8 | 3.0 | 3.3 |
| Down-regulated | | | | | | | | | | | |
| CG8579 | | 0.6 | 0.3 | 0.3 | 0.3 | 0.2 | 0.3 | 1.0 | 0.8 | 0.6 | 0.5 |
| CG4812 | | 0.2 | 0.2 | 0.3 | 0.4 | 0.6 | 0.6 | 1.1 | 0.8 | 0.7 | 0.9 |
| CG12385 | thetaTrypsin | 0.5 | 0.3 | 0.3 | 0.4 | 0.4 | 0.5 | 0.7 | 0.9 | 0.5 | 0.6 |
| CG8869 | | 0.4 | 0.3 | 0.2 | 0.3 | 0.2 | 0.2 | 1.2 | 0.8 | 0.6 | 0.4 |
| CG8867 | Ser4 | 0.6 | 0.6 | 0.4 | 0.3 | 0.2 | 0.2 | 1.2 | 1.0 | 0.6 | 0.6 |
| CG18180 | | 0.6 | 0.5 | 0.4 | 0.4 | 0.3 | 0.2 | 1.0 | 0.9 | 0.6 | 0.4 |
| CG18179 | | 0.5 | 0.4 | 0.4 | 0.4 | 0.3 | 0.3 | 1.0 | 0.5 | 0.3 | 0.4 |
| CG17951 | Ser99Dc | 0.7 | 0.5 | 0.3 | 0.2 | 0.2 | 0.2 | 0.9 | 0.8 | 0.6 | 0.7 |
| CG12351 | gammaTrypsin | 0.5 | 0.4 | 0.4 | 0.5 | 0.4 | 0.3 | 0.8 | 0.7 | 0.4 | 0.3 |
| CG9672 | | 0.8 | 0.6 | 0.5 | 0.4 | 0.4 | 0.4 | 0.9 | 0.6 | 0.7 | 0.6 |
| CG8871 | | 0.9 | 0.6 | 0.4 | 0.6 | 0.4 | 0.4 | 1.0 | 0.9 | 0.9 | 0.8 |
| CG16749 | | 0.7 | 0.5 | 0.4 | 0.5 | 0.5 | 0.5 | 1.0 | 0.8 | 0.9 | 0.8 |
| CG7532 | | 0.7 | 0.6 | 0.4 | 0.5 | 0.4 | 0.4 | 1.0 | 0.8 | 0.8 | 0.8 |
| CG6069 | | 0.8 | 0.7 | 1.0 | 0.9 | 0.4 | 0.5 | 1.2 | 1.0 | 0.6 | 0.5 |
| CG18030 | | 0.4 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 1.3 | 0.8 | 0.5 | 0.3 |
| CG10475 | | 0.5 | 0.3 | 0.2 | 0.2 | 0.1 | 0.2 | 0.9 | 0.5 | 0.5 | 0.3 |
| **Serpins (GO:0004868)** | | | | | | | | | | | |
| Up-regulated | | | | | | | | | | | |
| CG7219 | | 21.2 | 17.0 | 5.9 | 4.0 | 3.9 | 2.8 | 0.9 | 1.0 | 1.4 | 1.4 |
| CG11331 | | 5.8 | 6.5 | 4.6 | 2.3 | 2.1 | 1.9 | 0.9 | 1.3 | 1.1 | 2.1 |
| CG18525 | Sp5 | 2.8 | 3.2 | 3.3 | 2.9 | 2.4 | 3.4 | 0.8 | 1.6 | 1.5 | 1.5 |
| CG6687 | | 4.3 | 5.0 | 5.8 | 5.9 | 9.0 | 11.5 | 1.0 | 2.3 | 3.1 | 4.4 |
| CG9453 | Sp4 | 2.3 | 1.7 | 1.8 | 2.0 | 2.1 | 2.0 | 1.1 | 1.3 | 1.2 | 1.2 |
| Down-regulated | | | | | | | | | | | |
| CG6663 | | 1.1 | 0.8 | 1.0 | 0.9 | 0.6 | 0.5 | 0.8 | 0.8 | 0.5 | 0.4 |
| CG3801 | | 0.9 | 0.7 | 0.9 | 0.8 | 0.3 | 0.5 | 0.9 | 0.8 | 0.3 | 0.2 |
| CG8137 | Sp2 | 1.1 | 0.9 | 0.9 | 0.8 | 0.5 | 0.6 | 0.8 | 1.0 | 0.5 | 0.4 |
| **Kunitz-family ser-protease inhibitors (GO:0004867)** | | | | | | | | | | | |
| Up-regulated | | | | | | | | | | | |
| CG16713 | | 5.5 | 5.7 | 7.2 | 6.5 | 4.7 | 7.0 | 1.6 | 2.7 | 2.2 | 2.2 |
| CG3604 | | 2.5 | 4.5 | 3.7 | 2.4 | 1.3 | 2.0 | 1.2 | 1.3 | 1.2 | 1.3 |
| Down-regulated | | | | | | | | | | | |
| CG6953 | Fat-spondin | 0.8 | 0.4 | 0.4 | 0.5 | 0.9 | 1.3 | 0.9 | 1.0 | 1.0 | 1.3 |
| **Melanization and coagulation** | | | | | | | | | | | |
| Melanization (GO:0006582 ) | | | | | | | | | | | |
| CG10697 | Ddc | 7.3 | 11.1 | 4.9 | 1.9 | 1.6 | 1.6 | 0.9 | 1.1 | 0.9 | 0.9 |
| CG10118 | Pale | 2.2 | 2.4 | 1.8 | 1.3 | 2.0 | 1.6 | 1.0 | 1.3 | 2.1 | 2.0 |
| CG9441 | Punch | 3.2 | 4.3 | 4.7 | 2.5 | 1.3 | 2.2 | 0.9 | 1.0 | 0.8 | 0.7 |
| CG4665 | Dhpr | 2.1 | 3.2 | 3.1 | 2.2 | 1.5 | 1.7 | 1.1 | 1.3 | 1.0 | 1.3 |
| CG18550 | yellow f | 1.5 | 1.6 | 2.4 | 3.8 | 5.1 | 5.9 | 1.2 | 1.3 | 2.0 | 2.0 |

| | | Septic injury | | | | | | Fungal infection | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1.5 | 3 | 6 | 12 | 24 | 48 | 12 | 24 | 48 | 96 |
| **Melanization and coagulation (continued)** | | | | | | | | | | | |
| Melanization (GO:0006582) (continued) | | | | | | | | | | | |
| CG6524 | Cp19 | 1.5 | 2.0 | 2.1 | 1.7 | 2.3 | 3.4 | 1.3 | 1.0 | 2.1 | 2.1 |
| CG3759 | laccase-like | 2.0 | 1.8 | 2.2 | 1.5 | 1.9 | 2.2 | 0.8 | 0.8 | 1.5 | 1.4 |
| Coagulation (GO:0007596) | | | | | | | | | | | |
| CG5550 | Fibrinogen-like* | 9.5 | 2.5 | 1.4 | 1.4 | 1.1 | 1.8 | 1.2 | 1.3 | 1.4 | 0.9 |
| CG5730 | Annexin IX | 2.4 | 2.5 | 2.1 | 2.0 | 1.7 | 1.9 | 1.0 | 1.0 | 1.0 | 0.9 |
| **Antimicrobial peptides (GO:0003795)** | | | | | | | | | | | |
| CG10810 Drosomycin | | 6.1 | 6.7 | 6.2 | 8.3 | 8.0 | 7.0 | 2.0 | 6.4 | 7.0 | 6.7 |
| CG10812 Drosomycin B | | 1.4 | 1.4 | 2.1 | 3.2 | 2.7 | 3.1 | 1.2 | 1.8 | 3.2 | 3.7 |
| CG8175 Metchnikowin | | 5.5 | 7.4 | 3.5 | 1.9 | 2.3 | 1.7 | 1.4 | 4.4 | 6.1 | 7.1 |
| CG1385 Defensin | | 2.7 | 14.2 | 27.6 | 26.9 | 15.3 | 7.4 | 2.0 | 2.4 | 3.2 | 1.3 |
| CG10816 Drosocin | | 9.8 | 14.0 | 16.4 | 11.4 | 8.2 | 13.5 | 1.3 | 1.1 | 1.7 | 2.3 |
| CG12763 Diptericin | | 11.6 | 16.1 | 16.0 | 16.7 | 13.4 | 15.4 | 1.8 | 1.3 | 1.5 | 1.1 |
| CG10794 Diptericin B | | 7.2 | 6.3 | 7.6 | 6.0 | 5.3 | 5.3 | 1.2 | 1.0 | 1.2 | 1.4 |
| CG1365 CecropinA1 | | 8.5 | 5.5 | 8.3 | 10.8 | 9.4 | 8.5 | 1.3 | 1.2 | 1.4 | 1.1 |
| CG1367 CecropinA2 | | 21.1 | 20.8 | 25.8 | 22.6 | 18.3 | 15.0 | 1.3 | 1.2 | 1.3 | 0.9 |
| CG1878 Cecropin B | | 44.5 | 38.9 | 19.5 | 5.3 | 4.6 | 4.0 | 0.5 | 0.6 | 0.7 | 0.8 |
| CG1373 Cecropin C | | 92.2 | 110 | 32.0 | 8.5 | 9.0 | 7.7 | 1.2 | 0.9 | 1.0 | 1.2 |
| CG10146 Attacin A | | 18.4 | 16.3 | 16.4 | 16.9 | 16.9 | 16.9 | 2.1 | 3.4 | 3.7 | 4.2 |
| CG18372 Attacin B1 | | 11.7 | 9.5 | 12.0 | 12.2 | 12.5 | 13.2 | 2.1 | 2.5 | 3.3 | 3.1 |
| CG4740 Attacin C | | 27.1 | 31.7 | 26.8 | 31.7 | 30.9 | 30.2 | 1.3 | 1.5 | 1.7 | 2.5 |
| CG7629 Attacin D | | 4.6 | 22.5 | 36.5 | 10.3 | 9.6 | 7.5 | 0.9 | 0.8 | 1.3 | 1.0 |
| **Unknown peptides (40-134 aa)** | | | | | | | | | | | |
| CG18106 IM2 | | 3.9 | 4.8 | 6.0 | 6.5 | 4.9 | 6.9 | 2.0 | 3.7 | 4.1 | 3.4 |
| CG15065 IM2-like | | 3.2 | 3.3 | 2.6 | 2.0 | 1.0 | 1.3 | 2.2 | 2.7 | 2.6 | 2.2 |
| CG18108 IM2-like | | 5.6 | 8.1 | 9.3 | 11.3 | 9.5 | 10.3 | 2.7 | 7.2 | 7.6 | 7.4 |
| CG14027 (121aa) | | 1.0 | 3.9 | 17.9 | 31.1 | 13.8 | 8.4 | 1.6 | 3.6 | 2.4 | 1.2 |
| CG6429 (124 aa) | | 8.5 | 9.4 | 3.8 | 2.4 | 1.9 | 2.2 | 1.0 | 1.2 | 1.2 | 1.6 |
| CG15066 (134 aa) | | 6.3 | 9.7 | 10.3 | 10.0 | 9.4 | 8.5 | 2.6 | 7.7 | 9.6 | 10.0 |
| CG12965 (45 aa) | | 16.8 | 19.5 | 20.9 | 22.5 | 17.8 | 18.8 | 1.7 | 3.4 | 4.2 | 6.9 |
| CG9928 (106 aa) | | 1.5 | 1.4 | 1.3 | 2.6 | 3.4 | 4.2 | 1.1 | 1.9 | 1.9 | 1.9 |
| CG9080 (121 aa) | | 5.1 | 10.0 | 12.5 | 11.9 | 8.2 | 5.7 | 1.2 | 1.6 | 2.6 | 3.2 |
| CG13324 (112 aa) | | 6.3 | 6.7 | 4.9 | 2.6 | 1.4 | 1.5 | 1.3 | 1.3 | 1.1 | 1.6 |
| CG12494 (61aa) | | 1.3 | 1.7 | 3.4 | 4.1 | 4.2 | 4.2 | 1.3 | 1.2 | 1.3 | 1.2 |
| CG16978 (96 aa) | | 7.0 | 11.0 | 4.9 | 4.0 | 3.6 | 5.9 | 1.5 | 2.7 | 3.0 | 3.5 |
| CG5791 (98 aa) | | 1.3 | 1.6 | 4.8 | 7.7 | 7.3 | 5.6 | 2.3 | 6.0 | 7.5 | 6.9 |
| CG14957 (96 aa) | | 7.2 | 7.0 | 4.3 | 0.9 | 0.9 | 0.9 | 1.0 | 0.7 | 1.1 | 0.8 |
| CG4269 (102 aa) | | 4.5 | 5.3 | 5.1 | 4.0 | 2.7 | 4.1 | 0.9 | 1.6 | 1.0 | 1.2 |
| CG4250 (121 aa) | | 1.1 | 1.3 | 3.8 | 6.5 | 6.0 | 7.3 | 1.4 | 2.2 | 2.4 | 3.1 |
| CG16836 (78 aa) | | 1.5 | 2.0 | 3.4 | 4.9 | 4.8 | 5.4 | 1.6 | 2.9 | 3.5 | 3.5 |
| CG15126 (53 aa) | | 6.1 | 4.6 | 1.6 | 0.7 | 0.9 | 1.1 | 1.0 | 0.7 | 1.0 | 1.0 |
| CG17278 (49 aa) | | 3.4 | 2.9 | 1.9 | 1.7 | 1.2 | 2.0 | 1.0 | 1.2 | 1.3 | 1.0 |
| CG17107 (96 aa) | | 1.4 | 2.0 | 3.1 | 3.9 | 2.8 | 2.5 | 0.7 | 1.0 | 0.9 | 1.2 |
| CG13323 (112 aa) | | 2.8 | 2.8 | 3.1 | 2.2 | 1.3 | 1.8 | 1.2 | 1.7 | 1.5 | 1.6 |
| CG5773 (127 aa) | | 1.1 | 1.6 | 3.8 | 3.3 | 2.8 | 2.8 | 0.8 | 1.2 | 1.2 | 1.0 |
| CG7738 (104 aa) | | 1.3 | 1.1 | 1.4 | 2.6 | 3.4 | 3.8 | 1.2 | 1.5 | 2.0 | 3.8 |
| CG15829 (82 aa) | | 4.0 | 4.9 | 2.6 | 0.9 | 1.0 | 0.9 | 1.1 | 0.7 | 0.8 | 0.9 |
| CG9616 (127 aa) | | 1.4 | 1.5 | 1.7 | 2.7 | 2.3 | 2.8 | 1.5 | 1.5 | 1.3 | 1.1 |
| CG8157 (113 aa) | | 1.1 | 1.4 | 2.5 | 2.9 | 2.0 | 1.5 | 1.0 | 0.7 | 0.9 | 1.0 |
| CG5097 (43 aa) | | 0.9 | 0.8 | 1.3 | 2.3 | 2.6 | 2.7 | 1.5 | 1.1 | 1.0 | 2.9 |
| CG14599 (70 aa) | | 0.7 | 0.8 | 0.5 | 0.8 | 1.3 | 0.4 | 1.6 | 0.8 | 3.4 | 2.3 |
| **Others** | | | | | | | | | | | |
| Iron metabolism (GO:0006879 ) | | | | | | | | | | | |
| CG6186 | Transferrin | 0.9 | 1.1 | 1.2 | 1.3 | 2.0 | 1.3 | 0.8 | 1.6 | 2.1 | 2.3 |
| CG3666 | Transferrin | 10.8 | 6.3 | 3.0 | 1.8 | 2.3 | 2.3 | 1.1 | 1.6 | 1.3 | 1.6 |
| CG6898 | Zip3 | 2.0 | 2.8 | 3.4 | 3.0 | 3.2 | 2.9 | 1.0 | 1.1 | 1.2 | 1.9 |
| ROS | | | | | | | | | | | |
| CG8913 | Peroxidase | 3.2 | 4.0 | 5.9 | 5.8 | 7.7 | 9.0 | 1.2 | 2.2 | 2.6 | 2.9 |
| Lysosomal enzymes (GO:0005764) | | | | | | | | | | | |
| CG3132 | β-galactosidase | 1.8 | 2.2 | 4.7 | 2.2 | 3.4 | 3.8 | 1.3 | 1.2 | 0.9 | 1.2 |
| CG11459 | Cathepsin L | 0.9 | 0.8 | 1.8 | 6.5 | 4.5 | 5.0 | 1.0 | 1.6 | 2.0 | 3.7 |
| CG7780 | Lysosomal DNAse II | 0.9 | 0.6 | 1.1 | 2.5 | 3.8 | 4.9 | 1.2 | 2.0 | 2.2 | 2.2 |
| Lipases (GO:0016298 ) | | | | | | | | | | | |
| CG4267 | | 3.8 | 4.6 | 3.9 | 2.7 | 2.8 | 3.4 | 1.2 | 0.9 | 1.2 | 1.0 |
| CG4757 | Lipase,carboxylesteras | 0.9 | 1.2 | 5.2 | 11.1 | 19.6 | 35.6 | 0.7 | 5.5 | 13.9 | 20.1 |
| CG6675 | | 4.6 | 5.7 | 2.3 | 1.7 | 1.3 | 1.6 | 1.5 | 2.8 | 2.3 | 4.1 |
| CG7279 | Lip1 | 1.2 | 2.2 | 1.8 | 2.2 | 2.0 | 1.1 | 1.1 | 0.5 | 1.8 | 1.7 |
| JNK-pathway (GO:0007254) | | | | | | | | | | | |
| CG2275 | d-Jun* | 3.8 | 1.8 | 1.5 | 1.2 | 1.1 | 1.3 | 0.9 | 1.0 | 1.0 | 1.0 |
| CG7850 | Puc: JNK phosphatase | 2.5 | 1.4 | 1.5 | 1.3 | 1.1 | 1.7 | 0.8 | 1.2 | 1.1 | 1.0 |
| Toll-Pathway (GO:0008063) | | | | | | | | | | | |
| CG1857 | Necrotic | 4.2 | 4.4 | 4.6 | 4.9 | 4.8 | 5.9 | 1.1 | 2.3 | 3.3 | 3.4 |
| CG6134 | Spatzle | 3.9 | 3.8 | 2.5 | 2.0 | 1.8 | 2.6 | 0.8 | 1.5 | 1.2 | 1.3 |
| CG5490 | Toll | 3.1 | 2.0 | 1.6 | 2.0 | 2.3 | 3.1 | 0.9 | 1.3 | 1.5 | 1.2 |
| CG5974 | Pelle | 2.4 | 2.9 | 2.0 | 1.9 | 1.8 | 2.3 | 1.1 | 1.2 | 1.1 | 1.4 |
| CG5848 | Cactus | 7.8 | 7.5 | 3.4 | 3.4 | 3.7 | 4.2 | 1.4 | 2.1 | 1.8 | 2.4 |
| CG6794 | Dif | 2.7 | 1.8 | 1.7 | 1.5 | 1.4 | 2.3 | 1.5 | 2.0 | 1.7 | 1.5 |
| CG6667 | Dorsal | 1.5 | 1.7 | 2.2 | 1.9 | 2.2 | 2.9 | 1.3 | 1.0 | 0.9 | 1.1 |
| Imd -pathway (GO:0007249) | | | | | | | | | | | |
| CG11992 | Relish | 17.2 | 9.6 | 8.4 | 6.0 | 5.7 | 7.7 | 1.2 | 2.0 | 2.0 | 2.1 |
| CG5576 | imd* | 2.1 | 1.7 | 1.5 | 1.5 | 1.2 | 1.5 | 1 | 1.1 | 0.8 | 1.3 |

The fold change after septic injury and fungal natural infection compared to uninfected flies for selected DIRGs is shown. Time intervals after infection are indicated in hours at the top. The length of predicted small peptides (in amino acids) is shown. The gene ontology (GO) numbers are references to entries in the GeneOntology index of molecular functions and biological processes (www.geneontology.org). The four genes marked with an asterix do not fulfill the general threshold criteria we used to select DIRGs, because they were highly induced at only 1 time point. We have included them in this figure because their sequence suggests a role in the immune response.

with either no obvious similarity to known functional domains or that cannot presently be assigned to a specific aspect of the immune response (see Table 3, which is published as supporting information on the PNAS web site). Although the analysis of these new genes represents an important challenge for the future, they will not be discussed in this report. Instead, we will consider 134 DIRGs that have a known or predicted function consistent with a role in the immune response.

**Recognition.** Pathogen recognition by the innate immune system is believed to rely on interactions between conserved microbial determinants on the surface of pathogens and host recognition proteins. Peptidoglycan recognition proteins (PGRPs) can bind to peptidoglycan, a component of the Gram-positive bacteria envelope, and are found in many species including insects and mammals (7). In *Drosophila*, 12 *PGRP* genes have been identified, and 5 of them are up-regulated after the injection of either peptidoglycan or Gram-positive bacteria in adult flies (8). We have detected the induction by septic injury of the five reported inducible *PGRP* genes (*PGRP-SA*, *SC2*, *SB1*, *LB*, and *SD*; ref. 8) and of *PRGP-LC*, whose transcript was not detectable by previous Northern analysis (Table 1). In addition we identified a new inducible *PGRP*-like gene (*CG4437*), which could either be an independent transcript or a splicing variant of *PGRP-LC* (8). Consistent with their putative function in bacterial recognition, none of the *PRPG* genes are induced by fungal infection.

The Gram-negative binding proteins (GNBPs) have been identified for their affinity to lipopolysaccharides and β-1-3 glucan (9). In various insect species, including *Bombyx mori* and *Anopheles gambiae* (10, 11), *GNBP* genes are induced by bacterial infection. Our analysis confirms a previous study (9), showing that none of the three *Drosophila GNBP* genes are induced after infection. We do find that two uncharacterized genes (*CG13422* and *CG12780*), encoding short proteins with significant similarity to the N-terminal part of *Drosophila* GNBPs, are up-regulated after septic injury. Interestingly, *CG13422* is also strongly activated in response to *B. bassiana* infection, suggesting a role in fungal recognition (Table 1).

A third class of putative recognition proteins, the lectins, has been implicated in pathogen recognition in vertebrates and invertebrates (12). Unexpectedly, none of the 35 annotated genes encoding proteins with C-type lectin domains were induced by infection. However, two genes encoding members of the imaginal disk growth factor family (IDGF1 and -3; ref. 13) are up-regulated in response to septic injury (Table 1). IDGF proteins contain a putative chitin-binding lectin domain (14); in addition, they are known to stimulate cell growth in *Drosophila* cell lines and to be expressed in the fat body. None of the *IDGF* genes are induced by fungal infection, suggesting that they are not the receptors for fungal chitin. A possible role for IDGF proteins after septic injury could be to recognize *Drosophila* chitin at the wound site and stimulate the cell growth required for wound healing.

**Complement-Like Proteins and Phagocytosis.** In vertebrates, complement proteins like C3 and α-2-macroglobulin bind covalently to the surface of the invading microorganisms through a thiolester bond. This event triggers the activation of the complement terminal lytic complex and ultimately promotes phagocytosis (15). No such complex is present in insects, but several complement-like proteins called thiolester proteins (TEPs) have been identified in *A. gambiae* and *Drosophila* (16, 17). Recently, it has been shown that *A. gambiae* TEP1 can bind to the surface of bacteria and promote phagocytosis in a mosquito cell line (17). In *Drosophila*, four TEP-encoding genes have been identified in the genome, and three of them (*TEP1*, *-2*, and *-4*) are induced after septic injury of adult flies (16). Our study confirms that *TEP2* and *-4* are induced after septic injury and shows that they respond also to fungal natural infection (Table 1). In contrast with previous data (16), *TEP-1* did not seem to be induced in adults by either of the two infections. In addition to complement-like encoding genes, one gene induced by septic injury (*CG4823*) encodes an homologue of the α-2-macroglobulin receptor gene in vertebrates (18) that might be a receptor which recognizes opsonized microorganisms and triggers phagocytosis in *Drosophila*.

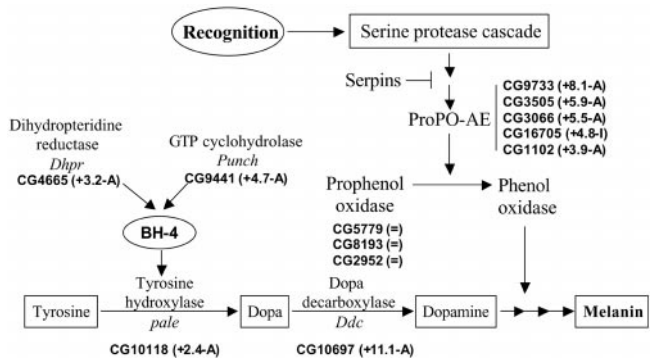


**Fig. 2.** *Drosophila* melanization cascade. Schematic representation of the melanization cascade (see text for description) The *Drosophila* genes involved in the melanization pathway are indicated. Previously uncharacterized genes have names comprised of CG followed by a number (28); more information about these genes can be found in the FlyBase database (www.flybase.org). The numbers in parentheses represent the peak of activation within the expression profile (fold change compared to uninfected flies). =, no change in the expression levels; A, acute-phase gene; I, intermediate-phase gene.

**Serpins and Serine Proteases.** Trypsin-like serine proteases and their inhibitors, serpins, play a central role in the insect immune response. Studies of the *Drosophila* Toll pathway show that at least some of these proteins are extracellular signaling molecules. The activation of the Toll ligand Spätzle is under the control of a still unknown serine protease cascade and of at least one serpin called Necrotic (19). Studies in other insects have extended the involvement of serine proteases and serpins to the activation of defense mechanisms such as coagulation (20) and melanization (21) (Fig. 2).

Table 1 shows the expression profile of genes encoding trypsin-like serine proteases and serpins that respond to infection. Of 206 trypsin-like serine protease genes present in the *Drosophila* genome, 19 are up-regulated by septic injury; 12 of these also respond to fungal infection. In addition, 16 genes encoding serine proteases are down-regulated by septic injury, of which 9 are also repressed by fungal infection.

There are 30 annotated genes encoding proteins with a serpin domain in the *Drosophila* genome. Two, *necrotic* and *TEP2*, were reported to be induced after infection (16, 19). An additional five uncharacterized serpins are up-regulated and three are down-regulated after septic injury, whereas six of these respond to natural fungal infection. Interestingly, three genes (*CG16713*, *CG3604*, and *fat-spondin*), encoding serine protease inhibitors of the Kunitz family, which has not previously been implicated in the immune response, are regulated by microbial infection (Table 1).

**Melanization and Coagulation.** Melanization is a common defense mechanism among invertebrates and it is involved in both pigmentation and wound healing (21). It requires the activation of phenoloxidase (PO), which catalyzes the conversion of dopamine into melanin. Melanin in turn is toxic to microorganisms. This reaction also leads to production of cytotoxic reactive oxygen species (ROS) that may also combat infection (22). PO is stored as an inactive precursor, proPO, which must be proteolytically activated by a serine protease cascade (21). The terminal serine proteases of this cascade have been identified in other insect species (21) and have been called proPO-activating

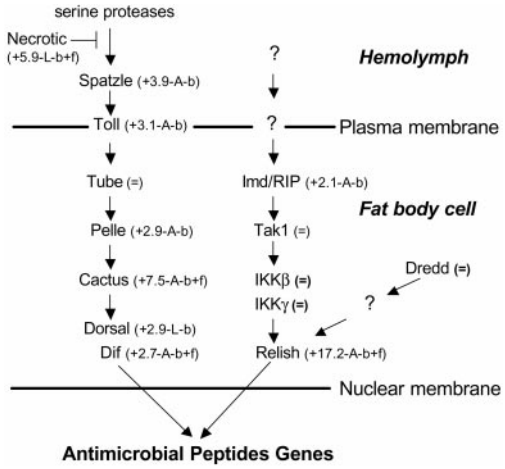


**Fig. 3.** *Drosophila* Toll and Imd pathways. Schematic representation of the Toll and Imd pathways (for more information see ref. 4). The numbers in parentheses correspond to the peak of activation within the expression profile (fold change compared to uninfected flies). A, acute-phase gene; L, late-response gene; b, gene regulated by bacterial infection; b + f, gene regulated by both bacterial and fungal infection. Imd encodes a protein with homology to RIP (P. Georgel, personal communication).

enzymes (proPO-AE; Fig. 2). Interestingly, five genes of *Drosophila* up-regulated after infection encode serine proteases closely related to proPO-AE from *Manduca sexta* and *Holotrichia dimophalia* (Table 1). Unlike the putative *proPO-AE* genes, none of the three *Drosophila proPO* genes are induced after immune response. Several genes encoding key upstream enzymes of the melanization reaction (22) are induced after infection. *pale* encodes tyrosine hydroxylase, which catalyses the conversion of tyrosine into dopa, and *Ddc* encodes dopa decarboxylase that catalyses the conversion of dopa into dopamine, which is the substrate of PO. The genes encoding dihydropteridine reductase (*Dhpr*) and GTP cyclohydrolase (*Punch*), which are involved in the biosynthesis of tetrahydrobiopterin (BH-4), an essential cofactor for tyrosine hydroxylase, are also induced after infection (see Table 1 and Fig. 2). Three other induced genes that may be involved in the melanization are *yellow f*, which encodes a homologue of the dopachrome conversion enzyme of *Anopheles aegypti* (GenBank accession no. AF288384, unpublished); *CG3759*, which encodes a laccase-like protein; and the structural protein-encoding gene *chorion protein 19* (Table 1).

The coagulation process is well studied in other arthropods like *Limulus* and crayfish (20, 23), but remains uncharacterized in *Drosophila*. This process involves serine protease processing of a clottable protein typically through a cascade. Curiously, the fly genome does not encode homologues of *Limulus* or crayfish clotting proteins (20, 23), but encodes 10 fibrinogen-like proteins and several serine proteases that present significant homology with vertebrate coagulation factors. We found one fibrinogen-like encoding gene (*CG5550*) that is strongly induced after septic injury, representing an obvious candidate for the clottable protein in *Drosophila*. Another gene induced by septic injury, *Annexin IX*, encodes a protein with homology to annexin V, a vertebrate anticoagulant factor (Table 1).

**Signaling.** In *Drosophila*, *AMP* genes are regulated by the Toll and Imd signaling pathways. These pathways culminate in the activation of the NF-κB-like factors Dif, Dorsal, and Relish (ref. 4; Fig. 3). In agreement with previous studies, we observed that most of the genes encoding components of the Toll pathway are up-regulated, whereas in the Imd pathway, only *Relish* and *imd* are significantly induced after infection (Table 1 and Fig. 3). Other *Drosophila* genes encoding homologues of NF-κB regulators in vertebrates (dTRAFs, DmMyd88, and DmIKKε) do not respond to infection (data not shown). We also confirmed previous observations (24) that, unlike *Toll*, none of the eight *Drosophila* Toll-like genes are significantly induced by infection in adults (data not shown).

Genes encoding components of the JAK-STAT pathway, which regulates both *TEP* expression and hemocyte differentiation (3, 16), did not seem to be transcriptionally regulated after infection (data not shown). One gene encoding a member of the JNK signaling pathway, which has been implicated in the lipopolysaccharide response, *D-Jun* (25), is rapidly induced in response to septic injury (Table 1), although it is not in the set of DIRGs (see *Concluding Remarks*).

**Antimicrobial Peptides.** The expression patterns of *AMP* genes in response to septic injury and fungal natural infection in our present study corroborate with previous Northern analyses (5, 26). For example, the antibacterial peptide genes *Diptericin* and *Cecropin* are not induced during natural infection by *B. bassiana*, whereas the peptides that display antifungal activity like Drosomycin and Metchnikowin respond to both septic injury and natural fungal infection (ref. 5; Table 1). We also confirmed that one class of *AMP* genes, the *lysozyme* genes (27), are not induced by infection (data not shown). Interestingly, among the three *drosomycin*-like genes revealed by the genome sequence (28, 29), we found one (*CG10812*) that we called *Drosomycin B*, which is induced both by septic injury and fungal natural infection, in agreement with its putative antifungal activity. These findings bring the number of characterized *AMP* genes induced in response to septic injury to 15.

We observed 28 immune-inducible genes that are predicted to encode small polypeptides of between 40 and 134 residues, the size range of most known antimicrobial peptides (Table 1). Only one gene in this group, *IM-2*, was previously identified although its precise function is unknown (30). *Drosophila* also possesses two genes encoding homologues of IM-2, which display a similar pattern of expression and, like *IM-2*, are induced by both bacterial and fungal infection. All three *IM-2* genes are in the same genomic region (2R-55C9), which also includes *imd* and two other inducible peptide-encoding genes (*CG16836* and *CG15066*). Many of these small polypeptide genes (for example, *CG14027*, *CG6429*, *CG12965*, *CG15066*, *CG9080*, *CG16978*, and *CG18108*) are strongly induced like the *AMP* genes, suggesting they encode effector molecules and possibly new classes of antimicrobial peptides. Alternatively, induced small peptides could participate in signaling as cytokines. One of these genes (*CG14599*) is specifically induced after natural infection by *B. bassiana*.

**Other Genes with Putative Roles in Immune Response.** The gene-expression profiles allow us to identify several other genes encoding factors potentially involved in the *Drosophila* host defense (Table 1). The mechanisms that control the concentration of reactive oxygen species (ROS) in response to infection still remain unclear. The melanization reaction, as stated above, contributes to the production of ROS, but peroxidases and nitric oxide synthases are also thought to play an important role (31). We identified one gene (*CG8913*) of 11 annotated genes encoding proteins with peroxidase domains that responds to both septic injury and fungal natural infection and might mediate the cytotoxic activity of hydrogen peroxide.

Iron is essential for most invading microorganisms during the course of infection, and both animals and plants have elaborate immune strategies that limit iron availability (31, 32). We found two *Drosophila* transferrin genes (*CG6186* and *CG3666*) and one iron transporter gene (*CG6898*) that are induced by septic injury and natural infection by *B. bassiana*. One of the transferrin genes (*CG3666*) is induced more than 13-fold just 1.5 h after septic injury, suggesting that sequestration of extracellular iron represents an important acute defense mechanism in *Drosophila*.

Other groups of immune-inducible genes encode proteins with related functions. For instance, three genes encode lysosomal enzymes (DNaseII, cathepsin, and β-galactosidase) that might be involved in phagocytosis of invading microorganisms. Four other genes encode lipases (Table 1). One of these genes (*CG4757*) is strongly induced (35-fold by septic injury and 20-fold by fungal infection), suggesting that lipases might be directly involved in a still-uncharacterized defense mechanism.

**Repressed Genes.** We identified a high number of genes that are repressed after microbial infection (Fig. 1). Among them, we found genes encoding for several serine proteases and serine protease inhibitors (Table 1), four cuticle protein genes, eight genes belonging to the cytochrome P450 superfamily, two larval serum protein genes, two actin encoding genes, several small peptides, and enzymes involved in general metabolism (data not shown). Besides serine proteases and their inhibitors, it is difficult to postulate a specific role in the immune response. It is possible that an efficient response to infection, which involves the intense synthesis of a high number of genes, requires the repression of dispensable metabolic pathways. Repression mechanisms might play a particularly important role in the fat body where most of the acute-phase genes (including *AMP* genes) are expressed.

## Concluding Remarks

We used high-density oligonucleotide arrays to catalog the genes whose mRNA levels change during the *Drosophila* immune response. Our data show a very strong correlation with the published literature. We also identified many genes that were not previously known to be immune-responsive. We observed that microbial infection induces a dramatic change in gene expression in the adult fly. Among the large number of genes displaying changes in mRNA levels in response to infection, we have selected over 200 that were up-regulated and over 150 that were repressed. It should be noted that our selection criteria, coupled with the decision to limit the number of DIRGs to 400, have the consequence that some number of truly immune-responsive genes are absent from the list of DIRGs. We discussed four examples of such genes that were not included in the list of DIRGs but may play important roles in the immune response (i.e., D-Jun, *imd*, and *CG5550*), but there are surely more. Additional experiments, such as the determination of the expression profiles in response to infection of flies mutant in key immune-response regulators, the determination of the response to other types of infection, and more finely graded time courses, will help to identify new DIRGs and to further subcategorize the DIRGs presented here.

One surprising feature of the *Drosophila* genome was the high number of trypsin-like serine protease-encoding genes (206 compared with 7 in *Caenorhabditis elegans*; ref. 29). Aside from four serine proteases involved in dorsal/ventral patterning during embryogenesis, the function of this large class of proteins has remained perplexing. Our study indicates that many of the serine proteases are involved in the immune response, ultimately suggesting targets for future analysis.

We also found several genes encoding small peptides that are strongly induced by microbial infection. Some of them may represent new effector molecules with antimicrobial activity. The analysis of the function of these genes may help to design new tools to cope with infection.

Finally, we found a large number of genes of unknown function that may be involved in control and execution of the immune response (see Tables 3 and 4, which are published as supporting information on the PNAS web site). A significant number of these genes has homologues in other species, including human, and their role in the immune response is likely conserved through evolution. The comparison of this dataset with the results from similar studies conducted by using different organisms will further help to identify genes involved in innate immunity and to characterize their function, ultimately leading to a more complete understanding of the immune response.



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