Current Biology 17, 67–72, January 9, 2007 ©2007 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2006.11.026

Report

Psidin Is Required in *Drosophila* Blood Cells for Both Phagocytic Degradation and Immune Activation of the Fat Body

Catherine A. Brennan,[1,2,*] Joseph R. Delaney,[3] David S. Schneider,[1] and Kathryn V. Anderson[2]
[1] Department of Microbiology and Immunology
Stanford University School of Medicine
299 Campus Drive
Stanford, California 94305
[2] Department of Molecular Biology
Sloan-Kettering Institute
1275 York Avenue
New York, New York 10021
[3] Brookdale Department of Molecular, Cell, and Developmental Biology
The Mount Sinai School of Medicine
One Gustave Levy Place, Box 1020
New York, New York 10029

Summary

Phagocytic blood cells are critical to innate immune defense: They internalize and destroy microbial invaders and produce signals that trigger other immune responses [1, 2]. Despite this central role, the in vivo contributions of phagocytosis to systemic immune activation are not well understood. *Drosophila* has proven a fruitful model for the investigation of evolutionarily conserved innate immune mechanisms, including NF-κB-dependent transcriptional induction, RNAi in antiviral responses, and phagocytosis [3–5]. The phagocytes of *Drosophila* encounter bacterial invaders early in infection and contribute to survival of infection [6–9]. Phagocytosis in flies and mammals is highly homologous: Both rely on scavenger receptors, opsonins, and actin rearrangements for engulfment; have phagosomal cysteine proteases active at low pH; and can be subverted by similar intracellular pathogens [9–13]. Although the role of *Drosophila* phagocytes in the activation of other immune tissues has not been clear, we show that induction of the antibacterial-peptide gene *Defensin* in the fat body during infection requires blood-cell contributions. We identify a gene, *psidin*, that encodes a lysosomal protein required in the blood cells for both degradation of engulfed bacteria and activation of fat-body *Defensin*. These data establish a role for the phagocytic blood cells of *Drosophila* in detection of infection and activation of the humoral immune response.

Results and Discussion

psidin Mutants Do Not Induce *Defensin* after Septic Injury

Genetic screens in *Drosophila* to uncover mechanisms controlling the activation of antimicrobial peptide (AMP) genes such as *Diptericin* and *Drosomycin* in the fat body have led to the elucidation of the Imd and Toll pathways, both of which culminate in the activation of NF-κB-like transcription factors in the fat body [3]. Similarly, the first allele of *psidin*, *ird16*, was recovered in a screen for mutant larvae unable to induce *Diptericin* in response to injected *E. coli* [14]. The *Diptericin* induction failure in *ird16* mutants was somewhat variable, whereas we found a consistent, strong impairment in the induction of *Defensin*, which encodes another AMP (Figure 1A). This specific defect differed from that of Imd-pathway mutants, such as *ird5* (IKKβ), which fail to induce a wide spectrum of antibacterial peptides (Figure 1A). *ird16* mutants eventually accumulated melanotic masses in the hemolymph and died after a prolonged third larval instar. A second ethyl methanesulfonate (EMS)-induced allele showed the same spectrum of mutant phenotypes.

Based on meiotic recombination mapping and sequencing (Supplemental Experimental Procedures in the Supplemental Data available online), both *ird16* mutations were associated with stop codons in the *CG4845* gene, which we renamed *psidin* (*p*hagocyte *s*ignaling *i*mpaired) (Figure 1B). Because neither allele showed a stronger phenotype when in *trans* to a deficiency, we inferred that both were null alleles. The *psidin* open reading frame has single homologs in yeast, mammals, and *C. elegans* and shares 22% identity and 45% similarity with predicted human protein FLJ13089. Psidin is 7% identical and 22% similar to Mdm20, the accessory subunit of the NatB N-acetyltransferase (NAT) of *Saccharomyces cerevisiae*; the homology is distributed across the length of the protein. NatB acetylates the N-terminal methionine of a small number of polypeptides as they are synthesized; however, neither the general role of N-terminal acetylation nor the biochemical function of the Mdm20 subunit is known [15–17]. One substrate of NatB is tropomyosin, whose binding of actin is strengthened by acetylation; other substrates are less well characterized [16–18].

Among the *Drosophila* AMPs, Defensin has the strongest activity against Gram-positive bacteria [19]. To test whether the *Defensin*-specific impairment correlated with a reduced ability to mount a response to Gram-positive infection, we challenged larvae with different types of bacteria. However, we found that *psidin* mutants were equally deficient in the induction of *Defensin* whether the infection was with a Gram-negative or Gram-positive micro-organism (Figure 1C).

psidin Function Is Not Required in the Fat Body for the Induction of *Defensin*

Ubiquitous expression of a *UAS-CG4845* transgene by using the *hsp70-* or *daughterless-GAL4* driver (Figure 2A) rescued all the *psidin* phenotypes, including lethality. However, expression of *psidin* in the fat body by using the *c564-GAL4* driver [20] rescued neither *Defensin* induction nor lethality (Figure 2A). The fat body was confirmed as the predominant site of *Defensin* expression

*Correspondence: cbrennan@stanford.edu

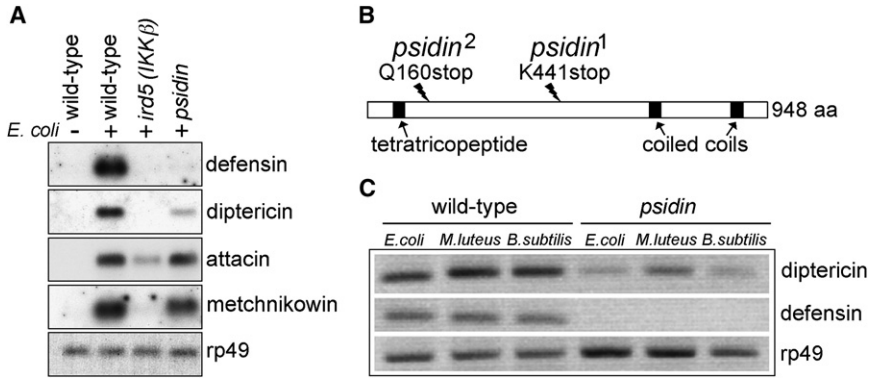

Figure 1. *psidin* Mutants Cannot Induce Defensin in Response to Infection

(A) Northern-blot analysis of AMP induction in wild-type, *ird5*^{F22}, and *psidin* mutant larvae [*psid*^{1}/Df(3R)H15], 3 hr after *E. coli* injection.

(B) The Psidin protein.

(C) RT-PCR-blot analysis of AMP induction in wild-type and *psidin* mutant larvae (psid^{1}/Df). Bacteria are *E. coli* (Gram neg) and *Micrococcus luteus* and *Bacillus subtilis* (Gram pos).

after immune challenge by comparing the levels of *Defensin* transcripts in whole larvae and dissected fat bodies (Figure 2B). *Defensin* was more than 20-fold enriched in fat-body RNA compared with whole larvae, which is consistent with the fat-body expression of a *Defensin*:*GFP* reporter construct [21]. Our results, therefore, suggest that *psidin* acts nonautonomously to control the induction of *Defensin* in the fat body.

psidin Function Is Required in the Blood Cells for the Induction of *Defensin*

We next tested whether *psidin* was required in the blood cells for the induction of *Defensin* during infection. Previous efforts to determine whether the blood cells play a role in the activation of AMP production in the fat body have relied on *domino* mutant larvae, in which proliferative tissues are disrupted and blood-cell numbers greatly reduced [7]. The activation of most AMPs, including *Diptericin*, in response to injected *E. coli* is normal in *domino* mutants, which suggested that Imd- and Toll-pathway activation are independent of blood-cell function [7]. However, *domino* mutants fail to induce *Diptericin* during a Gram-negative gut infection [22], suggesting that the blood cells could relay a signal from the gut to activate Imd signaling in the fat body.

Because the original analysis of the *domino* phenotype did not include *Defensin* [7], we re-examined the requirement for *domino* in the induction of AMP genes. We found that *domino* mutants, like *psidin* mutants, were specifically impaired in the induction of *Defensin* in response to septic injury (Figure 2C). These findings raised the possibility that *psidin* might act in the blood cells to promote *Defensin* induction in the fat body.

To test this hypothesis, we used two GAL4 drivers that are expressed in the blood cells. Collagen IV-GAL4 drives expression in both the fat body and the blood cells [23], and peroxidasin-GAL4 drives expression in the blood cells alone [24] (Figure S1A). Expression of UAS-*psidin* under the control of either CgIV-GAL4 or pxn-GAL4 was sufficient to rescue the inducibility of *Defensin* (Figure 2D). Although the *c564-GAL4* driver that failed to rescue the *Defensin* defect was originally reported to drive expression in both the fat body and the blood cells [20], we found that it does not drive detectable expression of GFP in the blood cells (data not shown). None of the tissue-specific promoters tested rescued the lethality of *psidin*, suggesting that expression in other tissues is necessary for successful entry into metamorphosis. These experiments demonstrate that the blood cells are required to activate systemic antimicrobial responses in the fat body in response to disseminated infection, and they identify a specific gene required for this function.

Defensin Induction Is Not Sensitive to Spätzle or Unpaired 3 Signaling

Mammalian phagocytes produce cytokines and other immunostimulatory signals upon exposure to microbes [1, 2]. *Drosophila* blood cells produce two cytokine-like proteins: Upd3, which activates JAK-STAT signaling, and Spätzle, a ligand for Toll [25, 26]. *spätzle* mutant larvae showed robust induction of *Defensin* (Figure 2E), and *totA* induction, which is dependent on Upd3 signaling [25], was normal in *psidin* mutants (Figure 2F). We therefore infer that neither Spätzle nor Upd3 is the blood-cell signal, and that an unknown signal produced by the blood cells in response to infection modulates the fat-body immune response. It will be interesting to determine whether this signal is a *Drosophila* cytokine or, alternatively, a bacterial product generated by phagocytic degradation.

Because *Diptericin* induction is variably impaired in *psidin* mutants (Figures 1A, 2A, and 2C), we infer that the blood-cell signal is not dedicated to *Defensin* induction. Studies of AMP regulation found that the *Defensin* promoter is unusual, and that both aberrant Rel-GATA binding-site spacing and low-affinity Rel binding sites contribute to low levels of expression of a reporter [27]. We propose that *Defensin*'s low level of expression relative to other AMPs renders it particularly sensitive to perturbations of immune signaling, including a blood-cell-dependent pathway that also activates other AMPs.

psidin Mutant Blood Cells Cannot Degrade Ingested Bacteria

We examined *psidin* mutant blood cells to learn how the gene might contribute to blood-cell signal production. Unlike *domino* mutants, which lack most blood cells [7],

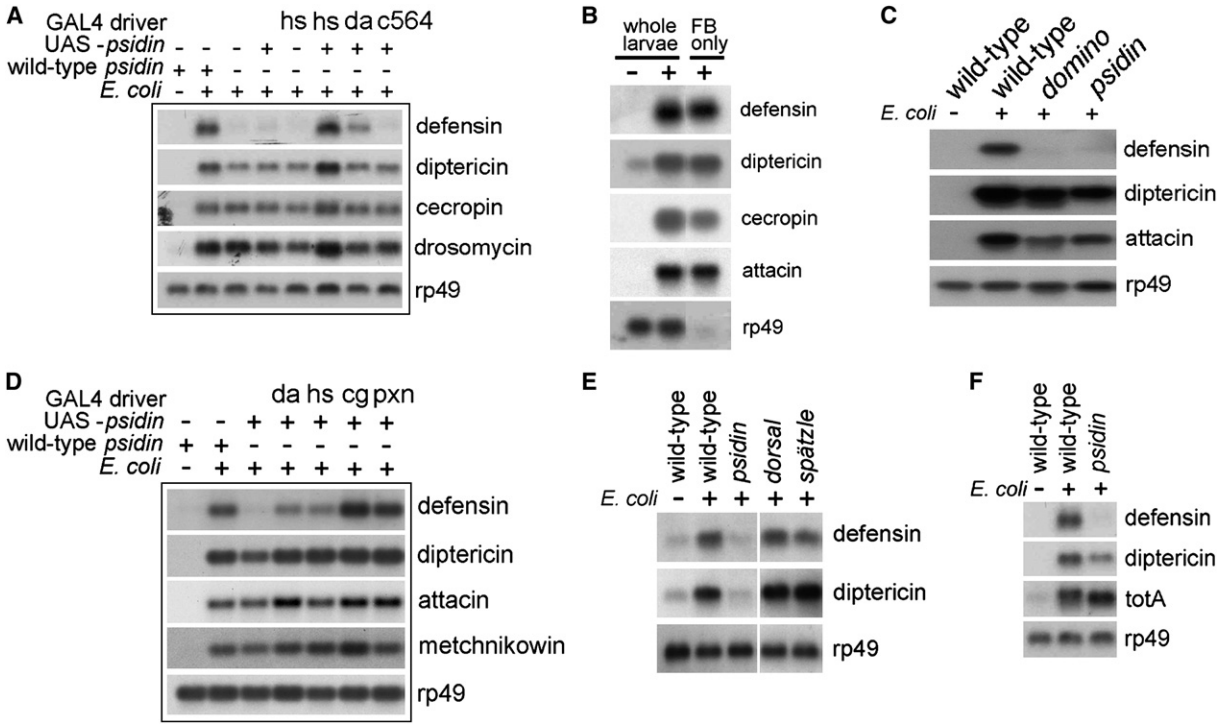

Figure 2. *Defensin* Induction in Larvae Requires a Blood-Cell Contribution

(A) Northern-blot analysis of AMP induction in wild-type and *psidin* mutant larvae (*psid*^{1}/Df) expressing a UAS-psidin rescue construct under the control of these GAL4 drivers: hs (heat shock; 37° for 2 × 1 hr, 48 hr, and 24 hr before infection), da (daughterless; ubiquitous), c564 (fat body, [20]).

(B) Northern-blot analysis comparing AMP induction in whole larvae and in dissected fat bodies. Ten micrograms of RNA was used in whole body lanes, 0.5 μg in the fat-body lane.

(C) Northern-blot analysis of AMP induction in wild-type, domino^{2}, and psidin^{1}/Df larvae.

(D) Northern-blot analysis of AMP induction in wild-type and *psidin* mutant larvae (*psid*^{1}/Df) expressing a UAS-psidin rescue construct under the control of these GAL4 drivers: da (daughterless; ubiquitous), hs (heat shock; 37° for 1 hr, 24 hr before infection), cg (collagen IV; fat-body and blood cells, [23]), and pxn (peroxidasin; blood cells, [24]).

(E) Northern-blot analysis of AMP induction. The genotypes are as follows: wild-type = Oregon R; *dorsal* = *dl*^{1}/Df(2L)J4; *psidin* = *psid*^{2}/*Df*; and *spätzle* = *spz*^{rm7}/Df(3R)AS637^{RX29}. A single blot is shown; some lanes have been deleted for clarity, as indicated with white line.

(F) Northern-blot analysis of AMP and *totA* induction in wild-type and *psid*^{2}/Df mutant larvae.

psidin mutants had normal blood-cell counts (data not shown). Ten to twenty percent of *psidin* blood cells were enlarged, but most were normal sized (Figure S1B). *psidin* mutants died after a prolonged third larval instar and developed melanotic masses two days after the normal time of pupariation. At this late time point, defects in the blood cells were detected, including necrotic and multinucleate cells. However, none of these defects was apparent at the early wandering stage when *Defensin* induction was assayed. Unlike *domino* mutants, *psidin* mutants melanized wound sites normally, suggesting that at least some blood-cell functions are intact [6] (Figure S1C).

Because phagocytosis is an important function of *Drosophila* blood cells, we tested the ability of *psidin* mutant cells to engulf fluorescein-conjugated, heat-killed *E. coli* and protect them from trypan blue, which quenches extracellular fluorescence. *psidin* blood cells were able to engulf these particles in a manner indistinguishable from that of the wild-type (WT) (Figure 3A). However, when we examined the fate of ingested GFP-expressing *E. coli*, we found that *psidin* mutant cells failed to digest them. In the first hour after injection of live *E. coli* into larvae, intracellular bacteria were seen in both WT and mutant blood cells. However, by 4 hr, whereas very few fluorescent bacteria remained in WT cells, *psidin* blood cells still contained many bacteria. At 6 hr, almost no bacteria were visible in WT cells, whereas bacteria in *psidin* mutant cells continued to persist, and even increase in number. In such cases, the bacteria remained clustered, suggesting they may have replicated within phagosomes (Figure 3B). We counted the number of green bacteria in WT and mutant blood cells 4 hr after infection. In 50 WT cells, only a single bacterium was visible. In contrast, 12 of 50 *psidin* mutant cells contained a single bacterium, and four had two or more intracellular *E. coli* (Figure 3C). Counts of colony-forming units (CFUs) showed that WT animals cleared an infection of 12,000 CFU within 5 hr, whereas *psidin* mutant animals had indistinguishable numbers of bacteria at 0 and 5 hr after infection (Figure 3D). The maintenance of stable numbers of bacteria in *psidin* mutants suggests that the bacterial replication observed within some blood cells may be balanced by some bacterial killing.

These blood-cell phenotypes raised the intriguing possibility that phagocytic degradation of microbes is required for the production of an immunostimulatory

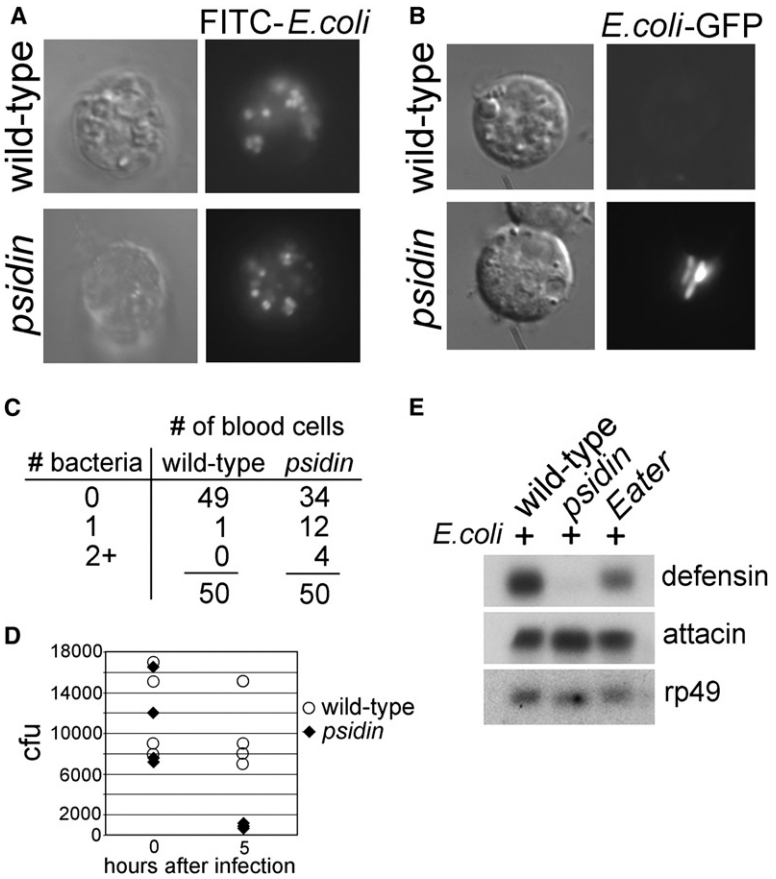

Figure 3. *Defensin* Induction Defects Are Associated with Phagocytosis Deficiencies

(A) Differential interference contrast (DIC) and fluorescence images of live blood cells 2 hr after injection of larvae with FITC-labeled *E. coli* Bioparticles. Blood cells suspended in trypan blue to quench extracellular fluorescence.

(B) DIC and fluorescence images of live blood cells 6 hr after injection of larvae with live, GFP-expressing *E. coli*.

(C) Quantitative comparison of the ability of wild-type and $psid^2$/Df blood cells to degrade GFP-expressing *E. coli*. Number of blood cells containing 0, 1, or 2 or more bacteria 4 hr after infection are indicated.

(D) CFUs in WT versus *psidin* at 0 and 5 hr after infection with *E. coli.* Each dot represents a single animal.

(E) Northern-blot analysis of AMP induction in *psidin* ($psid^2$/Df) and *Eater* [Df(3R)D605/Df(3R)TI-I] mutant larvae.

signal from the blood cells to the fat body to activate *Defensin*. To further test this hypothesis, we examined the ability of *Eater* mutants to induce *Defensin*. *Eater* encodes a transmembrane blood-cell receptor that binds bacteria and contributes to bacterial internalization [9]. *Eater* mutants showed normal induction of *Diptericin*, but induced *Defensin* to only 45% of WT levels (Figure 3E). This defect is not as pronounced as in *psidin* mutants, but is consistent with the finding that *Eater* mutants retain the ability to phagocytose Gram-negative bacteria at about 50% of WT levels [9]. The finding that two stages of phagocytosis, internalization and degradation, are both required for the blood cells to stimulate fat body *Defensin* induction indicates that phagocytosis plays a central role in activation of systemic immune responses in *Drosophila*.

Psidin Localizes to Blood-Cell Lysosomes

Immunostaining with antisera raised against an N-terminal fragment of Psidin showed a punctate distribution throughout the cytoplasm with an additional perinuclear localization in both larval blood cells and S2 cells, a macrophage-like cell line (Figure 4A). Specificity of the antibody was shown by increased staining when the *psidin* gene was overexpressed (Figure 4B) and reduced staining after RNAi gene silencing in S2 cells (Figure 4C). The perinuclear distribution is predicted for a homolog of Mdm20, which participates in the acetylation of proteins as they emerge from the ribosome [15–17]. The cytoplasmic Psidin-positive structures also were labeled with dArl8, a marker for lysosomes (Figure 4D) [28]. The lysosomal localization for a putative NAT subunit was unexpected and could indicate that Psidin has NatB-independent functions, or that N-terminal acetylation in metazoans is not exclusively cotranslational. In support of this latter possibility, San, the *Drosophila* homolog of another NAT subunit, also has a cytoplasmic distribution [29]. The lysosomal localization of Psidin does correlate with the defect in phagosomal degradation of ingested bacteria in *psidin* mutants. N-terminal acetylation of *Drosophila* Arl8 by the NatC complex is required for its lysosomal localization [28], and it is possible that Psidin acts in a NatB complex to localize other proteins essential for lysosome function.

In summary, we have shown that the *psidin* gene acts in blood cells, where it plays two roles in the larval immune response: It is required both for the phagocytic degradation of internalized bacteria and for the induction of *Defensin* in the fat body. Our inability to detect other defects in *psidin* mutant cells, coupled with the lysosomal localization of the Psidin protein as well as the impairment of *Defensin* induction in the phagocytic-internalization mutant *Eater*, leads us to favor the hypothesis that the phagocytic-degradation defect of *psidin* underlies the inability to activate the fat body. A model whereby phagosome maturation is required for activation of immunostimulatory signaling in *Drosophila* blood cells is consistent with the close relationship in mammals between endosome/phagosome function and immune activation. However, whereas the mechanisms underlying the processing and presentation of endosomal peptides and lipid antigens in adaptive immunity

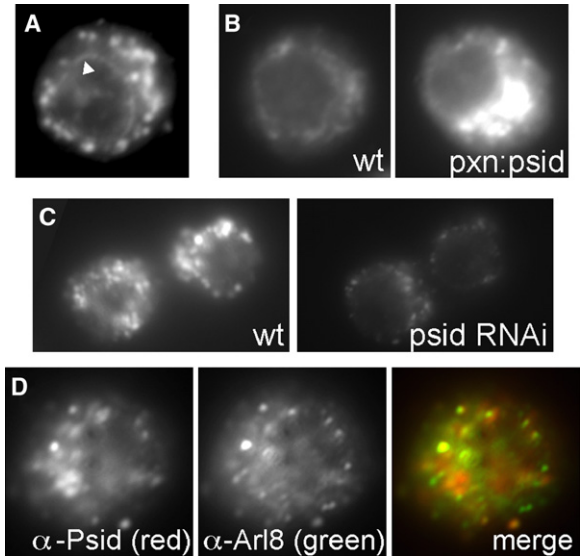

Figure 4. Psidin Is Expressed in Blood Cells in a Ring around the Nucleus and in Lysosomes

(A) Immunolocalization of Psidin protein in larval blood cells. Arrowhead indicates perinuclear staining.

(B) Immunostaining of Psidin protein in larval blood cells showing increased staining when *psidin* is overexpressed under the control of the peroxidasin GAL4 driver.

(C) Immunostaining of Psidin protein in S2 cells showing reduction of protein expression when *psidin* expression levels are knocked down by RNAi.

(D) Immunocolocalization of Psidin and dArl8 proteins.

have been studied extensively, the events linking phagosome maturation with initiation of innate immunity are still unclear. Future genetic studies on *Defensin* induction in *Drosophila* could be a useful system for the in vivo analysis of phagocytosis, including postengulfment steps, and its coupling to immunostimulatory mechanisms.

Supplemental Data

Supplemental Data include Experimental Procedures and one figure and are available with this article online at: http://www.current-biology.com/cgi/content/full/17/1/67/DC1/.

Acknowledgments

We wish to thank Louisa Wu and Kwang-Min Choe for isolation and preliminary mapping of the first *psidin* allele; Marie Meister for *domino* flies, Bill Pak for deficiency fly lines; Mike Galko for the peroxidasin:GAL4 flies; Angelike Stathopoulos for flies; Nina Matova and Michelle Arbeitman for help with experiments; Sean Munro for the dArl8 antibody; Marek Mlodzik for support and lab space; and Edan Foley, Ranjiv Khush, Bogdan Polevoda, Camille Diges, and members of the Anderson and Schneider laboratories for helpful suggestions. C.A.B. was the Keyser Family fellow supported by the Damon Runyon Cancer Research Foundation (DRG-3582). This work was also supported by National Institutes of Health grants AI45149 to K.V.A. and AI053080 to D.S.S.

Received: August 20, 2006
Revised: November 2, 2006
Accepted: November 3, 2006
Published: January 8, 2007

References

1. Underhill, D.M., and Ozinsky, A. (2002). Phagocytosis of microbes: Complexity in action. Annu. Rev. Immunol. *20*, 825–852.
2. Nathan, C. (2006). Neutrophils and immunity: Challenges and opportunities. Nat. Rev. Immunol. *6*, 173–182.
3. Brennan, C.A., and Anderson, K.V. (2004). Drosophila: The genetics of innate immune recognition and response. Annu. Rev. Immunol. *22*, 457–483.
4. Royet, J., Reichhart, J.M., and Hoffmann, J.A. (2005). Sensing and signaling during infection in Drosophila. Curr. Opin. Immunol. *17*, 11–17.
5. Wang, X.-H., Aliyari, R., Li, W.-X., Kim, K., Carthew, R., Atkinson, P., and Ding, S.W. (2006). RNAi interference directs innate immunity against viruses in adult Drosophila. Science *312*, 452–454.
6. Meister, M. (2004). Blood cells of Drosophila: Cell lineages and role in host defence. Curr. Opin. Immunol. *16*, 10–15.
7. Braun, A., Hoffmann, J.A., and Meister, M. (1998). Analysis of the Drosophila host defense in domino larvae, which are devoid of hemocytes. Proc. Natl. Acad. Sci. USA *95*, 14337–14342.
8. Elrod-Erickson, M., Mishra, S., and Schneider, D. (2000). Interactions between the cellular and humoral immune responses in Drosophila. Curr. Biol. *10*, 781–784.
9. 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 *123*, 335–346.
10. Rämet, M., Manfrueli, P., Pearson, A., Mathey-Prevot, B., and Ezekowitz, R.A. (2002). Functional genomic analysis of phagocytosis and identification of a Drosophila receptor for E. coli. Nature *416*, 644–648.
11. Kocks, C., Maehr, R., Overkleeft, H.S., Wang, E.W., Iyer, L.K., Lennon-Duménil, A., Ploegh, H.L., and Kessler, B.M. (2003). Functional proteomics of the active cysteine protease content in Drosophila S2 cells. Mol. Cell. Proteomics *2*, 1188–1197.
12. Stroschein-Stevenson, S., Foley, E., O'Farrell, P.H., and Johnson, A.D. (2006). Identification of Drosophila gene products required for phagocytosis of Candida albicans. PLoS Biol. *4*, e4.
13. Brandt, S.M., Dionne, M.S., Khush, R.S., Pham, L.N., Vigdal, T.J., and Schneider, D.S. (2004). Secreted bacterial effectors and host-produced Eiger/TNF drive death in a Salmonella-infected fruit fly. PLoS Biol. *2*, e418.
14. Wu, L.P., Choe, K.M., Lu, Y., and Anderson, K.V. (2001). Drosophila immunity: Genes on the third chromosome required for the response to bacterial infection. Genetics *159*, 189–199.
15. Polevoda, B., and Sherman, F. (2000). Nalpha-terminal acetylation of eukaryotic proteins. J. Biol. Chem. *275*, 36479–36482.
16. Polevoda, B., Cardillo, T.S., Doyle, T.C., Bedi, G.S., and Sherman, F. (2003). Nat3p and Mdm20p and required for function of yeast NatB Na-terminal acetyltransferase and of actin and tropomyosin. J. Biol. Chem. *278*, 30686–30697.
17. Singer, J.M., and Shaw, J.M. (2003). Mdm20 protein functions with Nat3 protein to acetylate Tpm1 protein and regulate tropomyosin-actin interactions in budding yeast. Proc. Natl. Acad. Sci. USA *100*, 7644–7649.
18. Caesar, R., Warringer, J., and Blomberg, A. (2006). Physiological importance and identification of novel targets for the N-terminal acetyltransferase NatB. Eukaryot. Cell *5*, 368–378.
19. Tzou, P., and Lemaitre, B. (2002). Constitutive expression of a single antimicrobial peptide can restore wild-type resistance to infection in immunodeficient Drosophila mutants. Proc. Natl. Acad. Sci. USA *99*, 2152–2157.
20. Harrison, D.A., Binari, R., Nahreini, T.S., Gilman, M., and Perrimon, N. (1995). Activation of a Drosophila Janus kinase (JAK) causes hematopoietic neoplasia and developmental defects. EMBO J. *14*, 2857–2865.
21. Tzou, P., Ohresser, S., Ferrandon, D., Capovilla, M., Reichhart, J.M., Lemaitre, B., Hoffmann, J.A., and Imler, J.L. (2000). Tissue-specific inducible expression of antimicrobial peptide genes in Drosophila surface epithelia. Immunity *13*, 737–748.
22. Basset, A., Khush, R.S., Braun, A., Gardan, L., Boccard, F., Hoffmann, J.A., and Lemaitre, B. (2000). The phytopathogenic bacteria Erwinia carotovora infects Drosophila and activates an immune response. Proc. Natl. Acad. Sci. USA *97*, 3376–3381.

23. Asha, H., Nagy, I., Kovacs, G., Stetson, D., Ando, I., and Dearolf, C.R. (2003). Analysis of Ras-induced overproliferation in Drosophila hemocytes. Genetics *163*, 203–215.
24. Stramer, B., Wood, W., Galko, M.J., Redd, M.J., Jacinto, A., Parkhurst, S.M., and Martin, P. (2005). Live imaging of wound inflammation in *Drosophila* embryos reveals key roles for small GTPases during in vivo cell migration. J. Cell Biol. *168*, 567–573.
25. Agaisse, H., Petersen, U.M., Boutros, M., Mathey-Prevot, B., and Perrimon, N. (2003). Signaling role of hemocytes in Drosophila JAK/STAT-dependent response to septic injury. Dev. Cell *5*, 441–450.
26. Irving, P., Ubeda, J.M., Doucet, D., Troxler, L., Lagueux, M., Zachary, D., Hoffmann, J.A., Hetru, C., and Meister, M. (2005). New insights into Drosophila larval haemocyte functions through genome-wide analysis. Cell. Microbiol. *7*, 335–350.
27. Senger, K., Armstrong, G.W., Rowell, W.J., Kwan, J.M., Markstein, M., and Levine, M. (2004). Immunity regulatory DNAs share common organizational features in Drosophila. Mol. Cell *13*, 19–32.
28. Hofmann, I., and Munro, S. (2006). An N-terminally acetylated Arf-like GTPase is localised to lysosomes and affects their motility. J. Cell Sci. *119*, 1494–1503.
29. Williams, B.C., Garrett-Engele, C.M., Li, Z., Williams, E.V., Rosenman, E.D., and Goldberg, M.L. (2003). Two putative acetyltransferases, San and Deco, are required for establishing sister chromatid adhesion in Drosophila. Curr. Biol. *13*, 2025–2036.

Supplemental Data

Psidin Is Required in *Drosophila* Blood Cells for Both Phagocytic Degradation and Immune Activation of the Fat Body

Catherine A. Brennan, Joseph R. Delaney, David S. Schneider, and Kathryn V. Anderson

Supplemental Experimental Procedures

Infection Protocols

Larvae were infected with *E. coli* by injecting 50 nl of an overnight culture into the body cavity. Infected larvae were grown for 3 hr at 25° before RNA extraction.

Isolating an Additional Allele of psidin

Five hundred males of genotype *ru h th st cu sr e ca* were fed 25 mM EMS and mated to virgins carrying the TM6C balancer. F1 males were mated singly to *psidin*^{1}/TM6 Tb virgins. Nine hundred and fifty lines were scored for melanotic masses and late larval lethality (absence of long pupae), and one allele was recovered.

Mapping and Cloning the psidin Mutation

The melanotic-mass phenotype of *psidin* larvae was used to map the gene by meiotic recombination between the visible markers *sr* and *e*, and then to the region deleted by Df(3R)*H15* (region 92B-D). Subsequent single-embryo PCR on mutants homozygous for deficiencies in the region with primers corresponding to DNA sequences in the region were used to define the endpoints of the deletions and to narrow the region of interest. The proximal limit was the distal endpoint of Df(3R)K9 (*psidin*$^{+}$) (near *hs6st*), and the distal limit was the distal breakpoint of Df(3R)*12* (*psidin*$^{-}$) (near *CG10887*). Twelve candidate genes in the interval were sequenced for both alleles, and only one sequence change was identified for each allele.

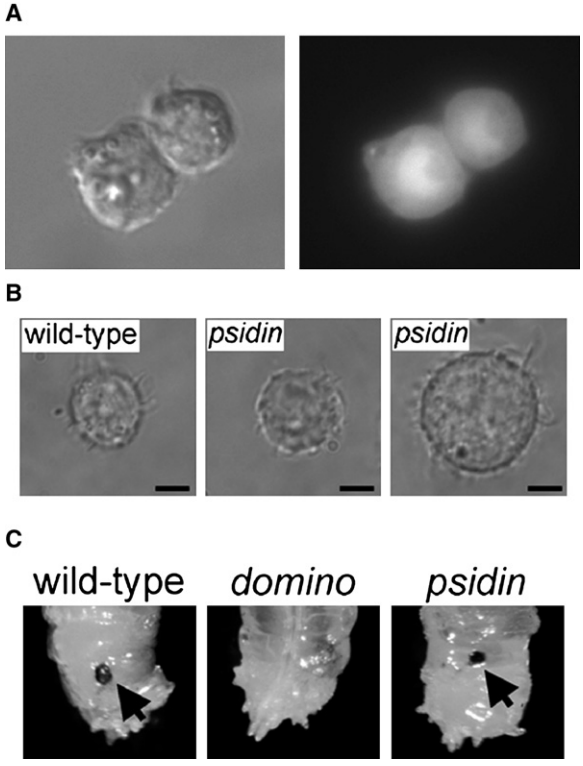

Figure S1. Blood Cells in *peroxidasin*-GAL4 and in *psidin* Larvae

(A) Brightfield and fluorescence microscopy images of blood cells from larva of genotype peroxidasin-GAL4/UAS-GFP. GFP is expressed in the blood cells and no other tissues.

(B) Live blood cells. Right-hand panel shows an oversized *psidin* (*psid*^{2}/Df) blood cell. Mutant cells up to twice the normal diameter were found. The scale bar represents 5 μm.

(C) Posteriors of third-instar larvae 30 min after septic injury. Arrows indicate melanization sites. The genotypes are *domino* (*dom*^{2}) and *psidin* (*psid*^{1}/Df).

Molecular Biology

A UAS-psidin rescue construct was generated as follows: A 3460 bp cDNA from EST GM28696 was excised from pOT2 in a partial XhoR1 digest and cloned into pUASt. Transgenic flies were obtained by using standard methods.

Northern-blot analysis was performed as follows: Total RNA was phenol-extracted from larvae. Three to ten micrograms were separated in 1.5% agarose MOPS [3-(N-morpholino)-propanesulfonic acid] formaldehyde gels and then transferred to Hybond N+ nylon membranes (Amersham Pharmacia Biotech). ^{32}P-labeled probes were made by random priming from DNA templates generated by PCR with the following primers: att, forward 5′-TGGGGCTGAT GCTCGTTT-3′ and reverse 5′-TCCACTTGTCCACTTGCT-3′; cec, forward 5′-CTCTCATTCTGGCCATCACC-3′ and reverse 5′-GGTC AACCTCGGGCAGTT-3′; def, forward 5′-TATCGCTTTTGCTCTGC TT-3′ and reverse 5′-CTTCTGGCGGCTATGCTG-3′; dipt, forward 5′-GAGATGCAGTTCACCATTG-3′ and reverse 5′-TTTCCAGCTCG GTTCTGAGT-3′; drom, forward 5′-GTACTTGTTCGCCCTCTTCG-3′ and reverse 5′-ATTTAGCATCCTTCGCACCA-3′; metch, forward 5′-TCTTGGAGCGATTTTTCTGG-3′ and reverse 5′TGTGTTAACGACA TCAGCAGTG-3′; totA, forward 5′-CTAGGTGCTTTGCACTGCTG-3′ and reverse 5′-GCCCTTCACACCTGGAGATA-3′; and rp49, forward 5′-CAGGCCCAAGATCGTGAA-3′ and reverse 5′-CAAATGTGTATTC CGACC-3′.

Hybridization was conducted at 68° in RapidHyb solution (Amersham Biosciences). Blots were stripped in boiling 0.1% SDS and then reprobed with rp49 as a loading control. Blots were exposed to film, and signals were quantified by measuring band densities with Scion Image analysis software. Northern-blot results shown are representative of multiple experiments.

Psidin antibodies

An N-terminal fusion protein of 6xHis and amino acids 1–147 of the *psidin* open reading frame was expressed in *E. coli* by using the *pET-28a* vector (Novagen). This was used as an antigen to generate rat antisera (Pocono Farms). For immunostaining, antisera were used at a 1:1000 dilution, with 2° goat anti-rat Alexa 594 (1:500) (Molecular Probes).

RNAi Experiments

A 460 bp PCR product amplified from the large 5th exon of *psidin*, and bearing the T7 promoter at each end, served as the template for dsRNA transcription and was generated with the primers 5′-TTAATACGACTCACTATAGGGAGACCTTCTATACGGCGCTCAAGC TGC-3′ and 5′-TTAATACGACTCACTATAGGGAGACGCAATGTAT TCCAGCCTTTCC-3′. RNA was synthesized with the Megascript kit (Ambion), annealed, and tested for size and yield by gel electrophoresis. Four micrograms of dsRNA was added to 50,000 S2 cells in 400 ml Schneider's media, and cells were cultured for 5 days at 25°.

Phagocytosis Experiments

For internalization assays, fluorescein-conjugated, heat-killed *E. coli* (Bioparticles, Molecular Probes E-2861) were washed and resuspended in a 2× volume of water. Fifty nanoliters was injected. After 2 hr, larvae were bled into trypan blue, which quenches the fluorescence of extracellular particles. For phagocytic-degradation assays, an overnight culture of GFP-expressing *E. coli* was diluted 5× in PBS. One hundred nanoliters (10,000–15,000 cfu) was injected. Following indicated incubation times, larvae were bled into Schneider's media, and blood cells were examined by light and fluorescence microscopy.