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Inducible Expression of Double-Stranded RNA Reveals a Role for dFADD in the Regulation of the Antibacterial Response in *Drosophila* Adults

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Summary

In *Drosophila*, the immune deficiency (Imd) pathway controls antibacterial peptide gene expression in the fat body in response to Gram-negative bacterial infection [1, 2]. The ultimate target of the Imd pathway is Relish, a transactivator related to mammalian P105 and P100 NF-κB precursors [3]. Relish is processed in order to translocate to the nucleus, and this cleavage is dependent on both Dredd, an apical caspase related to caspase-8 of mammals, and the fly Iκ-B kinase complex (dmIKK) [4–9]. dTAK1, a MAPKKK, functions upstream of the dmIKK complex and downstream of Imd, a protein with a death domain similar to that of mammalian receptor interacting protein (RIP) [10, 11]. Finally, the peptidoglycan recognition protein-LC (PGRP-LC) acts upstream of Imd and probably functions as a receptor for the Imd pathway [12–14]. Using inducible expression of *dFADD* double-stranded RNA, we demonstrate that dFADD is a novel component of the Imd pathway: *dFADD* double-stranded RNA expression reduces the induction of antibacterial peptide-encoding genes after infection and renders the fly susceptible to Gram-negative bacterial infection. Epistatic studies indicate that dFADD acts between Imd and Dredd. Our results reinforce the parallels between the Imd and the TNF-R1 pathways.

Results and Discussion

dFADD is a gene encoding a death domain protein with an overall structure similar to that of mammalian Fas-associated death domain-containing protein (FADD), an adaptor that is believed to interact with the TNF-R1 complex through homophilic death domain interactions with the TNF-R-associated death domain-containing protein (TRADD) [15]. FADD then recruits pro-caspase-8 through homophilic death effector domain associations (reviewed in [16]). Consequently, dFADD is an obvious candidate for linking the death domain protein Imd and the Dredd apical caspase in the Imd pathway. In this study, we have used the inducible expression of *dFADD* double-stranded RNA to determine if dFADD functions in the Imd pathway. This approach, which exploits the UAS/GAL4 binary system to drive expression of double-stranded RNA in a defined tissue is a form of RNA interference (RNAi) that has previously been shown to block the expression of defined genes [17–19].

We have generated transgenic flies carrying either *UAS-dTAK1-IR* or *UAS-dFADD-IR*. Both constructs consist of two 500 bp-long inverted repeats (IR) of the gene, separated by an unrelated DNA sequence that acts as a spacer, to give a hairpin-loop-shaped RNA. These transgenic flies were crossed to flies carrying various *GAL4* drivers in order to activate transcription of the hairpin-encoding transgene in the progeny. Three *GAL4* lines were used in this study: *daughterless-GAL4* (*da-GAL4*), which expresses GAL4 strongly and ubiquitously; *hs-GAL4*, which directs expression of GAL4 ubiquitously after heat shocks; and *yolk-GAL4*, which expresses the yeast transactivator in the fat body of female adults.

In mammals, double-stranded RNA is a potent activator of innate immune responses. Using transgenic flies carrying a *UAS-GFP-IR* construct, we have first checked that production of double-stranded RNA by itself does not affect the expression of the antimicrobial peptide genes after septic injury and the resistance to microbial infection in *Drosophila* adults (data not shown). As previously reported [11], Figures 1A and 1C show that *dTAK1*-deficient flies do not express the antibacterial peptide-encoding gene *Diptericin* upon immune challenge and are highly susceptible to infection by Gram-negative bacteria. A similar phenotype is generated by mutations affecting the other components of the Imd pathway [1, 2]. Interestingly, we now observe that the expression of *UAS-dTAK1-IR* induced by either the *hs-GAL4* or the *yolk-GAL4* drivers produces an immune deficiency phenotype similar to *dTAK1* mutants: *UAS-dTAK1-IR* flies fail to express antibacterial-encoding genes after infection and are highly susceptible to Gram-negative bacterial infection (Figures 1A and 1C; data not shown). Figure 1 shows, however, that the *UAS-dTAK1-IR* expression phenotype is weaker than the *dTAK1* null mutant phenotype, both in terms of survival and affect on AMP gene expression, suggesting that the inducible expression of RNAi mimics a partial loss-of-function mutation of the target gene. In agreement with what we observed in *dTAK1* null mutants [11], expression of *UAS-dTAK1-IR* using the ubiquitous driver *da-GAL4* does not lead to detectable developmental defects. This contrasts with the results obtained by expression of a dominant-negative construct of *dTAK1*, which lead to ectopic developmental defects [20, 21]. Taken together, our results demonstrate the suitability of the RNAi approach for functional studies of the antimicrobial response.

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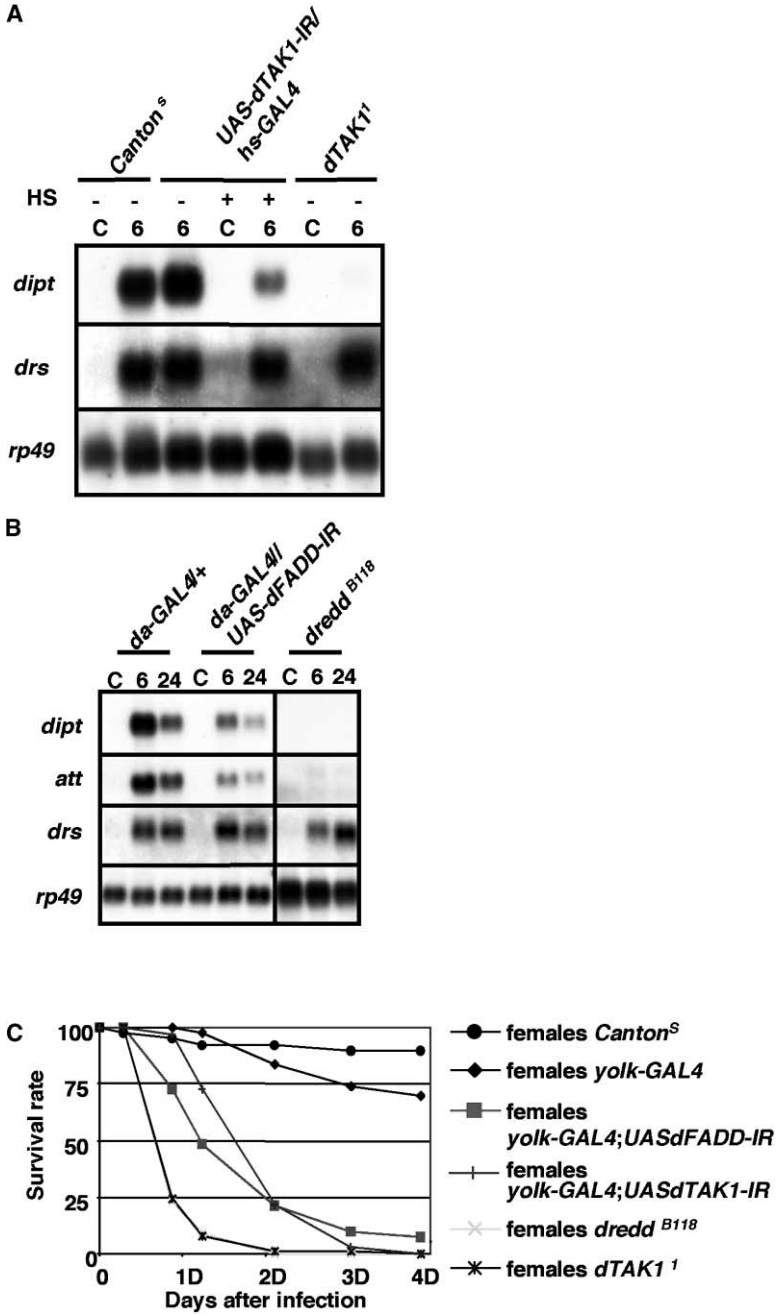

Figure 1. dFADD Mediates an Antibacterial Defense

(A) Inducible expression of *dTAK1-IR* mimics a strong mutation in *dTAK1*. Northern blot analysis shows that expression of *dTAK1-IR* with a *hs-GAL4* driver alters the expression of the antibacterial gene *Diptericin* (*dipt*), while the inducibility of the *Drosomycin* (*drs*) gene is unaffected. Similar results were obtained by using the *yolk-GAL4* driver (data not shown). The Northern blot was performed with total RNA extracted from wild-type (Canton^S), *dTAK1*^{1}, and *hs-GAL4/UAS-dTAK1-IR* adults infected with a mixture of Gram-negative (*Escherichia coli*) and Gram-positive (*Micrococcus luteus*) bacteria. Flies were incubated at 25°C and were collected at different time intervals after infection (indicated in hr). A plus indicates that the flies were incubated for 1 hr at 37°C before infection (HS, heat shock). The cDNA encoding the Ribosomal Protein 49 was used as a loading control (*rp49*). C: uninfected control flies. Total RNA extraction and Northern blotting experiments were performed as described in [6].

(B) dFADD regulates the expression of antibacterial peptide genes in adults. This Northern blot analysis shows that expression of *dFADD-IR* with a *da-GAL4* driver alters the expression of the antibacterial genes *Diptericin* (*dipt*) *and Attacin* (*att*) after septic injury, while the *Drosomycin* (*drs*) gene remains inducible. Quantitative measurements of the Northern blot experiment shows that, in *da-GAL4/UAS-dFADD-IR*, *Drosomycin* is induced to 85% of the wild-type level, *Diptericin* is induced to 20% of the wild-type level, and *Attacin* is induced to 35% of the wild-type level. Similar results were obtained by using *yolk-GAL4* and *hs-GAL4* (data not shown). The Northern blot was performed as in (A), with total RNA extracted from wild-type (*da-GAL4/+*), *Dredd* ^{B118}, and *da-GAL4/UAS-dFADD-IR* adults infected with a mixture of *E. coli* and *M. luteus*. Flies were incubated at 25°C and were collected at different time intervals after infection (indicated in hr). C: uninfected control flies.

(C) dFADD is required for resistance to Gram-negative bacterial infection. The survival rates of wild-type (Canton^S) (circle), *yolk-GAL4/+* (diamond), *yolk-GAL4; UAS-dTAK1-IR* (plus), *yolk-GAL4; UAS-dFADD-IR* (square), *dTAK1*^{1} (asterisk), and *Dredd*^{B118} ("x") flies after infection by *Erwinia carotovora carotovora 15* (*Ecc15*) are presented. A total of 80 adults, aged 2–4 days, were pricked with a needle previously dipped into *Ecc15*. The infected flies were incubated at 29°C and were transferred to fresh vials every 3 days. *dTAK1*^{1}, *Dredd*^{B118}, *yolk-GAL4; UAS-dTAK1-IR*, and *yolk-GAL4; UAS-dFADD-IR* adult flies are highly susceptible to *Ecc15* infection but are resistant to natural infection to *Beauveria bassiana* (data not shown). For more details on the infection procedure, see [27].

To address dFADD's role in the regulation of antimicrobial gene expression, we expressed the *UAS-dFADD-IR* transgene using the three GAL4 insertions. Flies that express *dFADD-IR* ubiquitously through *da-GAL4* show no detectable defects, suggesting that *dFADD* is not essential for development. These flies do, however, have phenotypes similar to those generated by mutations affecting the Imd pathway. The expression of antibacterial peptide genes *Diptericin* and *Attacin* are strongly reduced after septic injury, while the expression of the antifungal gene *Drosomycin* remains inducible (Figure 1B). In addition, these flies exhibit a high susceptibility to Gram-negative bacterial infection but resistance to fungal infection (Figure 1C, data not shown). This phenotype is identical to that generated by the *UAS-dTAK1-IR* construct and is similar, although slightly weaker, than those generated by null mutations in *dTAK1*, *kenny*, *ird5*, *Dredd*, *Relish*, and *imd* [3, 6,

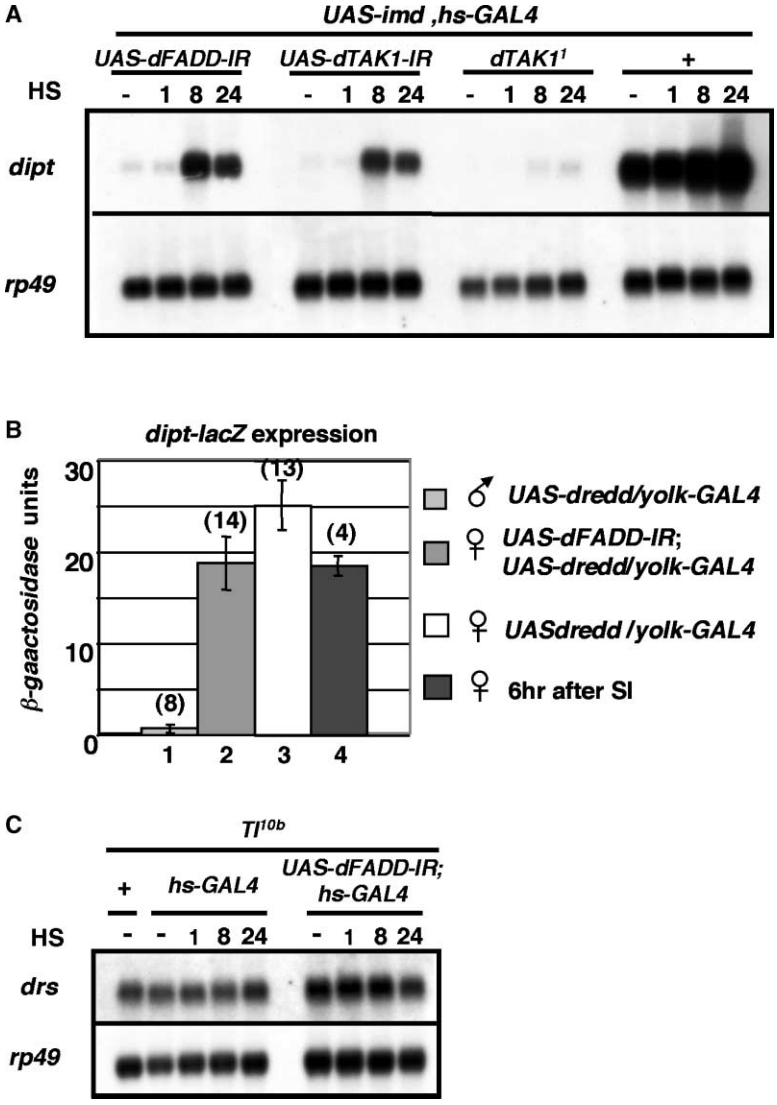

Figure 2. dFADD Is a Novel Component of the Imd Pathway

(A) dFADD and dTAK1 function downstream of Imd. Overexpression of the *UAS-imd* construct with a *hs-GAL4* or *yolk-GAL4* driver induces strong expression of the *Diptericin* gene in the absence of infection [10]. Although there is some constitutive *Diptericin* expression in these flies, the level of *Diptericin* increases after heat shock. This figure shows that the *Diptericin* expression induced by overexpressing *imd* is blocked by the $dTAK1^{1}$ mutation and is strongly reduced by coexpression of *UAS-dTAK1-IR* and *UAS-dFADD-IR*. Total RNA for Northern blot analysis was extracted from unchallenged adult flies collected without or at a different time interval (indicated in hr) after a 1-hr heat shock (37°C). Similar results were obtained using a *yolk-GAL4* driver (data not shown).

(B) dFADD functions upstream of Dredd. Overexpression of *UAS-Dredd* by the *yolk-GAL4* driver induced *Diptericin-lacZ* activity in the absence of challenge that is independent of the *dFADD* gene. All the tested flies carry one copy of the *Diptericin-lacZ* reporter gene on the X chromosome: (1) unchallenged *yolk-Gal4/UAS-Dredd* males, (2) unchallenged *UAS-dFADD-IR; yolk-GAL4/UAS-Dredd* females, (3) unchallenged *yolk-GAL4/UAS-Dredd* females, and (4) females collected 6 hr after bacterial challenge with *Ecc15* (SI, septic injury). Each column shows the level of *Diptericin-lacZ* expression monitored by *lacZ* titration [27]. Means for *Diptericin-lacZ* expression are shown with standard deviation, and the number of repeats is given in brackets. No expression is observed in males since the *yolk-GAL4* driver expresses GAL4 specifically in the female adult fat body.

(C) dFADD does not interfere with the Toll-mediated induction of *Drosomycin* expression. The high level of *Drosomycin* gene expression in uninfected Tl^{10b} mutant flies is not altered after expression of *UAS-dFADD-IR* with *hs-GAL4*. This demonstrates that *dFADD* is not required for the Tl^{10b}-driven constitutive expression of *Drosomycin* in adults. Total RNA for Northern blot analysis was extracted from unchallenged adult flies collected without or at a different time interval (indicated in hr) after a 1-hr heat shock (37°C).

8–11, 22]. These results demonstrate that, like the other components of the Imd pathway, dFADD is required for a full antibacterial response.

Overexpression of the *imd* gene leads to constitutive transcription of antibacterial peptide genes, and this induction requires the Dredd caspase [10]. Figure 2A shows that expression of both *dTAK1-IR* and *dFADD-IR* strongly reduces the Imd-mediated induction of antibacterial peptide-encoding genes, indicating that, genetically, dFADD and dTAK1 function downstream of Imd. We confirmed this result by demonstrating that the $dTAK1^{1}$ mutation also blocks the constitutive *Diptericin* expression induced by *imd* overexpression (Figure 2A). Overexpressing *Dredd* via the UAS/GAL4 system also leads to *Diptericin* expression in the absence of infection, which can be monitored with a *Diptericin-lacZ* transgene [11]. *lacZ* titration assays demonstrate that the *Diptericin* reporter gene expression induced by overexpressing the *UAS-Dredd* transgene is not affected by the coexpression of *dFADD-IR* (Figure 2B). Consequently, our epistatic studies place dFADD function upstream of the Dredd caspase. This result is in agreement with cell culture experiments showing that dFADD binds to Dredd through its N-terminal prodomain and promotes the proteolytic processing of Dredd [15].

Recent studies have shown that the *Drosophila* homolog of MyD88, dMyD88, is an essential component of the Toll pathway [23, 24]. In addition, dMyD88 has been shown to bind in vitro to dFADD, pointing to a possible interaction between dFADD and the Toll pathway [23]. Figure 2C shows, however, that expression of *dFADD-IR* does not block the constitutive *Drosomycin* expression induced by the dominant, gain-of-function $Toll^{10b}$ mutation (Figure 2C), and Figure 1B shows that *dFADD* RNAi does not block *Drosomycin* induction by infection. This result indicates that, like the other components of the Imd pathway, dFADD is not required for Toll pathway function.

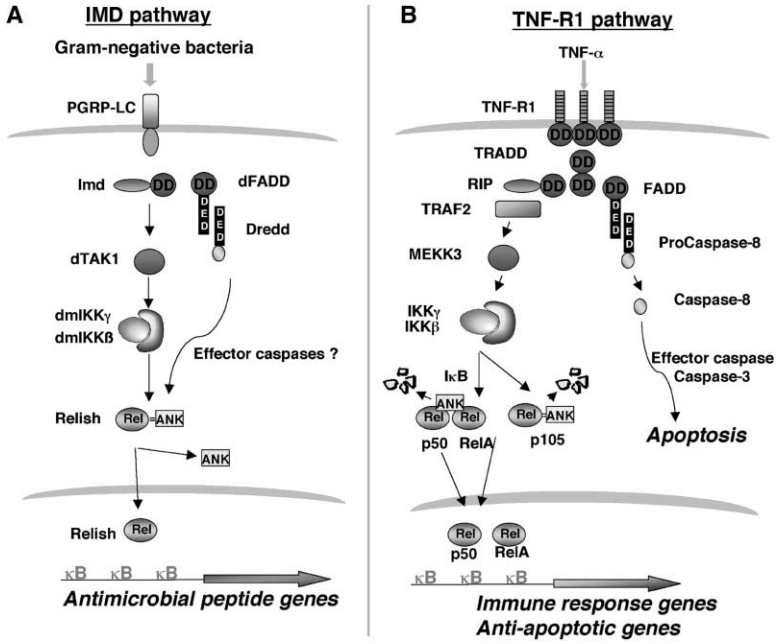

Figure 3. Parallels between the *Drosophila* Imd Pathway and the Mammalian TNF-R1 Pathway

(A and B) This model of the Imd pathway is based on independent epistatic studies showing that (i) dTAK1 functions upstream and Dredd functions downstream of the dmIKK complex [11], (ii) Imd functions upstream of dmIKK, Dredd, and dFADD [10] (this study), (iii) PGRP-LC functions upstream of Imd [13], and (iv) dFADD functions upstream of Dredd (this study). This model is supported by other studies that show that Relish activation requires dmIKK, Dredd, and PGRP-LC function [4, 7, 12], and the observation that dFADD binds to Dredd [15]. It cannot be definitively excluded that dFADD and Imd act at the same level in the cascade. Analogous to the TNF pathway, activated Dredd may cleave and activate an effector caspase that then cleaves Relish. Most of the components of the Imd pathway share similarities with cognate components of the TNF-R1 pathway: Imd, dFADD, Dredd, dmIKKβ, and dmIKKγ share homology with RIP, FADD, caspase-8, IKKβ, and IKKγ, respectively, and, finally, dTAK1 and MEKK3 are both MAPKKK.

Altogether, our analysis indicates that dFADD is a novel component of the Imd pathway that links Imd to Dredd. Biochemical studies show that dFADD contacts Dredd via homotypic dead effector domain interaction [15], and it is possible that dFADD interacts with Imd via its death domain. Consequently, dFADD, Dredd, and Imd may be components of a multiprotein adaptor complex functioning downstream of the receptor of the Imd pathway. Genetic studies suggest that the Imd pathway bifurcates downstream of Imd, with one branch leading to caspase activation via dFADD and the second branch leading to activation of the IKK complex via activation of dTAK1; both of these events are required for Relish processing (Figure 3).

Studies using loss-of-function mutations in the genes encoding components of the Imd pathway did not provide clear evidence for a role of this cascade in developmentally regulated apoptosis. Recently, it was, however, shown that the overexpression of *imd* with the *da-GAL4* driver in flies induces an early larval lethality that can be partially rescued by coexpression of the viral caspase inhibitor P35, suggesting that Imd can also promote apoptosis [10]. Interestingly, we noticed that the lethality induced by *imd* overexpression is totally suppressed in *Dredd* mutants but only marginally reduced in *dTAK1* mutants, suggesting that this effect is mediated through the dFADD/Dredd arm but not the dTAK1-dmIKK arm of the Imd pathway (see Table S1 in the Supplementary Material available with this article online).

In conclusion, the implication of dFADD in the *Drosophila* Imd pathway strengthens the parallels between the Imd and TNF-R1 pathways: both cascades regulate NF-κB via RIP-MAPKKK-IKK intermediates and promote caspase activation through the FADD adapter. In *Drosophila*, these two processes are required to activate Relish, while, in mammals, current models suggest that the TNF-R1 pathway leads to either NF-κB activation or programmed cell death activation [16] (Figure 3). Additional experiments are still required to demonstrate a clear role of the Imd pathway in the regulation of apoptosis. Finally, our study validates the use of the inducible expression of double-stranded RNA to address the in vivo function of genes that mediate the *Drosophila* antimicrobial response.

Experimental Procedures

RNAi transgenic fly lines of *dTAK1*, *dFADD*, and *GFP* were obtained using the inducible RNAi method ([17, 18]; unpublished data). A 500 bp-long cDNA fragment (nucleotide position 1–500 of the coding sequence) was amplified by PCR and was inserted as an inverted repeat (IR) into a modified Bluescript vector, pSC1, which possesses an IR formation site consisting of paired CpoI and SfiI restriction sites. In all cases, IRs were constructed in a head-to-head orientation. IR-containing fragments were cut out by NotI and were subcloned into pUAST, a transformation vector [25]. Detailed cloning procedures will be described elsewhere (R.U. and K.S., unpublished data). Transformation of *Drosophila* embryos was carried out in the w^{1118} fly stock. Each experiment was repeated using at least two independent *UAS-RNAi* insertions for each construct tested. The *GAL4* drivers have been described previously [10, 11, 26]. In this study, we used fly adults carrying one copy of the *UAS-RNAi* construct combined with one copy of the *GAL4* driver.

Supplementary Material

Supplementary Material including Table S1, which shows that the lethality induced by Imd overexpression is totally suppressed in *Dredd* mutants but only weakly affected in *dTAK1* mutants, is available at http://images.cellpress.com/supmat/supmatin.htm.

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References

1. Khush, R.S., Leulier, F., and Lemaitre, B. (2001). *Drosophila* immunity: two paths to NF-kappaB. Trends Immunol. *22*, 260–264.
2. Hoffmann, J.A., and Reichhart, J.M. (2002). *Drosophila* innate immunity: an evolutionary perspective. Nat. Immunol. *3*, 121–126.
3. Hedengren, M., Asling, B., Dushay, M.S., Ando, I., Ekengren, S., Wihlborg, M., and Hultmark, D. (1999). Relish, a central factor in the control of humoral but not cellular immunity in *Drosophila*. Mol. Cell *4*, 827–837.
4. Stöven, S., Ando, I., Kadalayil, L., Engström, Y., and Hultmark, D. (2000). Activation of the *Drosophila* NF-κB factor Relish by rapid endoproteolytic cleavage. EMBO Rep. *1*, 347–352.
5. Elrod-Erickson, M., Mishra, S., and Schneider, D. (2000). Interactions between the cellular and humoral immune responses in *Drosophila*. Curr. Biol. *10*, 781–784.
6. Leulier, F., Rodriguez, A., Khush, R.S., Abrams, J.M., and Lemaitre, B. (2000). The *Drosophila* caspase Dredd is required to resist gram-negative bacterial infection. EMBO Rep. *1*, 353–358.
7. Silverman, N., Zhou, J., Stöven, S., Pandey, N., Hultmark, D., and Maniatis, T. (2000). A *Drosophila* IkB kinase complex required for Relish cleavage and antibacterial immunity. Genes Dev. *14*, 2461–2471.
8. Rutschmann, S., Jung, A.C., Zhou, R., Silverman, N., Hoffmann, J.A., and Ferrandon, D. (2000). Role of *Drosophila* IKKγ in a Toll-independent antibacterial immune response. Nat. Immunol. *1*, 342–347.
9. Lu, Y., Wu, L.P., and Anderson, K.V. (2001). The antibacterial arm of the *Drosophila* innate immune response requires an IkappaB kinase. Genes Dev. *15*, 104–110.
10. Georgel, P., Naitza, S., Kappler, C., Ferrandon, D., Zachary, D., Swimmer, C., Kopczynski, C., Duyk, G., Reichhart, J.M., and Hoffmann, J.A. (2001). *Drosophila* immune deficiency (IMD) is a death domain protein that activates antibacterial defense and can promote apoptosis. Dev. Cell *1*, 503–514.
11. Vidal, S., Khush, R.S., Leulier, F., Tzou, P., Nakamura, M., and Lemaitre, B. (2001). Mutations in the *Drosophila* dTAK1 gene reveal a conserved function for MAPKKKs in the control of rel/NF-kappaB-dependent innate immune responses. Genes Dev. *15*, 1900–1912.
12. Choe, K.M., Werner, T., Stoven, S., Hultmark, D., and Anderson, K.V. (2002). Requirement for a peptidoglycan recognition protein (PGRP) in Relish activation and antibacterial immune responses in *Drosophila*. Science *296*, 359–362.
13. Gottar, M., Gobert, V., Michel, T., Belvin, M., Duyk, G., Hoffmann, J.A., Ferrandon, D., and Royet, J. (2002). The *Drosophila* immune response against Gram-negative bacteria is mediated by a peptidoglycan recognition protein. Nature *416*, 640–644.
14. Ramet, M., Manfruelli, 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.
15. Hu, S., and Yang, X. (2000). dFADD, a novel death domain-containing adapter protein for the *Drosophila* caspase DREDD. J. Biol. Chem. *275*, 30761–30764.
16. Baud, V., and Karin, M. (2001). Signal transduction by tumor necrosis factor and its relatives. Trends Cell Biol. *11*, 372–377.
17. Fortier, E., and Belote, J.M. (2000). Temperature-dependent gene silencing by an expressed inverted repeat in *Drosophila*. Genesis *26*, 240–244.
18. Kennerdell, J.R., and Carthew, R.W. (2000). Heritable gene silencing in *Drosophila* using double-stranded RNA. Nat. Biotechnol. *18*, 896–898.
19. Piccin, A., Salameh, A., Benna, C., Sandrelli, F., Mazzotta, G., Zordan, M., Rosato, E., Kyriacou, C.P., and Costa, R. (2001). Efficient and heritable functional knock-out of an adult phenotype in *Drosophila* using a GAL4-driven hairpin RNA incorporating a heterologous spacer. Nucleic Acids Res. *29*, E55.
20. Takatsu, Y., Nakamura, M., Stapleton, M., Danos, M.C., Matsumoto, K., O'Connor, M.B., Shibuya, H., and Ueno, N. (2000). TAK1 participates in c-Jun N-terminal kinase signaling during *Drosophila* development. Mol. Cell. Biol. *20*, 3015–3026.
21. Mihaly, J., Kockel, L., Gaengel, K., Weber, U., Bohmann, D., and Mlodzik, M. (2001). The role of the *Drosophila* TAK homologue dTAK during development. Mech. Dev. *102*, 67–79.
22. Lemaitre, B., Kromer-Metzger, E., Michaut, L., Nicolas, E., Meister, M., Georgel, P., Reichhart, J., and Hoffmann, J. (1995). A recessive mutation, immune deficiency (imd), defines two distinct control pathways in the *Drosophila* host defense. Proc. Natl. Acad. Sci. USA *92*, 9365–9469.
23. Horng, T., and Medzhitov, R. (2001). *Drosophila* MyD88 is an adapter in the Toll signaling pathway. Proc. Natl. Acad. Sci. USA *98*, 12654–12658.
24. Tauszig-Delamasure, S., Bilak, H., Capovilla, M., Hoffmann, J.A., and Imler, J.L. (2002). *Drosophila* MyD88 is required for the response to fungal and Gram-positive bacterial infections. Nat. Immunol. *3*, 91–97.
25. Brand, A.H., and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development *118*, 401–415.
26. Giebel, B., Stuttem, I., Hinz, U., and Campos-Ortega, J.A. (1997). Lethal of scute requires overexpression of daughterless to elicit ectopic neuronal development during embryogenesis in *Drosophila*. Mech. Dev. *63*, 75–87.
27. Tzou, P., Meister, M., and Lemaitre, B. (2002). Methods for studying infection and immunity in *Drosophila*. Methods Microbiol. *31*, 507–529.