Immune Activation of NF-κB and JNK Requires Drosophila TAK1*

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Stimulation of the Drosophila immune response activates NF-κB and JNK signaling pathways. For example, infection by Gram-negative bacteria induces the Imd signaling pathway, leading to the activation of the NF-κB-like transcription factor Relish and the expression of antimicrobial peptides. Relish is present in the cytoplasm as a full-length precursor protein and, upon infection, is endoproteolytically cleaved. The N-terminal Rel homology domain of Relish then translocates into the nucleus and activates antimicrobial gene expression, whereas the C-terminal IκB module remains in the cytoplasm. Signal-induced cleavage and activation of Relish requires a signaling pathway known as the Imd pathway, which includes the receptor PGRP-LC (peptidoglycan recognition protein LC), the intracellular signaling component IMD (a receptor interacting protein (RIP)-like death domain protein), TAK1 (a MAP3K), the Drosophila IKK complex (IKKβ/iridor5 and IKKγ/kenny), as well as the caspase Dredd and the adaptor known as the Fox-associated death domain (FADD) (6–16). The detailed biochemical mechanisms required for this intracellular signaling pathway are not understood.

Innate immune signaling pathways are highly conserved between insects and mammals (1–4). In both cases, pathogens are recognized through the detection of conserved molecules such as microbial cell wall components, which are shared by broad groups of microbes. For example, lipopolysaccharides (LPS) from Gram-negative bacteria are potent inducers of both insect and mammalian innate immunity. LPS and other microbial substances are recognized by germ line-encoded receptors such as Toll-like receptors (TLR) or peptidoglycan recognition proteins. Activation of these receptors stimulates conserved signaling pathways leading to the expression of antimicrobial proteins and immune stimulatory cytokines. For example, LPS treatment leads to the activation of NF-κB and c-Jun N-terminal kinase (JNK) signaling pathways in both insects and mammals (1). In mammals, the NF-κB pathway is required for the induction of cytokines in response to infection. Likewise, in the fly, the NF-κB homolog Relish is required for the induction of antimicrobial peptides in response to Gram-negative bacterial infection, and relish mutants are hypersusceptible to Gram-negative bacterial infections.

Relish is a bipartite protein with an NF-κB (Rel homology) domain and an inhibitory IκB domain. In unstimulated cells, Relish is present in the cytoplasm as a full-length precursor protein and, upon infection, is endoproteolytically cleaved. The N-terminal Rel homology domain of Relish then translocates into the nucleus and activates antimicrobial gene expression, whereas the C-terminal IκB module remains in the cytoplasm. Signal-induced cleavage and activation of Relish requires a signaling pathway known as the Imd pathway, which includes the receptor PGRP-LC (peptidoglycan recognition protein LC), the intracellular signaling component IMD (a receptor interacting protein (RIP)-like death domain protein), TAK1 (a MAP3K), the Drosophila IKK complex (IKKβ/iridor5 and IKKγ/kenny), as well as the caspase Dredd and the adaptor known as the Fox-associated death domain (FADD) (6–16). The detailed biochemical mechanisms required for this intracellular signaling pathway are not understood.

Another conserved immune signaling system is the JNK pathway. Activation of JNK (and other MAPK) signaling pathways in innate immune signaling is well documented in vertebrates. Three MAPK signaling pathways (JNK, ERK, and p38) are stimulated by LPS treatment (17). Pharmacological inhibition of the ERK and/or p38 pathways in monocyte cell lines inhibits the induction of cytokines such as IL-1, IL-8, and tumor necrosis factor α (TNFα) (17, 18). Also, in mouse embryonic fibroblasts JNK2 is required for induction of type I interferons in response to viral infection and for the induction of IL-12 and IL-6 in response to LPS (19). Although all the members of the JNK pathway are conserved in the fly, and Drosophila JNK signaling is activated in response to LPS stimulation (20), the role of this pathway in the insect immune responses has not been clearly delineated.

By contrast, the role of the JNK signaling pathway in Drosophila development has been extensively studied. The best understood role of JNK signaling is in dorsal closure during embryonic development (for review, see Ref. 21). The dorsal
Drosophila immunity, Tak1 activates JNK and NF-κB

Closure JNK pathway kinase cascade relies on the mixed line-
age kinase (MLK)-type MAP3K slipper (slipr), which is thought to function as a JNKK kinase (22), activating the JNK kinase hemipterous (hep), which, in turn, activates the JNK basket (bsk) (22). Bsk, in turn, phosphorylates and activates the trans-
cription factor d-Jun.

Dorsal closure is mechanistically similar to wound healing. Both processes involve pulling an epithelial sheet across an opening and sealing the hole. Recently, it was proposed that JNK signaling may be required for wound healing in the fly (23). Consistent with this idea, Perrimon and co-workers recently reported that the targets of JNK signaling during im-

mune activation include many cytoskeletal components (24) similar to those reported to be JNK-inducible genes during dorsal closure (25). Although this is a reasonable hypothesis, it has not yet been demonstrated that inhibition of JNK signaling, for example by inactivating bsk or hep, prevents proper wound repair. In addition to its potential role in wound healing, Drosophila JNK signaling has also been implicated in the activation of stress-protective proteins (25). Also, earlier re-
ports have suggested that AP-1-like transcription factors, which are the ultimate targets of the JNK signaling pathway, might regulate antimicrobial peptide gene expression (26). Thus, JNK may act at many different levels in the Drosophila immune response, but few mechanistic details have emerged.

In mammals, the MAP3K TAK1 plays a central role in the IL-1R/Toll-like receptor signaling pathways. In vitro biochemical studies showed that TAK1 could be activated directly by tumor necrosis factor receptor-associated factor 6 (TRAF6), which is known to be a critical component of this signaling pathway. Furthermore, these in vitro studies demonstrated that, once activated, TAK1 can activate both the IκB complex (and NF-κB signaling) as well as the JNKK MKK6 (and JNK signaling) (27). Recent studies in mammalian cell culture have demonstrated that TAK1 is required for NF-κB activation fol-

lowing tumor necrosis factor α or IL-1 stimulation (28). Simi-
larly, the Drosophila TAK1 is required for the Imd signaling pathway; Drosophila TAK1−/− flies have a severely compro-
mised immune response and do not express antimicrobial pep-
tides upon infection with Gram-negative bacteria (16). Also, gene expression profiling experiments have implied that Dro-
sophila TAK1 is responsible for activation of both JNK and NF-κB (Relish) signaling following immune stimulation (24). However, these studies provide no biochemical data supporting this hypothesis.

In this report we use Drosophila S2r+ cells to show that TAK1 is required for both LPS-induced JNK and IκB activation and for antimicrobial peptide gene induction. Surprisingly, the im-

mune activation of the JNK pathway is not required for anti-
microbial peptide gene induction. However, several other JNK-dep-

endent, LPS-inducible genes were identified in microarray studies, suggesting a role for JNK signaling in the cellular immune response as well as protection from stress.

EXPERIMENTAL PROCEDURES

S2r+ Cell Culture and Kinase Assays—S2r+ cells were grown as de-
scribed previously (10). Immune stimulation was with 10 μg/ml LPS from Escherichia coli 055:B5 (Sigma) for 3–5 h. Prior to LPS stimula-
tion, cells were differentiated with 1 μM 20-hydroxy-ecdysone (Sigma) for at least 24 h. RNA and protein extracts were prepared as described previously (10). IκB immunoprecipitation kinase assays were per-
fomed as described previously, except that 100 ng of recombinant baculovirus-produced Relish was used. For baculovirus construction, FLAG Relish from pSH-Relish (29) was cloned into the shuttle vector pBac-Hygro (30). JNK signaling activity was monitored with a c-Jun shift assay, as described previously (31).

Microarray Construction, Experimentation, and Analysis—cDNAs were amplified by PCR, with universal primers, from a collection of ~5900 plasmids from the Berkeley Drosophila Genome Project’s Dro-
sophila Gene Collection, version 1. This collection contains one re-

presentative DNA from every gene identified by the Berkeley Drosophila Genome Project’s expressed sequence tag (EST) project as of the year 2000. All products were analyzed by EtBr-stained agarose gel electro-
phoresis, and their product sizes were compared with the expected size determined as the Berkeley Drosophila Genome Project. A small number of amplification products were also analyzed by sequencing in order to verify the identity of the correct product. Tissue cDNAs were amplified by PCR, with universal primers, from a collection of ~15% of the samples. These problem samples

containing the probes, as described previously (31).

Flag Relish from pSH-Relish (29) was cloned into the shuttle vector Baculovirus-produced Relish was used. For baculovirus construction, the Berkeley Drosophila Genome Project (32).

S2r+ cell line (33). When these cells are treated with ecdysone, they differentiate and become responsive to LPS treatment, which leads to high levels of antimicrobial peptide gene expres-
sion (10). An additional advantage of this cell line is that RNAi can be used to target any gene of interest. Similar to the TAK1 mutant fly, targeting TAK1 with RNAi in S2r+ blocks LPS-

mediated activation of antibacterial peptide gene expression, similar to that observed with DmIκKα-RNAi (Fig. 1A). TAK1 is

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Drosophila immunity, Tak1 activates JNK and NF-κB

**Fig. 1. TAK1 is required for IKK activation and antimicrobial gene induction in Drosophila S2* cells.** A, RNAi-mediated targeting of TAK1 prevents the LPS-induced activation of antimicrobial peptide gene expression. RNAi-mediated inhibition of two other MAP3Ks, Slpr and Drosophila MEKK4 (dMEKK4), does not affect LPS-induced antimicrobial peptide gene expression. TAK1 RNAi was as effective as DmIKKγ RNAi in blocking the immune response, as monitored by Northern blotting with Diptericin, Cecropin, Attacin, or rp49 (as a loading control) probes. B, TAK1 is required for the LPS-stimulated activation of the IKK complex. Immunoprecipitation kinase assays were performed with anti-DmIKKγ antibody and recombinant Relish protein as a substrate. Inhibition of either DmIKKγ or TAK1 by RNAi prevents LPS-induced activation of the Drosophila IKK complex.

unique among the *Drosophila* MAP3Ks tested because it is required for LPS signaling, whereas *slpr* and *Drosophila MEKK4* are not.

In mammals, TAK1 has been shown to play a critical role in IL-1-induced NF-κB activation (27, 28). In *vivo*, TAK1 can directly phosphorylate and activate the human IKK complex. Thus, TAK1 has been proposed to function as the IKK activating kinase (IKK-K) in this signaling pathway (27). The immune-compromised phenotype of TAK1 mutant flies and S2* cells suggest that TAK1 may function as the IKK-K in the insect immune response. To determine whether TAK1 function is required for IKK activation, an immunoprecipitation kinase assay was utilized. The endogenous *Drosophila* IKK complex was immunoprecipitated with anti-DmIKKγ antisera from cell lysates prepared from LPS-treated or untreated cells. This immunoprecipitate was then tested for kinase activity *in vitro* using recombinant Relish as substrate. As demonstrated previously (10), LPS treatment leads to a significant increase in *Drosophila* IKK activity. Treatment of cells with TAK1 RNAi inhibits the LPS-induced IKK activation as much as targeting DmIKKγ itself (Fig. 1B). We conclude that TAK1 is required for LPS-induced activation of the *Drosophila* IKK complex and the subsequent expression of antimicrobial gene expression.

**TAK1 Is Required for JNK Activation**—In mammals, TAK1 has also been proposed to function as the JNKK kinase in the IL-1 and LPS signaling pathways (27). Therefore we sought to determine whether *Drosophila* TAK1 is also required for the activation of the JNK signaling pathway. JNK signaling was assayed by monitoring the phosphorylation-induced shift in c-Jun gel mobility following LPS stimulation. In this assay, *in vitro* translated 35S-labeled c-Jun was incubated with extract from untreated or LPS-treated cells. JNK activity is observed in the extracts from LPS-treated cells by the slower migration of c-Jun. RNAi targeting of TAK1 or the *Drosophila* JNKK or JNK (Hep and Bsk, respectively) prevents JNK activation. On the other hand, DmIKKγ and the MAP3Ks Slpr and MEKK4 are not involved in JNK activation.

extract from LPS-treated or untreated cells. The LPS-treated extract contains active JNK as evidenced by the decrease in mobility of the c-Jun protein after incubation with the extract from LPS-treated cells. A mutant c-Jun protein, with the JNK target serines (Ser-63 and Ser-73) mutated, is not phosphorylated in this assay (data not shown). This observation is consistent with a previous study showing that JNK activity was induced in response to LPS stimulation of S2* cells (20). RNAi was used to inhibit the function of *Drosophila* IKKγ, TAK1, and, as controls, the *Drosophila* homologs of JNKK and JNK, *hep* and *bsk*. As expected, both Hep and Bsk RNAi inhibited LPS-induced JNK activity as demonstrated by a lack of a c-Jun protein, whereas IKKγ and LacZ RNAi had no effect on JNK activation. TAK1 RNAi also inhibited LPS-induced JNK activity (Fig. 2). This demonstrates that TAK1, but not the *Drosophila* IKK complex, is required for LPS-induced JNK activation. Thus, it is likely that TAK1 functions as the JNKK kinase by phosphorylating and activating the *Drosophila* JNKK Hep.

**JNK Pathway Targets**—Previous studies have shown that LPS treatment activates JNK signaling (20) and that some antimicrobial genes have potential AP-1-like elements in their promoter/enhancer regions (26). However, it is not clear whether JNK signaling is involved in immune-induced antimicrobial gene expression in *Drosophila*. This is due, in part, to the fact that null mutants in the previously characterized JNK components, *hep* and *bsk*, are embryonic lethal and therefore do not survive to the age when the immune response can be readily measured. Therefore, RNAi was used to inhibit LPS-inducible JNK activation in S2* cells by targeting TAK1, Hep, and Bsk. The level of LPS-induced antimicrobial gene induction was then determined by Northern blotting (Fig. 3). Although TAK1 is required for induction of the antimicrobial genes, neither Hep nor Bsk appear to play a significant role in induction of *attacin*, *cecropin*, and *diptericin*. Note that the RNA analyzed in this experiment is from the same cells used in the JNK assay shown in Fig. 2, which demonstrated that TAK1, Hep, and Bsk are all required for JNK activation. Thus, TAK1, in its role as the IKK-K, is responsible for the activation of antimicrobial production, whereas the JNK pathway is not involved.

However, LPS-inducible JNK activation is likely to control the expression of other immune inducible genes. We used gene expression profiling to identify genes that are induced by LPS in a JNK-dependent manner. For these experiments, microarrays containing ~5900 unique full-length *Drosophila* cDNAs were used. In a series of 27 hybridizations (encompassing four independent experiments), we analyzed expression profiles of wild type cells (e.g. untransfected cells or cells transfected with LacZ dsRNA) and mutant cells (e.g. transfected with JNK and/or IKK dsRNAs) before and after immune stimulation. Eleven competitive hybridizations were performed comparing
cDNA prepared from the same cell line (i.e. wild type or mutant cells) before or after LPS stimulation. An additional sixteen hybridizations were performed comparing wild type LPS-stimulated cells to mutant LPS-stimulated cells. (See Supplemental Fig. 1 in the online version of this article for a diagram of this hybridization scheme.) To analyze this data set, which contains 10 conditions (or nodes), we used a newly developed tool for BAGEL (34). BAGEL uses Bayesian analysis and Markov chain Monte Carlo integration to generate estimates of the relative expression level of each gene in all conditions. This analysis also generates a 95% confidence interval for the expression level of each gene in each condition and a probability score (the Bayesian posterior probability) showing the likelihood that the expression level (of any gene) in each condition is greater or lesser than the expression level in any other condition. For our analysis, we considered the expression level of a gene to be significantly greater in one condition compared with another only if the Bayesian posterior probability was at least 0.95 (95%), which corresponds to a classical one-tailed p value of 0.05 or less.

The results of the BAGEL analysis were further parsed to select for genes whose expression profiles were of particular interest. For example, to identify LPS-inducible, JNK-specific genes, we selected those genes that are significantly induced by LPS in wild type and IKK RNAi-treated cells but are expressed at significantly lower levels in LPS-stimulated TAK, Bsk, or Hep RNAi-treated cells. This approach yielded a list of 25 genes, which were then sorted by their fold induction. To focus on those genes that are most strongly affected by LPS stimulation, we listed only those 15 genes which are induced at least 1.75-fold by LPS treatment (see Table I, panel A). Likewise, we identified 19 genes that are dependent on the IKK pathway (down-regulated in IKK and TAK1 mutant cells but not in Hep or Bsk mutant cells), 11 of which are induced at least 1.75-fold (Table I, panel B). Finally, we identified 15 genes (10 of which are induced >1.75-fold; Table I, panel C) that show a dependence on both IKK and JNK pathways. If a gene requires input from both pathways for maximal activation, it might be expected that blocking either pathway would reduce expression levels to intermediate levels, whereas blocking both pathways would have a more dramatic effect. In the data presented here, this would manifest by intermediate expression levels when IKK or JNK pathways are blocked (IKK, or Bsk or Hep RNAi, respectively) and very low expression levels when both pathways are blocked (TAK1 RNAi). This prediction is true for Mtol and CG9312, suggesting that maximal LPS induction of these genes requires input from both signaling pathways simultaneously.

To validate the results from these microarray studies, real time RT-PCR was used to quantify transcript levels of selected JNK-dependent genes. Fig. 4A shows that Punch is clearly an LPS-inducible gene in Drosophila S2* cells. LPS increases the expression level ~10-fold. Moreover, inhibition of the JNK signal inhibits activation of Punch. Likewise, real time RT-PCR verifies that sulfated is also an LPS-inducible gene that requires the JNK, but not the IKK, signaling pathway (Fig. 4B). Spatzle is also activated, weakly, in a JNK-depend-ent manner by LPS, suggesting that JNK signaling is involved in linking the Imd and Toll pathways (data not shown). Interestingly, the IKK pathway may have a small effect on Punch activation but not upon sulfated regulation. This is observed in the microarray data, where the level of Punch is reduced mildly in IKK mutant cells and in the real time analysis. The sulfated gene expression, however, does not require the IKK pathway.

**DISCUSSION**

The Drosophila TAK1 protein plays a critical role in the activation of the insect immune response. Genetic studies revealed that TAK1 mutant flies are unable to respond to Gram-negative infections and suggested that TAK1 functions upstream of the Drosophila IKK complex (16). Consistent with these results, we show that TAK1 is required for activation of the LPS-induced immune signaling pathways in Drosophila cells in culture. In addition, we show that TAK1 is required for activation of the Drosophila IKK complex in vitro. Thus, Drosophila TAK1 is likely to function as the IKK-K in the LPS signaling pathway, as has been proposed for human TAK1 (27).

JNK signaling is also activated during the immune response in both flies and humans. However, the exact mechanism by which LPS leads to JNK activation in Drosophila is unclear, as is the role of JNK signaling during the immune response. Boutros et al. (24) used gene expression profiling to infer that TAK1 is required for the activation of JNK signaling and that JNK signaling is important for wound healing. Here, we directly demonstrate that JNK activation requires TAK1. Thus, TAK1 appears to function both as a JNKK activating kinase and an IKK activating kinase, as proposed for mammalian TAK1 (27). Furthermore, our microarray results suggest that JNK signaling may have important functions in cellular immunity and the stress response.

The gene expression profiling data presented here identifies a relatively small number of genes that specifically require the JNK signaling pathway for their LPS-induced expression. The expression of two genes (Punch and sulfated) identified in these experiments has been validated by real time RT-PCR. Punch is an immune inducible gene in cells in culture, as shown here, and in adult flies as shown by De Gregorio et al. (35). However, these authors found that, in adult flies, the immune induction of Punch requires Relish, whereas the data presented here demonstrate that Punch induction in S2* cells requires JNK pathway components (hep, bsk, and TAK1) but not the Relish-activating kinase IKK. The experiments presented here were performed in an embryonic Drosophila cell line (that has macrophage-like qualities), whereas the data from De Gregorio et al. was generated from entire adult flies. Thus, it is possible that the signaling pathways required for Punch induction vary depending on the developmental stage and cell type examined. In fact, Punch has at least two promoters that direct developmentally specific expression (36).

*Punch* encodes the enzyme for GTP cyclohydrolase I, which is the first enzyme (and rate-determining step) in the formation of the cofactor tetrahydrobiopterin (BH4). This cofactor is required for the conversion of tyrosine to dopamine (37), which has at least two possible roles in immunity. First, dopamine is one of two main Drosophila catecholamines, which are important for the stress response in both insects and mammals (38). Second, dopamine is the precursor of melanin, which is pro-
duced during wound healing and encapsulation processes in the fly. In fact, it has been proposed that increased Punch activity could lead to increased melanization (35).

The cofactor BH4 is also an essential cofactor for nitric oxide synthase (NOS). NO itself has at least two possible roles in the immune response. First, NO is known to be a major microbicidal compound in mammalian phagocytic cells and is likely to function similarly in Drosophila macrophages (39). Second, NO has also been implicated in immune signaling in Drosophila. Foley and O'Farrell recently reported that NO is required for transmitting a signal from the site of infection to the fat body, the major organ of immune responsive gene expression (40). Thus, Punch may contribute to the insect immune response in several ways, including protection against stress, melanization of wound sites, and activation of cellular and humoral immunity.

The potential role of sulfated in the immune response is less obvious. sulfated encodes an extracellular sulfatase that removes sulfate groups from heparin sulfate proteoglycans (HSPGs) (41). In avian and Drosophila systems, it is thought that sulfated activity is crucial for the regulation of Wnt signaling, possibly by controlling the extracellular milieu in which the Wnt ligand travels (41, 42).

One of the most intriguing targets of both the JNK and IKK pathways is Mvl, the Drosophila NRAMP-1 homolog. Mvl mutants were first identified in the fly because they display gustatory behavioral defects caused by the inability to properly process sensory neuronal input (43). Mvl is expressed in both
the nervous system and circulating hemocytes. In the mouse, NRAMP-1 is expressed in macrophages, and mutations in the NRAMP-1 gene are responsible for the sensitivity of some inbred mice strains to the *Mycobacterium bovis* bacille Calmette-Guérin (BCG) and other intracellular bacterial pathogens. NRAMP-1 is thought to control the levels of cations, possibly Fe²⁺ or Mn²⁺, in lysosomal compartments of mouse macrophages. A current model suggests that NRAMP-1 pumps cations out of the phagolysosome, thereby starving microbes of cations required by the enzymes (superoxide dismutase and catalase), which protect the bacteria from reactive oxygen intermediate (ROI)- and reactive nitrogen intermediate (RNI)-induced damage (44). In the fly, the role of Mvl in immunity is not yet characterized, but its induction during an immune response coupled with the activity of this protein in vertebrate macrophages suggests that it may play an important role in the cellular immune response.

A recent microarray study provided evidence that LPS-induced JNK activation is important for the stimulation of a gene expression program similar to that seen during dorsal closure. Thus, JNK may be important for wound healing (24). We have detected the expression of only a few of the JNK target genes reported in this study (for example, *Filamin*). Instead, we identified a number of genes implicated in cell-cell interactions, stress responses, and macrophage activation. The differences between these studies could be the consequence of one or more of the following reasons. First, the experiments of Boutros et al. targeted two MAP2Ks (Hep and dMKK4) by RNAi (24). As shown in the biochemical data presented here, *Drosophila* JNKK (*hep*) is responsible for LPS-induced phosphorylation of...
c-Jun. Thus, it is likely that in the experiments of Boutros et al. (24) additional MAPK pathways were inhibited. Second, we used edcsyone-treated S2* cells instead of undifferentiated S2 cells. Ecdysone treatment causes significant changes in the S2* cells, including altered morphology and adherence, cell cycle arrest, and, most importantly, a greater level of LPS-induced antimicrobial peptide gene expression (data not shown and Ref. 45). Also, in our experiments we used printed cDNA arrays that include only ~40% of the predicted genes in the fly, whereas Boutros et al. used oligo-based arrays that include features for all ~14,000 predicted genes (24). Therefore, our data argue that JNK signaling is required for the activation of cellular immunity and stress protection, whereas a connection to wound healing cannot be excluded by our data.

Earlier studies suggested that certain antimicrobial genes (e.g. diptericin) require a combination of transcription factors for their proper induction. It was suggested, based on DNA footprinting and DNA sequence analysis, that Diptericin activation requires a kB binding site (now believed to be the site of Relish binding) as well as putative NF-IL6-like, and interferon regulatory factor (IRF)-like binding sites (26). However, the last decade of research has identified only Relish as being required for the immune inducible expression of diptericin. The data presented here show that the JNK signaling pathway and the AP-1-like factors activated by Drosophila JNK signaling are not involved in antimicrobial peptide gene induction in phagocytes. This would be quite different from immune activation of many mammalian cytokine genes, which require the coordination of several signaling pathways and the activity of several transcription factors for full immune induction. For example, IFN-γ induction requires the activation of three independent signaling cascades and the cooperative binding of three transcription factors, NF-κB, c-Jun/activating transcription factor 2 (ATF-2), and the interferon regulatory factor, to the enhancer region. Together, these transcription factors form a higher order complex known as the enhanceosome (46). Control of the insect antimicrobial genes may not require this complex enhanceosome architecture.

These studies clearly demonstrate that activation of the innate immune response in Drosophila leads to the activation of JNK and NF-κB signaling pathways through a branched signal transduction cascade. The MAP3K TAK1 lies at the branch point of this cascade and likely functions as the JNKK activating kinase and the IKK activating kinase. These signaling pathways are highly conserved. TAK1 also serves similar functions in mammalian innate immune signaling. Furthermore, we have identified novel immune-induced targets of the JNK pathway, which may function in cellular immunity and stress protection.

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REFERENCES

1. Silverman, N., and Maniatis, T. (2001) Genes Dev. 15, 2321–2342
2. Hoffmann, J. A., Kaufatos, F. C., Janeway, C. A., and Ezekowitz, R. A. (1999) Science 284, 1313–1318
3. Hoffmann, J. A., and Reichhart, J. M. (2002) Nat. Immunol. 3, 121–126
4. Tzou, P., De Gregorio, E., and Lemaître, B. (2002) Curr. Opin. Microbiol. 5, 102–110
5. Stéven, S., Ando, I., Kadalayil, L., Engstrom, Y., and Hultmark, D. (2000) EMBO J. 19, 347–352
6. Ramet, M., Manfruelli, P., Pearson, A., Clauder-Munster, S., Ansorge, W., and Bohmann, D. (2001) Dev. Cell 1, 503–514
7. Silverman, N., Zhou, R., Stéven, S., Pandey, N., Hultmark, D., and Maniatis, T. (2000) Genes Dev. 14, 2461–2471
8. Lu, Y., Wu, L. P., and Anderson, K. V. (2001) Genes Dev. 15, 104–110
9. Burchmann, S., Jung, A. C., Zhou, R., Silverman, N., Hoffmann, J. A., and Ferrandon, D. (2000) Nat. Immunol. 1, 342–347
10. Leulier, F., Rodríguez, A., Khusn, R. S., Abrams, J. M., and Lemaître, B. (2000) EMBO Rep. 1, 353–358
11. Leulier, F., Vidal, S., Saigo, K., Ueda, R., and Lemaître, B. (2002) Curr. Biol. 12, 996–1000
12. Naitza, S. Rosse, C., Kappler, C., Georgel, F., Belvin, M., Gubb, D., Camonis, J., Hoffmann, J. A., and Reichhart, J. M. (2002) Immunity 17, 575–581
13. Vidal, S., Khus, R. S., Leulier, F., Tsou, P., Nakamura, M., and Lemaître, B. (2001) Genes Dev. 15, 1900–1912
14. Guba, M., and Mackman, N. (2001) Cell. Signal. 13, 85–94
15. Zhu, W., Downey, S. J., Gu, D., Di Padova, F., Gram, H., and Han, J. (2000) J. Immunol. 164, 6349–6358
16. Chu, W. M., Ostar, D., Li, Z. W., Chang, L., Chen, Y., Hu, Y., Williams, B., Perrault, J., and Karin, M. (1999) Immunity 11, 721–731
17. Sluss, H. K., Han, Z., Barrett, T., Davis, R. J., and Ip, Y. T. (1996) Genes Dev. 10, 2745–2758
18. Stronsach, B. E., and Perrimon, N. (1999) Oncogene 18, 6172–6182
19. Stronsach, B., and Perrimon, N. (2002) Genes Dev. 16, 377–387
20. Ramet, M., Lanot, R., Zachary, D., and Manfruelli, P. (2002) Dev. Biol. 241, 145–156
21. Boutros, M., Agaisse, H., and Perrimon, N. (2002) Dev. Cell 3, 711–722
22. Jasper, H., Benes, V., Schwager, C., Sauer, C., Auge, A., and Bohmann, D. (2001) Dev. Cell 1, 579–586
23. Meister, M., Braun, A., Kappler, C., Reichhart, J. M., and Hoffmann, J. A. (1994) EMBO J. 13, 5958–5966
24. Wang, C., Dong, L., Hong, M., Akkaraju, G. R., Inoue, J., and Chen, Z. J. (2001) Nature 412, 346–351
25. Takaesu, G., Surabhi, R. M., Park, J. K., Ninomiya-Tsujii, J., Matsumoto, K., and Geymonat, R. B. (2003) J. Mol. Biol. 328, 105–115
26. Han, Z. Z., and Ip, Y. T. (1999) J. Biol. Chem. 274, 23155–23161
27. Gravelle, B. R., Hertel, K. J., and Maniatis, T. (1998) EMBO J. 17, 6674–6756
28. Lee, F. S., Hagler, J., Chen, Z. J., and Maniatis, T. (1997) Cell 88, 213–222
29. DeNesi, J. L., Iyer, V. R., and Brown, P. O. (1997) Science 278, 680–686
30. Samakovlis, C., Åslöng, B., Román, G. H., Gateff, E., and Hultmark, D. (1992) Biochem. Biophys. Res. Commun. 188, 1169–1175
31. Townsend, J. P., and Hartl, D. L. (2002) Genome Biology http://genomebiology.com/2002/3/12/research/0071
32. De Gregorio, E., Spellman, P. T., Rubin, G. M., and Lemaître, B. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 12590–12595
33. McLean, J. R., Krishnakumar, S., and O’Dunnell, J. M. (1993) J. Biol. Chem. 268, 27191–27197
34. Krishnakumar, S., Burton, D., Rascio, J., Chen, X., and O’Dunnell, J. (2000) J. Neurogenet. 14, 1–23
35. Stathakis, D. B., Burton, D., McVror, W. E., Krishnakumar, S., Wright, T. R., and O’Dunnell, J. M. (1999) Genetics 153, 361–382
36. Bogdan, C. (2001) Nat. Immunol. 2, 907–916
37. Foley, E., and O’Farrell, P. H. (2003) Genes Dev. 17, 115–125
38. Dhoet, G. K., Gustafsson, M. K., Ai, X., Sun, W., Standiford, D. M., and Emerson, C. P., Jr. (2001) Science 293, 1663–1666
39. Blair, S. S. (2001) Science’s STKE http://stke.sciencemag.org/cgi/content/full/sigtrans;2001/101/pe32
40. Rodríguez, V., Cheah, P. Y., Ray, K., and Chia, W. (1995) EMBO J. 14, 3007–3020
41. Dhoet, G. K., Gustafson, M. K., Ai, X., Sun, W., Standiford, D. M., and Emerson, C. P., Jr. (2001) Science 293, 1663–1666
