Drosophila Thor participates in host immune defense and connects a translational regulator with innate immunity

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Thor **has been identified as a new type of gene involved in *Drosophila* host immune defense. *Thor* is a member of the *4E-binding protein* (*4E-BP*) family, which in mammals has been defined as critical regulators in a pathway that controls initiation of translation through binding eukaryotic initiation factor 4E (eIF4E). Without an infection, *Thor* is expressed during all developmental stages and transcripts localize to a wide variety of tissues, including the reproductive system. In response to bacterial infection and, to a lesser extent, by wounding, *Thor* is up-regulated. The *Thor* promoter has the canonical NFκB and associated GATA recognition sequences that have been shown to be essential for immune induction, as well as other sequences commonly found for *Drosophila* immune response genes, including interferon-related regulatory sequences. In survival tests, *Thor* mutants show symptoms of being immune compromised, indicating that *Thor* may be critical in host defense. In contrast to *Thor*, *Drosophila eIF4E* is not induced by bacterial infection. These findings for *Thor* provide the first evidence that a *4E-BP* family member has a role in immune induction in any organism. Further, no gene in the translation initiation pathway that includes 4E-BP has been previously found to be immune induced. Our results suggest either a role for translational regulation in humoral immunity or a new, nontranslational function for *4E-BP* type genes.**

Analysis of immunity in *Drosophila* and other insects has increasingly shown similarities with other organisms and revealed that innate immunity is an ancient defense mechanism (for review, see refs. 1–4). The insect immune response has cellular and humoral components hallmarked, respectively, by the mobilization of hemocytes and production of antimicrobial proteins. Focusing on the humoral component, molecular genetic investigations in *Drosophila* have revealed that the *Toll* dorso–ventral-signaling pathway is used not only in *Drosophila* immunity but in mammalian and plant immunity as well (for review, see ref. 4). *Drosophila* differentiates between types of infections, and the *Toll* pathway is critical for antifungal defense, whereas the gene *immune deficiency* (*imd*) is critical for antibacterial defense (5, 6). The antimicrobial genes induced through these pathways follow accordingly, being antifungal, such as *Drosomycin*, or antibacterial, such as *Cecropin*, *Diptericin*, and *Defensin*, and many of these genes have similar family members in other organisms as well (for review, see refs. 3 and 4).

The situation in *Drosophila* is not nearly so straightforward, however, as both *Toll* and *18wheeler* (18w), which are similar to the mammalian interleukin-1 receptor (IL-1R), also are involved in the antibacterial response (6, 7). In addition, the response varies by the individual antibacterial protein gene, with the extent of induction modulated by the type of bacteria (8). Further, some signaling pathway components are also up-regulated by infection to some degree, such as *Toll*, *18w*, and the NFκB homolog *Dorsal-related immunity factor* (*Dif*) (6, 7, 9). These observations indicate a highly complex and sophisticated response to immune challenges.

To identify new genes involved in the immune response, we undertook a genetic screen in *Drosophila* designed to detect genes that are up-regulated by bacterial infection without a bias for the type of gene selected or the phenotype of a corresponding mutant (10). We selected for strains carrying a single *P*-element-enhancer trap insertion that showed an increase in B-galactosidase after an infection. One new gene was identified by the screen, named *Thor*, and postulated to encode a nonantibacterial protein (10). We now report the molecular characterization and mutant phenotype of *Thor*.

Thor is a member of the 4E-binding protein (4E-BP) family. Mammalian 4E-binding proteins have been defined as critical regulators in a pathway that controls initiation of translation (for review, see refs. 11 and 12). The role of 4E-BP hinges on its binding and sequestration of eukaryotic initiation factor 4E (eIF4E). When 4E-BP is bound to eIF4E, eIF4E cannot bind appropriately to form the translation initiation complex; phosphorylation of 4E-BP releases eIF4E, and translation is then permitted. The results with *Thor* provide evidence of a *4E-BP* family member being involved in immune induction of *Drosophila* or other organisms. Further, no gene in this translation initiation pathway has been previously found to be immune induced. Our results suggest either a role for translational regulation in humoral immunity or a new, nontranslational function for *4E-BP* type genes.

Materials and Methods

Drosophila **Stocks.** Oregon R flies were used as the standard wild-type strain. $Thor^1$ carries an insertion of *P*{*lacW*}, as described in ref. 10 and *Results*, and *imd* was identified from a laboratory stock as described in refs. 5 and 6. To produce stocks to test for the effects of mutation of *Thor*, both a wild-type revertant, $Thor^{1rv1}$, and an additional *Thor* mutant, $Thor^2$, were derived from $Thor^1$ by *P*-element mobilization as in ref. 10.

Infection Experiments. Bacteria used were *Escherichia coli*, *Enterobacter cloacae* B_{12} (*E. cloacae* B_{12}), *Staphylococcus epidermidis*, and *Micrococcus roseus*. Bacterial growth and injections of flies were handled as in ref. 10, and survival experiments were conducted as described in ref. 6 with the exception that flies were tested for 4 days.

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Abbreviations: 4E-BP, 4E-binding protein; Dif, dorsal-related immunity factor; eIF4E, eukaryotic initiation factor 4E.

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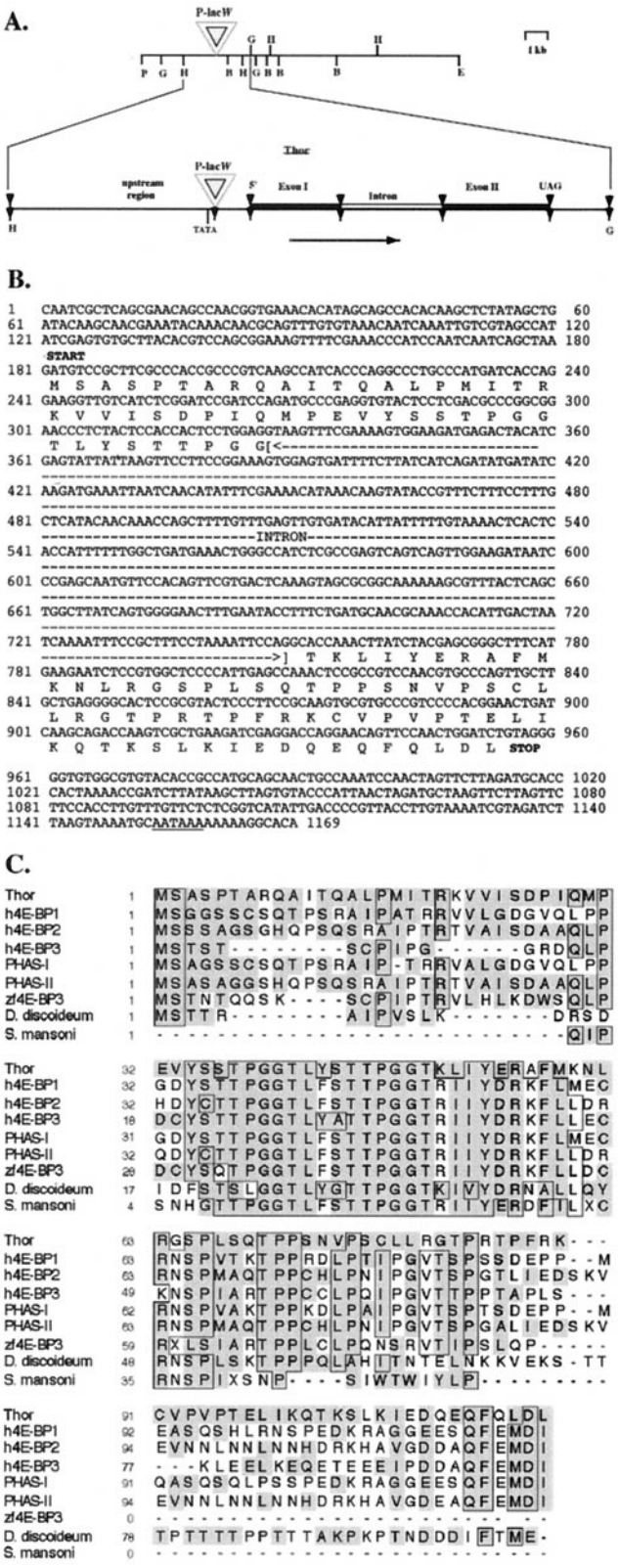

Fig. 1. Characterization of *Thor* sequences. (*A*) Restriction map of the genomic DNA surrounding the *Thor P*-element insertion (*P-lacW*) at polytene chromosome position 23F–24A. Arrangement of transcribed region and placement of insertion between the TATA box sequence and start codon also is indicated. Restriction enzyme abbreviations: G (*Bgl*II), P (*Pst*I), H (*Hin*dIII), B (*Bam*HI), and E (*Eco*RI). (*B*) Nucleotide sequence (top row) and predicted amino acid sequence (bottom row) of THOR. The start and stop codons are shown in bold with the intron represented by a dashed line. The predicted PolyA signal sequence is underlined. GenBank accession no. AF244353. (*C*) Amino acid alignment of THOR to 4E-BP sequences. Additional GenBank accession nos. are for human 4E-BP: h4E-BP1 (NM004095), h4E-BP2 (NM004096), and h4E-BP3 (NM003732); for mouse PHAS-I (U28656.1) and PHAS-II (U75530); for zebrafish: zf4E-BP3 (AI722723); *Dictyostelium discoideum* (C94507); and *Schistosoma mansoni* (AI014205).

Molecular Genetic Analysis. Genomic DNA flanking the *Thor P*-element insertion site was recovered by the plasmid rescue technique (13). DNA preparation, restriction mapping, and high stringency Southern blots were performed as described in ref. 14. DNA fragments for sequencing were subcloned into the Bluescript vector (Stratagene) and both strands sequenced using external (T7 and T3) and internal primers (DNAgency) on an automated sequencer at the Molecular Genetics Core Facility of the University of Texas Medical School. Sequence similarity searches were carried out with BLAST and BEAUTY postprocessing by using the *Drosophila* genome database FLYBASE, the National Center for Biotechnology Information, and the Human Genome Center at Baylor College of Medicine. Nucleotide and amino acid sequences were aligned with CLUSTAL W. Amino acid identity and homology comparisons were performed using the program SEQVU 1.0.1 (GES scale). Analysis of promoter region sequence was carried out using MACVECTOR and the TRANSFAC database.

Histology and Tissue *in Situ* Hybridization. B-galactosidase staining of whole mount and dissected tissues was as in ref. 10. Tissue *in situ* hybridizations were performed using digoxigenin-labeled probes of *Thor*-coding sequence, prepared as described in the Boehringer-Mannheim DNA labeling and detection kit and as in ref. 7 for embryos, larvae, and adults.

Northern Blot Analysis. The Totally RNA Kit (Ambion) was used to extract RNA from each developmental stage of the Oregon R strain. The Totally RNA kit also was used to extract RNA from Oregon R and *Thor* mutant adults that were untreated, wounded with a sterile needle or injected with *E. cloacae* B_{12} through puncture with a septic needle. Wounded and infected flies were allowed to recover for 6 h at 25°C and visually checked for wound response by characteristic melanization around the puncture site before RNA was extracted. Formaldehyde gels and Northern blots were made as in ref. 15. Probes were for *Cecropin*, *actin5C*, and *rp49* as in ref. 7, *eIF4E* (16) and *Thor*. *Thor* probes were both DNA and RNA, corresponding to cDNA and genomic DNA sequence, i.e., the 1.7-kb *Bgl*II plasmid rescue subclone. Radioactively labeled DNA probes were made using the Prime-a-gene kit (Promega) and antisense RNA probes were made using the RNA transcription kit (Ambion).

Results

Molecular Identification and Expression of *Thor*. The *Thor* gene was initially identified based on a *P*{*lacW*} insertion located at cytological position 23F-24A that shows increased reporter gene expression in response to bacterial infection (10). To determine the molecular identity of *Thor*, we used plasmid rescue (13) to isolate genomic DNA fragments flanking the *P*{*lacW*} insertion, for a total of 14 kb (Fig. 1*A*). Restriction enzyme fragments of this DNA were used in tissue *in situ* hybridizations to whole mount embryos to define candidates for the region encoding *Thor*. Only the 1.7 kb immediately downstream of the insertion hybridized, and expression showed in the central nervous system, same as the B-galactosidase localization of the enhancer trap (10). This DNA fragment was then tested in a Northern blot analysis comparing RNA from wild-type adults with and without a bacterial infection. A band of 0.85 kb was detected that is

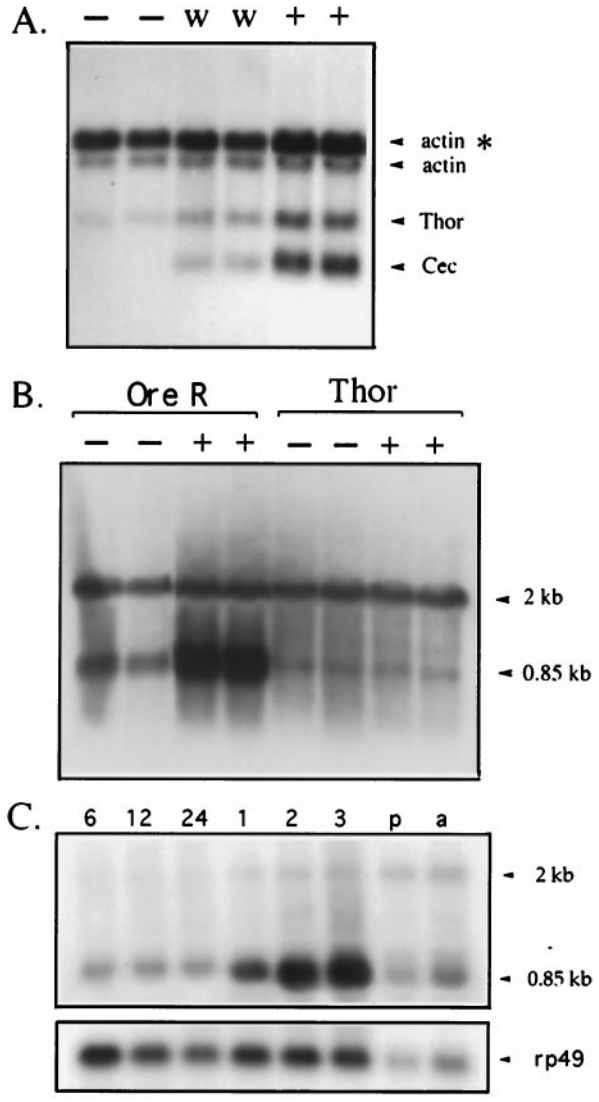

Fig. 2. Northern analysis of *Thor* expression. (*A*) Oregon R adults untreated (−), wounded (w) and infected (+). The control probe to show wounding and infection is *Cecropin* (Cec). The *Thor* probe is a DNA probe made from the 1.7-kb *Bgl*II fragment downstream of the *P{lacW}* insertion, as seen in Fig. 1*A*. Antisense RNA probes give the same results as DNA probes, and RNA probes were used for the results shown in *B* and *C*. The loading control probe is for *actin*, and the * refers to a transcript initially noticed because it changed the intensity of one of the *actin* bands. Reprobing confirmed hybridization of *Thor* to this 2-kb transcript (*B* and *C*). (*B*) Oregon R and mutant *Thor*^{1} adults untreated (−) and with an infection (+). (*C*) Developmental profile of *Thor* expression in Oregon R. Stages are: 0–6 h (6), 6–12 h (12), and 12–24 h (24) embryos; first (1), second (2), and third (3) instar larvae; pupae (p); and adults (a). The blot was reprobed with *rp49* as the loading control.

present without an infection and strongly induced by infection (Fig. 2*A*). DNA sequencing analysis revealed that the 1.7 kb does include the entire coding region of *Thor*, and that one 423-bp intron interrupts the ORF that encodes a 117-aa protein (Fig. 1*B* and *C*). FLYBASE clone GH19868 is a full-length *Thor* cDNA, starting at base 14 and ending at base 1139 of Fig. 1*B*, and Clot 7548 is a set of partial *Thor* cDNAs. The *Thor* protein is similar to the mammalian translational regulators known as 4E-BPs (Fig. 1*C*). Sequences corresponding to 4E-BPs also have been identified in zebrafish, the slime mold *Dictyostelium discoideum* and the parasite *Schistosoma mansoni* (Fig. 1*C*). THOR is most similar to mouse PHAS-II and human 4E-BP2, with 39% and

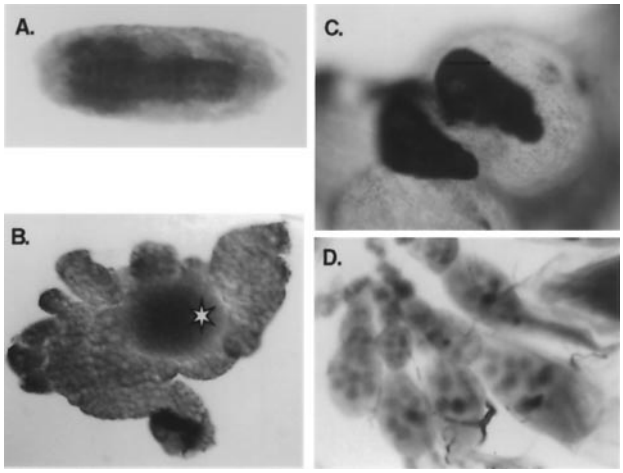

Fig. 3. Tissue-specific localization of *Thor* expression. *Thor* expression is detected only in the central nervous system in embryos (*A*), but in many tissues in larvae and adults, e.g., the fat body (*B*, *), testes (*B*, *), and ring gland (*C*) of third instar larvae and the ovarian nurse cells (*D*) of adult females. Probes of *A-C* are digoxigenin-labeled, whereas *D* shows nuclear B-galactosidase staining of the *Thor*-enhancer trap as this shows more clearly that nurse cells as early as stage 4 express *Thor*.

38% identity respectively, and 67.5% similarity for both. Of importance is the high conservation in the central region, residues 34–59 for THOR, as this includes the binding region of 4E-BPs to eIF4E. THOR has 66.5–70% identity with all sequences in this region, except for *D. discoideum*, which has 55.5% identity.

In mouse and human, two and three 4E-BPs, respectively, have been identified. Standard genome Southerns do not suggest another gene in *Drosophila* (data not shown), however, we do not have an explanation for the 2-kb transcript that shows on Northern blots with *Thor* probes (Fig. 2). It may be that the 2-kb transcript results from differential splicing or that it reflects cross-hybridization to transcripts from a different locus with sequence similarity. This transcript is, however, clearly not induced by infection (Fig. 2). In contrast, the 0.85-kb *Thor* transcript is not only induced by infection, it also is up-regulated to a lesser degree by wounding, as has been found for other immune-regulated genes (Fig. 2*A*). The main site of induction of the immune response is the fat body, which corresponds to the mammalian liver (for review, see ref. 3), and we have found that *Thor* is also up-regulated in the fat body by comparing dissected third instar larval fat bodies with and without infection (data not shown).

The developmental profile of wild-type *Thor* expression shows transcripts present throughout all stages of development, with a noticeable increase in the larval stages, especially the third instar (Fig. 2*C*). We have previously shown that B-galactosidase in the *P{lacW} Thor* strain localizes to the embryonic nervous system and to many tissues in larvae and adults (10). We have found the same results by tissue *in situ* hybridizations with *Thor* probes and extended the analysis to the reproductive system (Fig. 3). Fig. 3 shows *Thor* expression in the testes of third instar males and in the ovaries of adult females. This expression in the testes and ovarian nurse cells is part of a more complex pattern of expression in both the female and male reproductive systems (B. Andruss, S. Meller, and D.A.K., unpublished observations). The general expression of *Thor* and in particular the expression in the ovaries, in which there are intricate systems of translational regulation (17), are consistent with the role of *Thor* as a 4E-BP, but the role of a 4E-BP in the immune response is not consistent with or predicted by current views in immunity. Two important

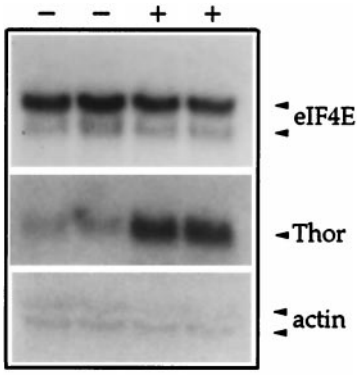

Fig. 4. Test for immune induction of *eIF4E*. A Northern blot of RNA from Oregon R adults untreated (−) and with an infection (+) was successively washed and reprobed for *eIF4E*, *Thor* and, as loading control, *actin*.

initial concerns are thus: (*i*) is *Thor* induction regulating cell growth as a secondary effect of the immune response? and (*ii*) Is *Thor* induction specifically immune induced?

Test for eIF4E Immune Induction. An important question with regard to the possibilities of *Thor* induction being involved in cell growth regulation is whether or not *eIF4E* also is induced by infection. In mammalian cells, overexpression of *eIF4E* is associated with malignant transformation, and concomitant overexpression of *4E-BP* has been shown to negate this overgrowth (for review see ref. 18). In the *Drosophila* immune response, large quantities of antimicrobial proteins are produced and one possibility is that *eIF4E* could be up-regulated to increase translation. In this scenario, *Thor* up-regulation would have the homeostatic function of producing more 4E-BP to keep growth regulation in balance. We therefore tested the hypothesis that *eIF4E* also is up-regulated by bacterial infection. We found that this is not the case, as *Thor* is up-regulated upon infection while the levels of *eIF4E* mRNA remain the same (Fig. 4). Therefore, if these two components are coordinately regulated, it is not at the level of transcription.

***Thor*-Promoter Analysis.** All of the promoter regions studied for *Drosophila* antimicrobial genes induced by infection have been found to have elements similar to those of immune inducible genes in mammals (for review, see refs. 2 and 3). Sequence analysis of the 5′-flanking region has determined that *Thor* has an array of these types of elements (Fig. 5). First of all, the *Thor* promoter has the canonical NFκB recognition sequence that has been shown to be essential for immune induction (for review, see refs. 2 and 3). Further, *Thor* has the GATA sequence associated with NFκB elements that has been also shown to be important for immune induction (19) and conserved in other *Drosophila* species (14). Additional sequences found upstream of *Drosophila* immune response genes have been also identified, in particular those involved in vertebrate cytokine regulation and liver specific expression (Fig. 5). TRANSFAC analysis (20) has identified more sequences related to liver-specific regulation, such as hepatocyte nuclear factor/forkhead, and also interferon-related regulatory sequences (Fig. 5).

Response of Mutant *Thor* Flies to Bacterial Infection. The most critical test of immune response is for survival after infection. To determine the effect of *Thor* mutation on immune response, we identified *Thor* mutations and then subjected control and *Thor* mutant flies to different types of bacterial infection and observed their survival for 4 days.

The *P*{*lacW*} insertion that led to identifying *Thor* also

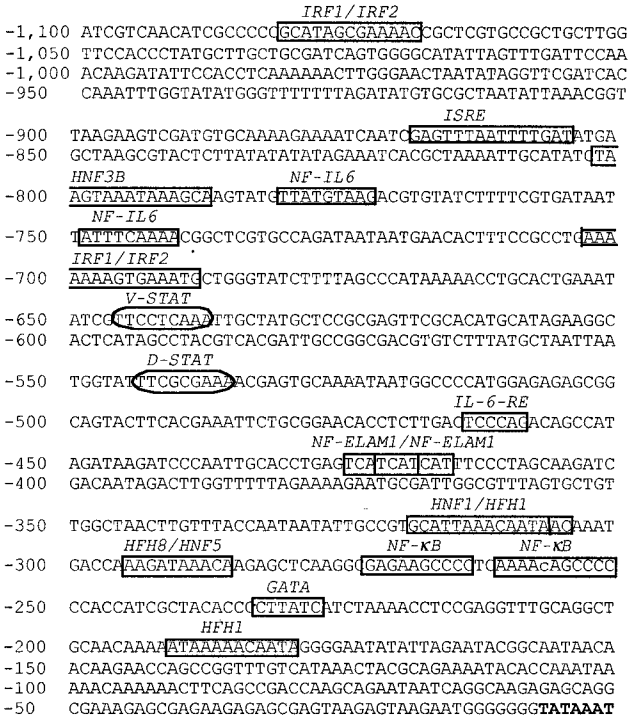

Fig. 5. Thor upstream region. Regulatory motifs are outlined in the sequence 5′ of the TATA box (bold). Consensus sequences used to identify these motifs are as follows, and referenced in 14, except as indicated: NFκB response elements, GGGRNTYYYY (31); GATA, WGATAR; IL-6 response elements (NF-IL6), TKNNGNAAK, (IL-6-RE) CTGGGA; Nuclear factor endothelial leukocyte adhesion molecule 1 (NF-Elam1), WCAKCAK; Hepatic nuclear factor 5 response element (HNF5), TRTTTGY. A single mismatch in one of the NFκB elements is indicated by a lowercase letter. Additional sequences with core identity and at least a 0.8 outside core match to the TRANSFAC database (20) also are indicated. These Transfac sequences are the elements for: interferon regulatory factors IRF1, SNAAAGYGAAACC, and IRF2, GNAAAGYGAAASY; interferon-stimulated response element IRSE, CAGTTTCWCTTTYCC; signal transducers and activators of transcription, *Drosophila* STAT (D-STAT), TTTCCSGGAAA and vertebrate STAT (V-STAT), TTCCCRKAA; hepatic nuclear factor HNF1, GGTTAATNWTTAMMN; hepatocyte nuclear factor/forkhead homolog HNF3B, KGNANTRTTTRYTTW, HFH1, NAWTGTTTATWT and HFH8, TGTTTATNYR. The core sequences are underlined. Only the GATA near NFκB is indicated.

resulted in the *Thor*^{1} mutation. *P*{*lacW*} inserted adjacent to the TATA box, separating the coding region from the promoter region (Fig. 1*A*), and Northern analysis of flies with this insertion reveals that only a small amount of the 0.85-kb *Thor* transcript is present, and it does not increase after infection (Fig. 2*B*). The *P*-element insertion has thus resulted in the production of the *Thor*^{1} mutation, a noninducible, very weakly expressing hypomorph. To control for background effects, we used a wild-type revertant of the *Thor*^{1} mutation, *Thor*^{1rv1}, which was produced by precise excision of the *P*{*lacW*} insertion. To confirm that any differences between *Thor*^{1} and *Thor*^{1rv1} flies are the result of mutation of *Thor*, we also used a second *Thor* mutation, *Thor*^{2}. In *Thor*^{2}, *P*{*lacW*} also is excised, but leaving *Thor* still mutant because of imprecise excision that deleted bases between *P*{*lacW*} and the first B site (Fig. 1*A*). *Thor*^{1} and *Thor*^{2} mutants are homozygous viable and fertile, and in noninfected and sterile wounding controls, survival is similar to wild-type Oregon R flies (10; data not shown), *Thor*^{1rv1} and *imd* (Fig. 6*A*). *Thor*^{1rv1} survives like Oregon R and is referred to here as the designated wild-type control (wt in Fig. 6). As a control for susceptibility to infection, we used *imd* mutants. In similar

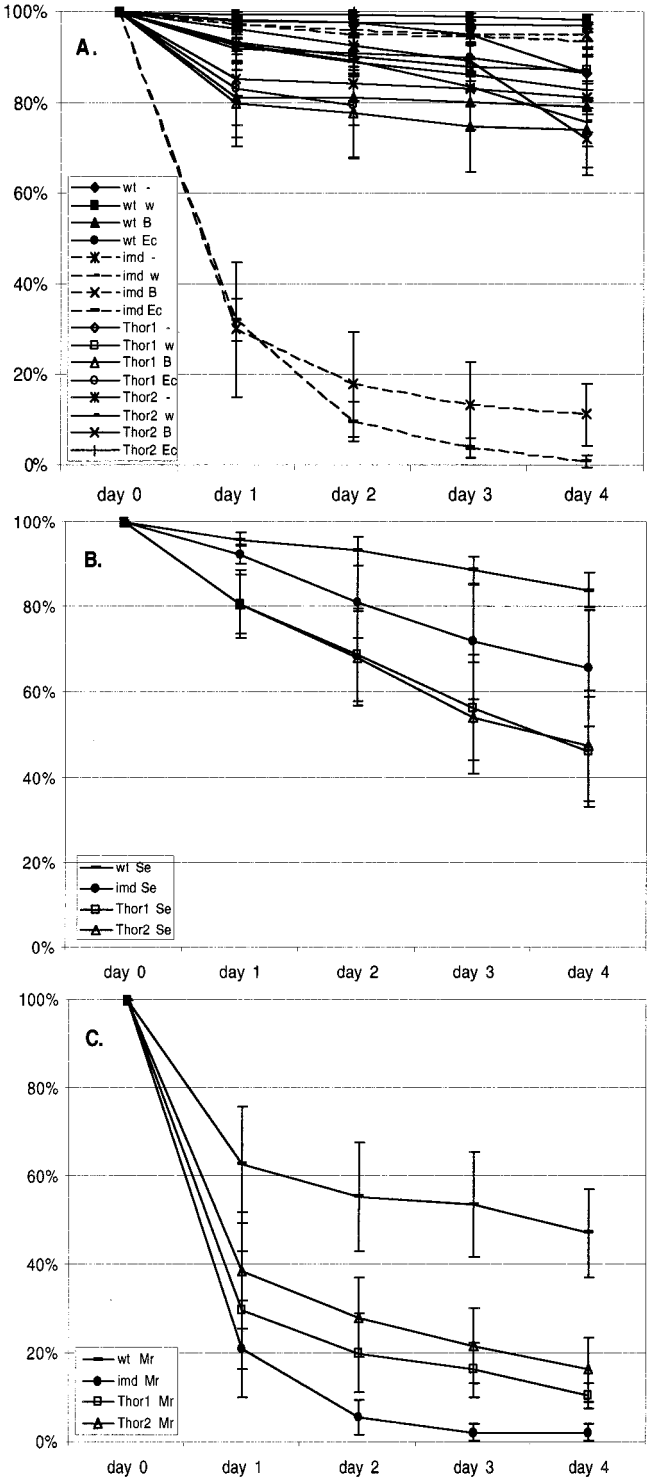

Fig. 6. Survival of adults after bacterial infection. The survival rates (%) after injection of different types of bacteria for *Thor* flies (*Thor*^{1} and *Thor*^{2}) compared with *imd* flies and a wild-type revertant, *Thor*^{1rv1} (wt). (*A*) Flies without an injection (−) and with a sterile wound (w) are presented for each stock as controls to compare the effect of infection. Bacteria used are *E. cloacae* B_{12} (*B*) and *E. coli* (*Ec*). Additional types of bacteria used are presented separately in (*B*), *S. epidermidis* (*Se*), and (*C*), *M. roseus* (*Mr*). Flies were kept at 29°C, and data points are means and corresponding standard error for at least five replicates of samples of 20 flies.

experiments, flies with the *imd* mutation have been shown to be highly susceptible to bacterial infection, and *imd* clearly operates in controlling induction of antibacterial genes (5, 6, 8).

We found that survival of mutant *Thor* adults was similar to wild-type adults when infected by *E. coli* and *E. cloacae* B_{12} (Fig. 6*A*). Both bacterial types affected *imd* flies as expected, with on average 11% surviving 4 days after infection with *E. cloacae* B_{12} and 1% surviving with *E. coli* infection (Fig. 6*A*). After infection (4 days) with *S. epidermidis*, however, on average only 46% of *Thor*^{1} and 47% of *Thor*^{2} flies survived (Fig. 6*B*). Surprisingly, 66% of *imd* flies survived, which is much higher than *Thor* mutants and also the previously reported survival for *imd* flies, which was approximately 8% after *E. coli* infection (6). Tests with *M. roseus* showed that even wild-type flies are susceptible, with 47% surviving on average (Fig. 6*C*). *Thor* mutants and *imd* flies were both more susceptible, with 11% for *Thor*^{1}, 16% for *Thor*^{2}, and 2% for *imd* surviving on average 4 days after infection. The antibacterial response provides a strong defense, and failure of survival by mutants indicates that a critical facet of the response has been disrupted. Our results show that *Thor* mutants are immune compromised, and when subjected to some types of bacteria, the disruption of the immune response leads to failure of survival.

Discussion

Our initial screen for immune response genes was carried out without bias in the selection process, leading to the identification of a new type of gene involved in immunity. *Thor* is a member of the *4E-BP* family, and it is induced in response to bacterial infection and, to a lesser extent, by wounding. Without an infection, *Thor* is expressed during all developmental stages, and transcripts localize to a variety of tissues. Promoter region sequences and infection induced expression of *Thor* in the fat body are consistent and correspond with the fat body being the major organ of the humoral response, but other tissues may also up-regulate *Thor*. The presence of potential interferon related regulatory sequences is interesting, particularly given the roles of interferon and translational regulation in viral infection. Further, the interferon consensus response element GAAANN portion of the *Thor* interferon-related regulatory sequences has been previously identified in the *Diptericin* promoter and specifically shown to enhance *Diptericin*-promoter activity (21).

In contrast to humoral immunity, translational regulation in cellular immunity is already indicated. For example, in *Drosophila*, translation has been studied by Watson *et al.* on *lethal(1)aberrant immune response*[8], which encodes the homolog of human S6 ribosomal protein and causes cancerous overgrowth upon mutation (22), and on *S6k*, the kinase of RpS6 (23). In mouse T cells, activation correlates with changes in translation as well as transcription (24). In immunosuppressant drug studies, rapamycin has been found to block cell cycle progression and involve inhibiting 4E-BP1 phosphorylation and cap-dependent translation (see ref. 25).

The extent of the protective role of *Thor* up-regulation in the immune response was tested by using *Thor* mutants in infection survival experiments. While this type of experiment appears simple, it is actually difficult to perform and does not indicate the specific defect caused by a mutation. However, the function of the entire immune response to an infection is tested, and as such this is the best available method to begin assessing the role of *Thor* in immune function. We found that *Thor* mutants are immune compromised, with some bacterial types having little or no effect on survival and others strongly reducing survival capacity. Although four bacterial types is insufficient for a conclusion, the difference in effect does correlate with whether the bacteria are Gram-positive or Gram-negative. Lemaitre *et al.* (8) have demonstrated that *Drosophila* is sensitive to bacterial infection type and shows varying levels of induction for the

different antibacterial protein genes, both for individual strains and generally for Gram-positive vs. Gram-negative. *imd* flies were expected to survive poorly with all types, yet *imd* survived well when infected with *S. epidermidis* and better than did *Thor* flies. This result is consistent with the observation that the *imd* pathway is preferentially activated by Gram-negative bacteria (8), and therefore a Gram-positive bacterial strain may be less likely to cause lethality in an *imd* mutant. *Thor* flies were the opposite, being more resistant to Gram-negative bacteria.

The sequence similarity of *Thor* with *4E-BP* and the conservation of *4E-BP* family members implicate a role for translational regulation in the immune response. The most straightforward hypotheses involve a role based on binding to eIF4E, as that is the most conserved region and defines what is known of the family in other systems. The fact that *eIF4E* is not also up-regulated by infection is intriguing in terms of the relative functions of these genes in regulating cell growth, and now in responding to infection. Because a *4E-BP* or other translational component has not been previously found to be induced by infection, our results with *Thor* indicate that an unexpected system is operative in the immune response. As the death of *imd* flies appears to reflect the necessity of the induction of *imd*-regulated genes (5, 6), *Thor* lethality can be postulated to reflect the necessity of the translation of the relevant immune transcripts for a given type of bacterial infection. Thus, the function of *Thor* in immunity may be the preferential translation of immune transcripts. A primary mode used by viruses to subvert cellular translational machinery is an alternate, 5′-cap-independent initiation of translation through the use of specific 5′-untranslated region sequences, the internal ribosome entry sites, and dephosphorylation of eIF4E (26, 27). A model from other studies is thus that THOR would act to inhibit cap-dependent translation by eIF4E and to promote cap-independent translation. Given that in the immune response massive production of antimicrobial proteins is required, this leads to the testable hypothesis that translation of immune transcripts involves cap-independent mechanisms. Also, translation of mRNAs encoding proteins with counteractive effects could be suppressed. Alternatively, if translation of *Thor* is delayed after immune induction, then THOR could function to block translation, thereby shutting off the immune response.

Thor may function in immunological memory. A second infection has been observed to produce a stronger response than the first (28), and *Thor* may provide the mechanism to explain this form of, as is now termed here, immunological memory. This could be, for example, through sequestration of immune transcripts or blocking translation, as described above, with immune transcripts remaining stable for later translation. Immunological memory and preferential translation are also not mutually exclusive. For example, *Dif* has a 5′-untranslated region typical for alternate translational regulation, and *Dif* up-regulation already has been postulated to provide an explanation for a stronger second response (9).

In terms of other models, the heat shock response studied in many organisms provides an excellent model of both transcriptional and translational regulation for comparing to the *Drosophila* immune response. Heat shock mRNA is induced via heat shock sequence promoter elements, and translation of nonheat shock mRNA is repressed, whereas translation of heat shock mRNA is preferential, mediated by specific 5′-untranslated region sequences and appearing to involve cap-independent translation (for review, see refs. 29 and 30). *Thor* is a candidate member of an old, fundamental system that has developed to cope with stress and infections of various types.

Although *Thor* is most likely to function as a 4E-BP in the immune response, it may be that *Thor* has functions that are immune specific and not necessarily related to translational regulation. It is an open question as to whether additional *4E-BPs* or other components in the 4E-BP pathway will also be found to be like *Thor* in having immune function. However, given the phylogenetic conservation of innate immunity, it is expected that this new component of immunity will also be conserved.

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1. Kimbrell, D. A. (1991) *BioEssays* **13,** 657–663.
2. Hultmark, D. (1993) *Trends Genet.* **9,** 178–183.
3. Meister, M., Lemaitre, B. & Hoffmann, J. A. (1997) *BioEssays* **19,** 1019–1026.
4. Hoffmann, J. A., Kafatos, F. C., Janeway, C. A. & Ezekowitz, R. A. B. (1999) *Science* **284,** 1313–1318.
5. Lemaitre, B., Kromer-Metzger, E., Michaut, L., Nicolas, E., Meister, M., Georgel, P., Reichhart, J. M. & Hoffmann, J. A. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 9465–9469.
6. Lemaitre, B., Nicolas, E., Michaut, L., Reichhart, J. M. & Hoffmann, J. A. (1996) *Cell* **86,** 973–983.
7. Williams, M. J., Rodriguez, A., Kimbrell, D. A. & Eldon, E. D. (1997) *EMBO J.* **16,** 6120–6130.
8. Lemaitre, B., Reichhart, J.-M. & Hoffmann, J. A. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 14614–14619.
9. Ip, Y. T., Reach, M., Engstrom, Y., Kadalayil, L., Cai, H., Gonzalez-Crespo, S., Tatei, K. & Levine, M. (1993) *Cell* **75,** 753–763.
10. Rodriguez, A., Zhou, Z., Tang, M. L., Meller, S., Chen, J., Bellen, H. & Kimbrell, D. A. (1996) *Genetics* **143,** 929–940.
11. Hershey, J. W. B., Matthews, M. B. & Sonenberg, N. (1996) *Translational Control* (Cold Spring Harbor Lab. Press, Plainview, NY).
12. Preiss, T. & Hentze, M. W. (1999) *Curr. Opin. Genet. Dev.* **9,** 515–521.
13. Wilson, D., Bellen, H. J., Pearson, R. K., O'Kane, C. J., Grossniklaus, U. & Gehring, W. (1989) *Genes Dev.* **3,** 1301–1333.
14. Zhou, X, Nguyen, T. & Kimbrell, D. A. (1997) *J. Mol. Evol.* **44,** 272–281.
15. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
16. Lavoie, C. A., Lachance, P. E. D., Sonenberg, N. & Lasko, P. (1996) *J. Biol. Chem.* **271,** 16393–16398.
17. Stebbins-Boaz, B. & Richter, J. D. (1997) *Crit. Rev. Eukaryot. Gene Expr.* **7,** 73–94.
18. Clemens, M. J. & Bommer, U. A. (1999) *Int. J. Bioc. Cell Biol.* **31,** 1–23.
19. Kadalayil, L., Petersen, U. M. & Engstrom, Y. (1997) *Nucleic Acids Res.* **25,** 1233–1239.
20. Heinemeyer, T., Chen, X., Karas, H., Kel, A. E., Kel, O. V., Liebich, I., Meinhardt, T., Reuter, I., Schacherer, F. & Wingender, E. (1999) *Nucleic Acids Res.* **27,** 318–322.
21. Georgel, P., Kappler, C., Langley, E., Gross, I., Nicolas, E., Reichhart, J. M. & Hoffmann, J. A. (1995) *Nucleic Acids Res.* **23,** 1140–1145.
22. Watson, K. L., Konrad, K. D., Woods, D. F. & Bryant, P. J. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 11302–11306.
23. Watson, K. L., Chou, M. M., Bienis, J., Gelbart, W. M. & Erikson, R. L. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 13694–13698.
24. Garcia-Sanz, J. A., Mikultis, W., Livingstone, A., Lefkovits, I. & Muellner, E. W. (1998) *FASEB J.* **12,** 299–306.
25. Scott, P. H., Brunn, G. J., Kohn, A. D., Roth, R. A. & Lawrence, J. C. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 7772–7777.
26. Matthews, M. B. (1996) in *Translational Control*, eds. Hershey, J. W. B., Matthews, M. B. & Sonenberg, N. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 505–548.
27. Sachs, A. B., Sarrow, P. & Hentze, M. W. (1997) *Cell* **89,** 831–838.
28. Boman, H. G., Nilsson, I. & Rasmussen, B. (1972) *Nature (London)* **237,** 232–235.
29. Duncan, R. F. (1996) in *Translational Control*, eds. Hershey, J. W. B., Matthews, M. B. & Sonenberg, N. (Laboratory Press, Cold Spring Harbor), pp. 271–294.
30. Vries, R. G. J., Flynn, A., Patel, J. C., Wang, X. M., Denton, R. M. & Proud, C. G. (1997) *J. Biol. Chem.* **272,** 32779–32784.
31. Kappler, C., Meister, M., Lagueux, M., Gateff, E., Hoffmann, J. A. & Reichhart, J. M. (1993) *EMBO J.* **12,** 1561–1568.