EUKARYOTIC CELL, Apr. 2007, p. 658–663 Vol. 6, No. 4
1535-9778/07/$08.00+0 doi:10.1128/EC.00346-06
Copyright © 2007, American Society for Microbiology. All Rights Reserved.

Drosophila melanogaster Thor and Response to *Candida albicans* Infection▽†

A. Levitin,[1]* A. Marcil,[1] G. Tettweiler,[2] M. J. Laforest,[1] U. Oberholzer,[1]
A. M. Alarco,[1] D. Y. Thomas,[3] P. Lasko,[2] and M. Whiteway[1,2]

Genetics Group, Biotechnology Research Institute, National Research Council, Montreal, Quebec H4P 2R2, Canada[1]; Department of Biology, McGill University, Montreal, Quebec H3A 1B1, Canada[2]; and Department of Biochemistry, McGill University, Montreal, Quebec H3G 1Y6, Canada[3]

Received 31 October 2006/Accepted 23 January 2007

We used *Drosophila melanogaster* macrophage-like Schneider 2 (S2) cells as a model to study cell-mediated innate immunity against infection by the opportunistic fungal pathogen *Candida albicans*. Transcriptional profiling of S2 cells coincubated with *C. albicans* cells revealed up-regulation of several genes. One of the most highly up-regulated genes during this interaction is the *D. melanogaster* translational regulator 4E-BP encoded by the *Thor* gene. Analysis of *Drosophila 4E-BP*^{null} mutant survival upon infection with *C. albicans* showed that 4E-BP plays an important role in host defense, suggesting a role for translational control in the *D. melanogaster* response to *C. albicans* infection.

Candida albicans is a part of normal microbial flora that can be found in mucocutaneous surfaces of the oral cavities, gastrointestinal tracts, and vaginas of many mammals, including humans. Although *C. albicans* does not normally cause severe disease in immunocompetent hosts, this pathogen can trigger life-threatening systemic infections in immunocompromised individuals. Mammals respond to *C. albicans* infection through activating both acquired and innate immune responses (3, 30), with the innate immune response as the first defense. Since the innate immune response is evolutionarily highly conserved, *Drosophila melanogaster* is a promising system for studying virulence characteristics of medically important pathogens such as *C. albicans* (7, 32). The *Drosophila* immune response is composed of both humoral and cellular components (2). The innate immune system consists of two major networks defined by the Imd (immune deficiency) and Toll pathways that are activated by fungal and bacterial infections (7). These pathways initiate humoral antimicrobial defenses in the *Drosophila* fat body, the analog to a mammalian liver. The cellular immune response involves plasmatocytes (blood cells) that can phagocytose microbes and encapsulate parasites; transcription activation of a variety of pathways is necessary for these responses.

In eukaryotes, regulation of gene expression at the translational level is a very complex process. It allows for very rapid adaptive changes in global protein synthesis levels and for selective mRNA translation during the regulation of the cell cycle, development, apoptosis, the response to cell proliferation conditions, and cellular stress conditions, such as infection. During translation initiation, the 40S preinitiation complex is recruited to mRNA by interactions with the cap-binding complex eIF4F (eukaryotic initiation factor 4F). The eIF4F complex consists of 3 subunits: eIF4E, the cap binding protein; eIF4A, a RNA helicase; and eIF4G, a scaffolding protein.

The activity of eIF4E is regulated by the eIF4E-binding proteins (4E-BPs). These repressor proteins inhibit cap-dependent translation by preventing the association of eIF4E with eIF4G and thereby suppressing the formation of the cap-binding complex. The binding of 4E-BPs to eIF4E is modulated by the phosphorylation status of the 4E-BPs at several serine and threonine residues. Under active growth conditions, 4E-BPs are hyperphosphorylated, remain dissociated from eIF4E, and are inactive in blocking cap-dependent translation. However, under conditions that block cell proliferation or induce apoptosis, hypophosphorylated 4E-BPs sequester eIF4E and inhibit cap-dependent, but not cap-independent, translation (9, 13, 15, 34).

Recent studies have shown that *Drosophila* has a single d4E-BP (21), in contrast to mammals, which express three distinct 4E-BP proteins (24, 26). *Drosophila* 4E-BP is an effector of cell growth (21). The phosphorylation of d4E-BP is stimulated by insulin via the conserved insulin receptor (dInR-PI3K-Akt-TSC-dTOR) pathway. In starved *Drosophila* S2 cells, most of the d4E-BP consists of the nonphosphorylated isoform (α), which is active in binding deIF4E. Treatment with insulin induces a shift to another isoform (β), hyperphosphorylated at Thr37 and Thr46, which causes d4E-BP dissociation from deIF4E (20).

Among the first-line defense players of the innate immune system are the macrophages, which can phagocytose pathogens. In the present work, we used S2 cells, which share many characteristics with mammalian macrophage cells, to study pathogen-host interactions. It was recently described by Stroschein-Stevenson et al. (32) that S2 cells engulf *C. albicans* as early as 30 min after they encounter each other. We showed that phagocytosis of *C. albicans* cells induces differential expression of immune response genes. Microarray analysis of the host-pathogen interaction identified several genes involved in innate response to *C. albicans* infection, including *Thor*, which encodes d4E-BP. Subsequently, we investigated the importance of d4E-BP in vivo and observed an increased sensitivity of *d4E-BP*^{null} flies to *Candida* infection. Our data suggest that d4E-BP is important for fly survival after *Candida* infection.

MATERIALS AND METHODS

Yeast, bacterial, and *Drosophila* strains and cell lines. The *C. albicans* strains used in this study were SC5314 (12) and CAI4-GFP, expressing a soluble intra-

* Corresponding author. Mailing address: 6100 Royalmount Ave, Montreal, Quebec H4P 2R2, Canada. Phone: (514) 496-6146. Fax: (514) 496-6213. E-mail: anastasia.levitin@cnrc-nrc.gc.ca.
† This is National Research Council publication 47513.
▽ Published ahead of print on 2 February 2007.

cellular green fluorescent protein (GFP) (*ura3*::*imm434*/*ura3*::*imm434 pAM5.6*) (2, 10). These strains were grown in YPD (1% yeast extract, 2% peptone, 2% dextrose, 0.05% uridine, pH 5.5) or SD-ura (0.15% dropout uracil, 0.05% uridine, 0.67% yeast nitrogen, 2% dextrose) media, respectively. *Drosophila* Schneider 2 (S2) cells (Invitrogen) were grown in Schneider's media (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (S-10 medium) according to the supplier's specifications (ATCC). The *d4E-BP^null^* (*Thor*^{2}), revertant (*Thor*^{1Rv1}), and Oregon-R wild-type flies are as described previously (5, 6, 33). The *Saccharomyces cerevisiae* strain used was MLY40 (17).

Time-lapse microscopy. The day before introducing the fungal cells, 10^6 *Drosophila* S2 cells were seeded in a Bioptechs petri dish. S2 cells were then incubated with live or 4% paraformaldehyde-fixed *Candida* cells or with 3.53-μm latex beads (Estapor Microspheres). Phase-contrast as well as epifluorescence pictures were taken at a ×400 magnification every 15 min with a DMIRE2 inverted microscope (Leica Microsystems Canada) equipped with a Hamamatsu cooled charge-coupled-device camera, a Bioptechs temperature-controlled stage adapter, and a Ludl motorized stage. Openlab software (Improvision) was used for image acquisition.

Immunofluorescence. *Drosophila* S2 cells (10^7) were seeded in six-well plates (Becton-Dickinson), and *Candida* cells (strain CAI4-GFP) were added at a multiplicity of infection (MOI) of 1. At the indicated incubation time, cells were washed two times in S-10 medium and stained with an anti-*Candida* antibody as previously described (29), except the secondary antibody was a Rhodamine red-X-conjugated F(ab)′2 donkey anti-rabbit antibody (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) diluted 1:200 in S-10. Epifluorescence was monitored using the appropriate filters, at ×400 magnification.

Total RNA and mRNA extractions. Total RNA was extracted by the hot phenol extraction method (8). mRNA isolation was performed using the Micro-FastTrack mRNA isolation kit from Invitrogen according to the manufacturer's recommendations.

Microarrays. The microarrays used in this study were purchased from the Drosophila Microarray Center (d12k v1) (23). Transcription profiles for each condition represent the average of at least 4 to 9 independent hybridizations. These include dye swap hybridizations (Cy3/Cy5 and Cy5/Cy3) from at least three independently produced RNA preparations. The DNA microarray slides were scanned with a ScanArray 5000 scanner (version 2.11; GSI Lumonics, then Packard BioScience, now Perkin Elmer-Cetus, Wellesley, CA) at a 10-μm resolution. Quantitation and normalization of DNA microarrays were performed as described previously (22). The resulting 16-bit TIFF files were quantified with QuantArray software (versions 2.0 and 3.0; Perkin Elmer-Cetus). Statistical analysis and visualization were performed with GeneSpring software (Silicon Genetics, Redwood City, CA) as described previously (22).

Northern blot analyses. The Northern blot analyses were performed as described previously (18). Probes for *d4E-BP* and *RpL4* genes were synthesized as follows. Fragments from the *d4E-BP* (*Thor*) gene were amplified by PCR using a forward primer, 5′-TGGGGACGGGCACGCACTTG-3′, and a reverse primer, 5′-GTGGTCCCCTGGTGGTCT-3′. The *RpL4* probe was used as an internal control to monitor RNA loading and transfer. Fragments for the *RpL4* gene were amplified using a forward primer, 5′-GGCGGCGACCTTCTTCTT-3′, and a reverse primer, 5′-GTGTGCCGACAGCTAGGATT-3′.

Antibodies and Western blot analyses. Anti-d4E-BP was a generous gift from N. Sonenberg (21). Anti-phospho-4E-BP1 (Thr37/46) antibodies were obtained from Cell Signaling Technology, Inc. Anti-actin antibodies were obtained from Chemicon/Millipore.

Protein extracts (50 μg) were loaded on a 15% acrylamide gel, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred to nitrocellulose membranes (Bio-Rad) for Western blot analysis. Membranes were incubated with anti-d4E-BP primary antibody (1:2,000) in Tris-buffered saline containing 0.05% Tween 20 plus 5% bovine serum albumin or with a 1:2,000 dilution of anti-actin monoclonal antibody, followed by a 1:2,000 dilution of anti-rabbit or anti-mouse horseradish peroxidase-conjugated immunoglobulin G (Santa Cruz Biotechnology). The proteins were detected using Lumi-Light Western blotting substrate (Roche).

***Drosophila* infection.** Infection of flies (1- to 3-day-old virgin females or males; 30 per experimental group) was performed as described previously (16) with a thin needle dipped in a concentrated cell pellet containing 200 optical density of the yeast cells used in our study. The inoculum size was evaluated at approximately 10^3 cells per fly. Following infection, flies were maintained at 25°C on regular fly medium. Infection experiments were performed at least three independent times, and standard deviations were calculated.

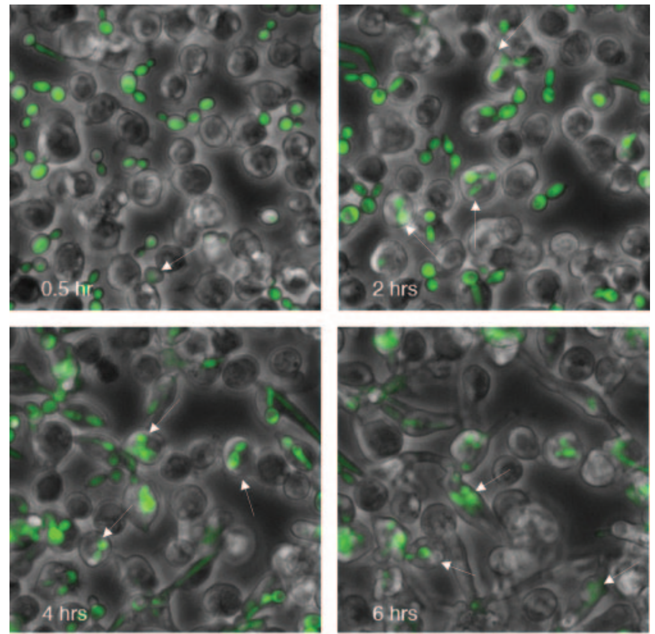

FIG. 1. Interaction of *Drosophila* S2 cells with *Candida* strain CAI4-GFP. *Drosophila* S2 cells were incubated at 25°C with *Candida* at an MOI of 1 and monitored by time-lapse microscopy at ×400 magnification for the indicated times (bottom left, in hours). Arrows point to representative *Candida* cells engulfed by the S2 cells.

RESULTS

Phagocytosis of *Candida albicans* by S2 cells. *Drosophila* plasmatocytes are responsible for the phagocytosis and destruction of apoptotic cells and microorganisms (11, 19). The plasmatocytes share several properties with mammalian macrophages at the structural and molecular levels (1, 25). In this study, we used S2 cells to analyze cell-mediated innate immunity and phagocytosis. This cell line, which is derived from *Drosophila* embryos, expresses macrophage-like genes (*croquemort*, *dSR-CI*, and *PGRP-LC*) and possesses macrophage-like phagocytic properties (25, 28, 32). The goal of the present work was to analyze the effect of the internalized *Candida* on S2 cells. To establish whether S2 cells phagocytize *C. albicans*, we monitored the internalization of GFP-labeled *C. albicans* CAI4 cells (CAI4-GFP) in coincubation experiments with S2 cells for up to 6 h (Fig. 1). The results indicated that *C. albicans* cells were indeed engulfed by S2 cells. We also took a double-labeling approach to distinguish between *C. albicans* cells internalized versus attached to the surface. Figure 2 shows that *Candida* cells associated with S2 cells are efficiently internalized, with the percentage of engulfed *Candida* increasing with the incubation time. It is noteworthy that S2 cells are loosely adherent and many cells are lost during the immunofluorescence procedure.

Endpoint dilution analysis of *Candida* interaction with *Drosophila* S2 cells. To assess the antifungal activity of *Drosophila* S2 cells, we conducted an endpoint survival assay where we monitored the survival of *Candida* SC5314 and GFP-labeled CAI4 strains in the presence of *Drosophila* S2 cells. Survival was measured as the number of colonies in the presence of S2 cells divided by the number of colonies in the absence of the S2 cells. In the

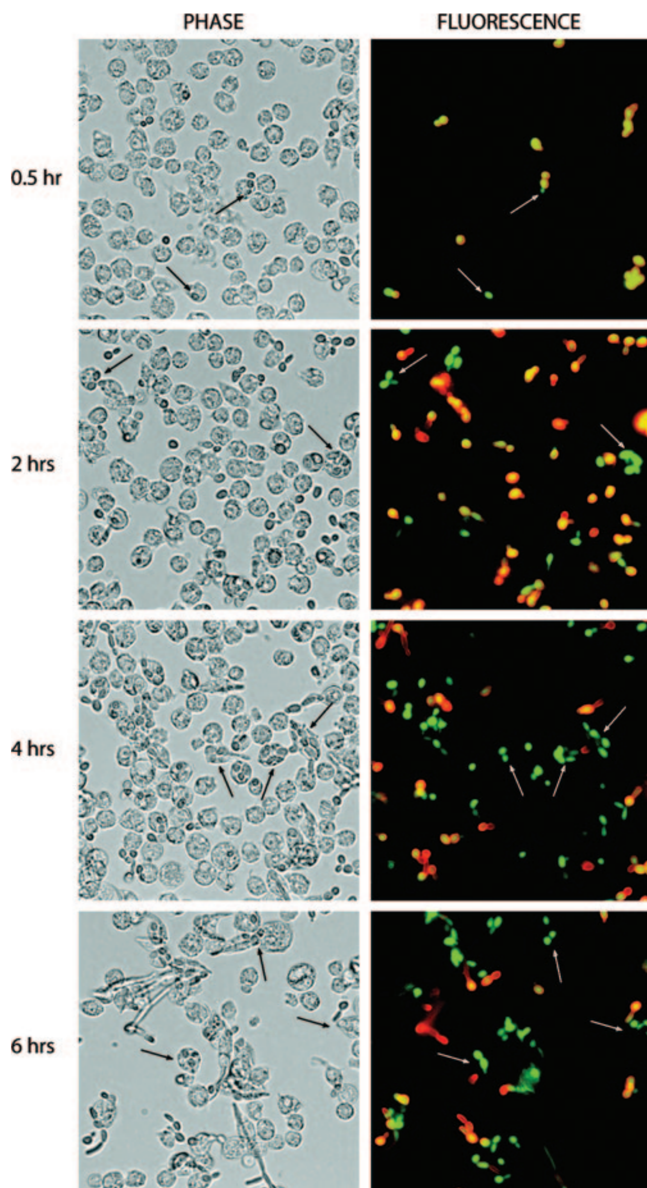

FIG. 2. Phagocytosis of *Candida* strain CAI4-GFP by *Drosophila* S2 cells. *Drosophila* S2 cells were incubated at 25°C with *Candida* CAI4-GFP at an MOI of 1 for the indicated time. They were then stained with an anti-*Candida* polyclonal antibody (in red), as described in Materials and Methods. Engulfed *Candida*, protected from primary antibody binding, remained green, whereas nonphagocytosed *Candida* became yellow-red.

presence of *Drosophila* S2 cells, the number of colonies formed was 57.4% ± 9.2% lower in the case of strain SC5314 and 61.3% ± 4.5% lower in the case of strain CAI4-GFP than the number of *Candida* cells in the absence of S2 cells.

Transcriptional analysis of S2 cells' response to the presence of *C. albicans*. We used high-density microarrays representing 10,500 *Drosophila* genes to study global gene expression changes of the S2 cells in response to *C. albicans* infection. *Drosophila* S2 cells were coincubated with the *C. albicans* wild-type strain SC5314, at a starting MOI of 1, in Schneider medium supplemented with 10% fetal bovine serum at 25°C. A comparison of mRNA levels at 3 h and 6 h after infection, respectively, revealed relatively few changes in gene expression. In Table 1, we show a list of 27 genes differentially regulated by the presence of *C. albicans* in S2 cells (with a cut-off of 1.5-fold at 6 h postinfection). Among the translational repressors, *Thor* was one of the most strongly induced genes in the presence of *C. albicans* (5.6-fold after a 6-h treatment). A representative of secreted proteins, *lox* (lysyl-oxydase like or *dLOXL-1*) was observed to be induced 4.5-fold at the same time point. A member of the immunoglobulin superfamily, the *Impl2* (CG15009) gene was also induced 2.6-fold as well as the *fok* gene (3.3-fold). A negative regulator of translation, poly(A)-binding protein-interacting protein 2a (4, 31), was also induced 1.5-fold after a 6-h *Candida* infection. *Attacin A*, encoding an antibacterial peptide, showed a 1.6-fold up-regulation. Among detoxification or stress-related proteins in S2 cells infected with *C. albicans*, the *Hph* gene was up-regulated 1.6-fold. We also observed the up-regulation of genes involved in sterol and lipid metabolism, such as *Fpps* (2.2-fold) and *ifc* (1.6-fold). Four genes were down-regulated upon *Candida* infection of S2 cells. The *String* gene involved in the mitotic cell cycle was down-regulated 1.8-fold in 6 h upon infection. A myoblast fusion gene, *rolling stone*, was also down-regulated 1.7-fold, along with an exonuclease-like gene and an unknown gene, CG30457 (down-regulated 1.6- and 1.5-fold, respectively).

***d4E-BP* mRNA and protein levels increase upon *C. albicans* infection.** Northern blot analyses were performed to confirm the induction of *d4E-BP* detected by the microarray analyses. As shown in Fig. 3, *d4E-BP* mRNA levels increase in the presence of live *C. albicans*; up-regulation of *d4E-BP* expression is not detected with coincubation with latex beads or with fixed *C. albicans* cells. Therefore, the induction of *d4E-BP* expression appears specific to the immune function in S2 cells and not to the phagocytosis of fixed pathogen or latex particles.

We also analyzed the presence of the d4E-BP protein in total extracts from S2 cells and *C. albicans*-coincubated cells (Fig. 4). Consistent with our microarray and Northern blot results, we observed increased levels of d4E-BP protein after a 6-h coincubation with live *C. albicans*. Using a phosphospecific antibody, we found that most of the increased d4E-BP was in the hypophosphorylated (α), active form, although a small increase in the hyperphosphorylated (β) form of d4E-BP was also observed. These results establish that, in S2 cells in the presence of live *C. albicans*, active d4E-BP protein is present at a higher level.

Response of *d4E-BP^{null}* flies to infection by *C. albicans*. To determine whether d4E-BP contributes to antifungal immunity, we analyzed the survival of *Drosophila 4E-BP^{null}* mutant flies following their infection with *C. albicans*. In separate experiments, 1- to 3-day-old male and female virgin flies were pricked with a very thin needle coated with concentrated *C. albicans* SC5314 or *S. cerevisiae* strain pellets or with a sterile needle as a negative control. We measured the survival of Oregon-R, revertant (*Thor^{1rev1}*), and *d4E-BP^{null}* (*Thor^{2}*) flies 6 h after infection (Fig. 5). We found that *d4E-BP^{null}* mutant flies are about 50% less resistant to *Candida* infection than controls, indicating that d4E-BP is involved in conferring immunity to this fungal species. Although *d4E-BP^{null}* mutant flies showed a decreased resistance to *Candida* infection in both

TABLE 1. Genes regulated in S2 cells in the presence of *Candida albicans* strain SC5314[a]

CG no.	FlyBase	Full name	Gene product	Avg fluorescence ratio at time (h): 3	6	*P* value (6 h)
CG8846	Thor	d4E-BP1	eIF4E binding protein	2.8	5.6	2.07E−05
CG11335	lox	Lysyl oxidase	Lysyl oxidase and Scavenger receptor cysteine-rich (SRCR) domains	1.7	4.5	0.00532
CG10746	fok	Fledgling of Kpl38B	129-amino-acid peptide	1.7	3.3	0.0118
CG15009	ImpL2	Ecdysone-inducible gene L2	Cell adhesion, extracellular	1.5	2.6	0.00308
CG1600			Zinc-containing alcohol dehydrogenase superfamily	1.5	2.5	0.000133
CG7224			118-amino-acid peptide	1.2	2.4	0.00494
CG12389	Fpps	Farnesyl pyrophosphate synthase	Cholesterol metabolism (EC 2.5.1.1)	1.5	2.2	0.000256
CG12818			288-amino-acid peptide	1.5	2.1	0.00132
CG4427	cbt	Cabut	Transcriptional activator/JNK cascade/autophagy cell death	1.3	1.7	0.00253
CG31543	Hph	HIF prolyl hydroxylase	Oxygen sensor	1.2	1.6	0.000776
CG11652			Diptaminde synthesis domain/diphtheria toxin resistance	1.3	1.6	5.03E−05
CG17836			HMG-1, HMGY DNA binding domain/transcription regulation	1.3	1.6	0.0286
CG9078	ifc	Infertile crescent	Sphingolipid delta-4 desaturase	1.3	1.6	0.0272
CG7741			Similar to yeast Cwfj/RNA splicing	1.2	1.6	2.93E−05
CG12317	JhI-21		L-Amino acid transporter	1.3	1.6	0.00682
CG10146	AttA	Attacin-A	Antimicrobial peptide	1.2	1.6	0.0322
CG3424	path	Pathetic	Amino acid/polyamine transporter	1.2	1.5	0.0289
CG12358	Paip2	Poly(A)-binding protein-interacting protein 2	Negative regulator of translation	1.2	1.5	0.00815
CG3767	JhI-26	Juvenile hormone-inducible protein 26	Putative CHK domain (choline kinase)	1.2	1.5	0.0103
CG1882			Alpha/beta hydrolase flod/aromatic compound metabolism	1.2	1.5	0.0106
CG17534	GstE9	Glutathione *S*-transferase E9	Glutathione transferase (EC 2.5.1.18)	1.2	1.5	0.0225
CG17533	GstE8	Glutathione *S*-transferase E8	Glutathione transferase (EC 2.5.1.18)	1.2	1.5	0.0608
CG5729	Dgp-1		GTP binding domain/putative translation factor	1.0	1.5	0.0227
CG1395	stg	String	Tyrosine/serine/threonine phosphatase (EC 3.1.3)	−1.3	−1.8	0.000200
CG7670			3′–5′ exonuclease and RNase H-like domains	−1.1	−1.7	0.00103
CG9552	rost	Rolling stone	Myoblast fusion	−1.1	−1.6	0.00548
CG30457				−1.3	−1.5	0.0344

[a] Genes whose expression was either up-regulated (positive values) or down-regulated (negative values) in *Candida*-treated cells compared to the *Candida*-free S2 cells at indicated time points.

sexes, it is noteworthy that female flies showed a higher resistance than the males. In contrast, the *d4E-BP*^{null} mutation had no effect on survival after infection with *S. cerevisiae*.

DISCUSSION

We used hemocyte-like *Drosophila* S2 cells, in coculture experiments with *C. albicans*, as a model representing cell-mediated innate immunity against infection by this pathogen. The transcriptional profiling of the S2 cells during the phagocytosis of *C. albicans* revealed a number of differentially expressed *Drosophila* genes in response to the pathogen. Eight of

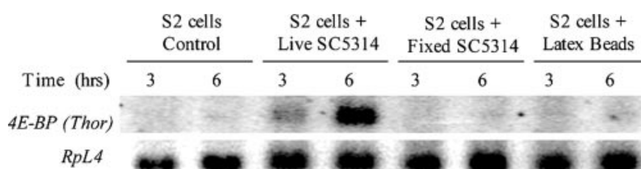

FIG. 3. Northern blot analysis of *Thor* gene induction in S2 cells. Lanes from left to right: S2 cells (control), S2 cells infected with live wild-type *C. albicans* (SC5314), S2 cells in the presence of paraformaldehyde-fixed *C. albicans*, and S2 cells ingesting latex beads, for 3 and 6 h.

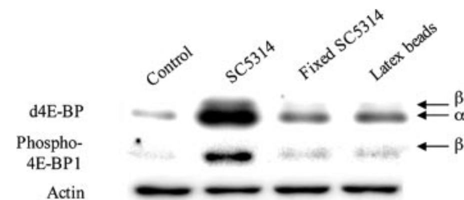

FIG. 4. Western blot of d4E-BP protein in S2 cells. Lanes from left to right: S2 cells alone (control), S2 cells infected with live *C. albicans* (SC5314), S2 cells in the presence of paraformaldehyde-fixed *C. albicans*, and S2 cells ingesting latex beads for 6 h. Identical amounts of total protein (30 μg) were analyzed by Western blotting with 1868 antibody to d4E-BP or phospho-4E-BP1 (thr37/46). α, active, nonphosphorylated isoform; β, hyperphosphorylated isoform; actin, loading control.

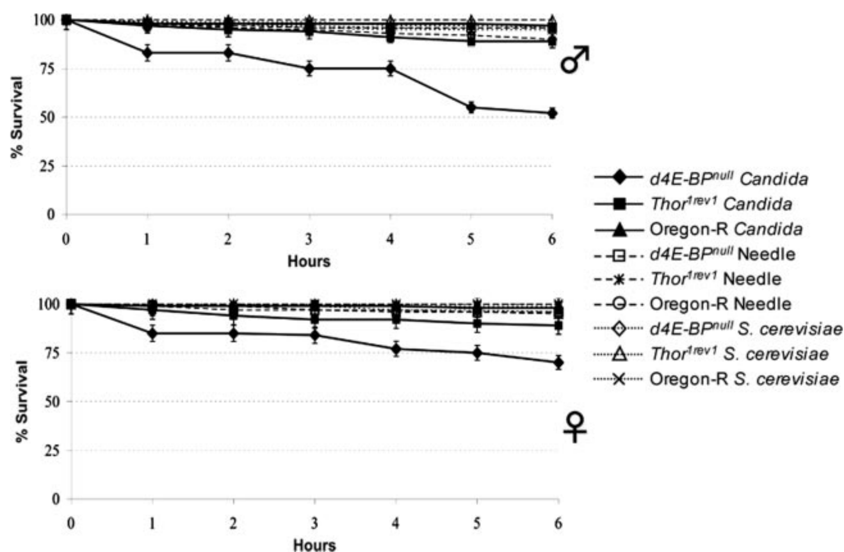

FIG. 5. Survival of *D. melanogaster* infected with *C. albicans* strain SC5314 is affected by the 4E-BP mutation. Survival of needle-pricked *d4E-BP*^{null} virgin flies was compared to the Oregon-R (wild type) and *Thor*^{1rev1} (revertant) flies. (A) The *d4E-BP*^{null} male mutant flies were approximately two times more susceptible to the infection with *C. albicans* during the first 6 h than the wild-type and revertant flies. (B) The *d4E-BP*^{null} virgin female flies were 1.5 times more sensitive to the *Candida* infection than the wild-type and revertant female flies. As a control, *d4E-BP*^{null}, Oregon-R, and *Thor*^{1rev1} flies of both genders were pricked with a sterile needle or with a needle coated with *S. cerevisiae*, which had no effect on the survival rate of *Drosophila* flies. Survival rates did not change significantly after 6 h. Each data point represents the mean of results from three independent experiments.

these genes were up-regulated more than 2-fold after 6 h of infection, and the remaining 19 genes were up- or down-regulated at least 1.5-fold. Among the differentially expressed genes, there were known immune-related genes, such as *AttA* or *Thor*, and several new candidates for genes involved in cell-based immunity. However, the relatively small number of genes modulated by the interaction with *C. albicans* indicates that *Drosophila* S2 cells do not rapidly regulate the transcription of a large number of genes in response to the presence of this fungus. In particular, several known immune-related genes, such as those encoding the antifungal peptides *Drosomycin* and *Metchnikowin* or other components of the Toll pathway, are not represented in this profile. Either their regulation might have occurred at times outside the scope of our experiments, or S2 cells lack the capacity to transcriptionally regulate them. Interestingly, the strong and rapid induction of the *d4E-BP (Thor*) gene suggests that regulation of translation could be a significant mechanism in *Drosophila* cell-based immunity. *Drosophila* 4E-BP is homologous to 4E-BPs from other species, and the phosphorylation sites in mammalian 4E-BP1 are conserved in d4E-BP (21). Recent studies have shown that *Drosophila* has a single d4E-BP (21), in contrast to mammals, which express three distinct 4E-BP proteins (24, 26). This makes *Drosophila* an excellent model to study the function of 4E-BP in the immune response to pathogens.

In addition to the Northern blots confirming the activation of *d4E-BP* in S2 cells "infected" by live *C. albicans* (Fig. 3), Western blot analysis (Fig. 4) confirmed the increase in d4E-BP protein level. We have shown that this protein also remains mostly in its active α form in the S2 cells in the presence of live *C. albicans*. Such an increase in the level of the active hypophosphorylated d4E-BP protein would compete with the formation of the cap-binding complex and inhibit cap-dependent translation.

To date, the only transcription factor known to regulate the transcription of *d4E-BP* is dFOXO, which is negatively regulated by insulin and positively regulated by different cellular stresses, through the forkhead response element in the *d4E-BP* gene promoter (14, 33). However, an additional signaling pathway might target d4E-BP in response to infection in flies, since our transcriptional analysis also failed to detect a change in expression of the *FOXO* gene (the gene was up-regulated by a maximum of 1.1-fold at 6 h post-*Candida* infection) as well as *dInr*, the insulin receptor gene positively regulated by FOXO (27) (Table 1).

In this work, we have established the S2 cell line as a model for the study of gene expression during host-*Candida* interaction. We used transcriptional profiling to identify several candidates for new immune-related genes in *Drosophila* and placed d4E-BP as an important player in defense against *C. albicans* infection. In further studies, the signaling pathway directing the expression and phosphoregulation of d4E-BP in the *Drosophila* immune response should be of particular interest.

ACKNOWLEDGMENTS

We thank N. Sonenberg for the anti-d4E-BP antibodies.

This work was supported by Canadian Institutes of Health Research (CIHR) grant to M.W. and D.Y.T. A.L. gratefully acknowledges a Canadian Government Laboratory Visiting Fellowship.

REFERENCES

1. **Abrams, J. M., A. Lux, H. Steller, and M. Krieger.** 1992. Macrophages in Drosophila embryos and L2 cells exhibit scavenger receptor-mediated endocytosis. Proc. Natl. Acad. Sci. USA **89:**10375–10379.

2. **Alarco, A. M., A. Marcil, J. Chen, B. Suter, D. Thomas, and M. Whiteway.** 2004. Immune-deficient Drosophila melanogaster: a model for the innate immune response to human fungal pathogens. J. Immunol. **172:**5622–5628.
3. **Ashman, R. B., and J. M. Papadimitriou.** 1995. Production and function of cytokines in natural and acquired immunity to *Candida albicans* infection. Microbiol. Rev. **59:**646–672.
4. **Berlanga, J. J., A. Baass, and N. Sonenberg.** 2006. Regulation of poly(A) binding protein function in translation: characterization of the Paip2 homolog, Paip2B. RNA **12:**1556–1568.
5. **Bernal, A., and D. A. Kimbrell.** 2000. Drosophila Thor participates in host immune defense and connects a translational regulator with innate immunity. Proc. Natl. Acad. Sci. USA **97:**6019–6024.
6. **Bernal, A., R. Schoenfeld, K. Kleinhesselink, and D. A. Kimbrell.** 2004. Loss of *Thor*, the single 4E-BP gene of *Drosophila*, does not result in lethality. Drosoph. Inf. Serv. **87:**81–84.
7. **Brennan, C. A., and K. V. Anderson.** 2004. Drosophila: the genetics of innate immune recognition and response. Annu. Rev. Immunol. **22:**457–483.
8. **Carlson, M., and D. Botstein.** 1982. Two differentially regulated mRNAs with different 5′ ends encode secreted with intracellular forms of yeast invertase. Cell **28:**145–154.
9. **Clemens, M. J.** 2001. Translational regulation in cell stress and apoptosis. Roles of the eIF4E binding proteins. J. Cell. Mol. Med. **5:**221–239.
10. **Fonzi, W. A., and M. Y. Irwin.** 1993. Isogenic strain construction and gene mapping in Candida albicans. Genetics **134:**717–728.
11. **Franc, N. C., P. Heitzler, R. A. Ezekowitz, and K. White.** 1999. Requirement for croquemort in phagocytosis of apoptotic cells in Drosophila. Science **284:**1991–1994.
12. **Gillum, A. M., E. Y. Tsay, and D. R. Kirsch.** 1984. Isolation of the Candida albicans gene for orotidine-5′-phosphate decarboxylase by complementation of S. cerevisiae ura3 and E. coli pyrF mutations. Mol. Gen. Genet. **198:**179–182.
13. **Gingras, A. C., S. P. Gygi, B. Raught, R. D. Polakiewicz, R. T. Abraham, M. F. Hoekstra, R. Aebersold, and N. Sonenberg.** 1999. Regulation of 4E-BP1 phosphorylation: a novel two-step mechanism. Genes Dev. **13:**1422–1437.
14. **Junger, M. A., F. Rintelen, H. Stocker, J. D. Wasserman, M. Vegh, T. Radimerski, M. E. Greenberg, and E. Hafen.** 2003. The Drosophila forkhead transcription factor FOXO mediates the reduction in cell number associated with reduced insulin signaling. J. Biol. **2:**20.
15. **Kleijn, M., G. C. Scheper, M. L. Wilson, A. R. Tee, and C. G. Proud.** 2002. Localisation and regulation of the eIF4E-binding protein 4E-BP3. FEBS Lett. **532:**319–323.
16. **Lemaitre, B., J. M. Reichhart, and J. A. Hoffmann.** 1997. Drosophila host defense: differential induction of antimicrobial peptide genes after infection by various classes of microorganisms. Proc. Natl. Acad. Sci. USA **94:**14614–14619.
17. **Lorenz, M. C., and J. Heitman.** 1997. Yeast pseudohyphal growth is regulated by GPA2, a G protein alpha homolog. EMBO J. **16:**7008–7018.
18. **Martchenko, M., A. M. Alarco, D. Harcus, and M. Whiteway.** 2004. Superoxide dismutases in Candida albicans: transcriptional regulation and functional characterization of the hyphal-induced SOD5 gene. Mol. Biol. Cell **15:**456–467.
19. **Meister, M., and M. Lagueux.** 2003. Drosophila blood cells. Cell. Microbiol. **5:**573–580.
20. **Miron, M., P. Lasko, and N. Sonenberg.** 2003. Signaling from Akt to FRAP/TOR targets both 4E-BP and S6K in *Drosophila melanogaster*. Mol. Cell. Biol. **23:**9117–9126.
21. **Miron, M., J. Verdu, P. E. Lachance, M. J. Birnbaum, P. F. Lasko, and N. Sonenberg.** 2001. The translational inhibitor 4E-BP is an effector of PI(3)K/Akt signalling and cell growth in Drosophila. Nat. Cell Biol. **3:**596–601.
22. **Nantel, A., D. Dignard, C. Bachewich, D. Harcus, A. Marcil, A. P. Bouin, C. W. Sensen, H. Hogues, M. van het Hoog, P. Gordon, T. Rigby, F. Benoit, D. C. Tessier, D. Y. Thomas, and M. Whiteway.** 2002. Transcription profiling of Candida albicans cells undergoing the yeast-to-hyphal transition. Mol. Biol. Cell **13:**3452–3465.
23. **Neal, S. J., M. L. Gibson, A. K. So, and J. T. Westwood.** 2003. Construction of a cDNA-based microarray for Drosophila melanogaster: a comparison of gene transcription profiles from SL2 and Kc167 cells. Genome **46:**879–892.
24. **Pause, A., G. J. Belsham, A. C. Gingras, O. Donze, T. A. Lin, J. C. Lawrence, Jr., and N. Sonenberg.** 1994. Insulin-dependent stimulation of protein synthesis by phosphorylation of a regulator of 5′-cap function. Nature **371:**762–767.
25. **Pearson, A. M., K. Baksa, M. Ramet, M. Protas, M. McKee, D. Brown, and R. A. Ezekowitz.** 2003. Identification of cytoskeletal regulatory proteins required for efficient phagocytosis in Drosophila. Microbes Infect. **5:**815–824.
26. **Poulin, F., A. C. Gingras, H. Olsen, S. Chevalier, and N. Sonenberg.** 1998. 4E-BP3, a new member of the eukaryotic initiation factor 4E-binding protein family. J. Biol. Chem. **273:**14002–14007.
27. **Puig, O., M. T. Marr, M. L. Ruhf, and R. Tjian.** 2003. Control of cell number by Drosophila FOXO: downstream and feedback regulation of the insulin receptor pathway. Genes Dev. **17:**2006–2020.
28. **Ramet, M., P. Manfruelli, A. Pearson, B. Mathey-Prevot, and R. A. Ezekowitz.** 2002. Functional genomic analysis of phagocytosis and identification of a Drosophila receptor for E. coli. Nature **416:**644–648.
29. **Rocha, C. R., K. Schroppel, D. Harcus, A. Marcil, D. Dignard, B. N. Taylor, D. Y. Thomas, M. Whiteway, and E. Leberer.** 2001. Signaling through adenylyl cyclase is essential for hyphal growth and virulence in the pathogenic fungus Candida albicans. Mol. Biol. Cell **12:**3631–3643.
30. **Romani, L., and S. H. Kaufmann.** 1998. Immunity to fungi: editorial overview. Res. Immunol. **149:**277–281.
31. **Roy, G., M. Miron, K. Khaleghpour, P. Lasko, and N. Sonenberg.** 2004. The *Drosophila* poly(A) binding protein-interacting protein, dPaip2, is a novel effector of cell growth. Mol. Cell. Biol. **24:**1143–1154.
32. **Stroschein-Stevenson, S. L., E. Foley, P. H. O'Farrell, and A. D. Johnson.** 2006. Identification of Drosophila gene products required for phagocytosis of Candida albicans. PLoS Biol. **4:**e4.
33. **Tettweiler, G., M. Miron, M. Jenkins, N. Sonenberg, and P. F. Lasko.** 2005. Starvation and oxidative stress resistance in Drosophila are mediated through the eIF4E-binding protein, d4E-BP. Genes Dev. **19:**1840–1843.
34. **von der Haar, T., J. D. Gross, G. Wagner, and J. E. McCarthy.** 2004. The mRNA cap-binding protein eIF4E in post-transcriptional gene expression. Nat. Struct. Mol. Biol. **11:**503–511.