Supplementary Note 11: Traf6 proteins

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Introduction

TRAF6 is an adaptor protein that acts in several signaling pathways in the immune system of vertebrates (see Walsh et al. 2015 for review). This multifunctionality stems from its ability to interact with many different proteins. TRAF6 also acts as a non-conventional E3 ubiquitin ligase. In the Toll pathway, TRAF6 interacts with the IRAK complex to activate the TAK1 complex by ubiquitination (reviewed by Valanne et al. 2011).

There have been mixed reports as to whether the Drosophila ortholog of TRAF6, also known as dTRAF2, functions in Drosophila innate immunity. Shen et al. (2001) showed that TRAF6 can interact with Pelle *in vitro* and that the two proteins act synergistically to activate Dorsal when transfected into Schneider cells. Moreover, a null mutant of TRAF6 appears to have a deficient immune system, showing reduced transcription of the antimicrobial peptide genes *diptericin and drosomycin* in response to infection (Cha et al. 2003). In contrast, Sun et al. (2002) reported that RNAi against TRAF6 in cultured cells with constitutively active Toll receptors had no effect on the expression of a *drosomycin* reporter construct, suggesting that TRAF6 is not required for Toll signaling.

As in vertebrates, *Drosophila* TRAF6 has been shown to function in other pathways besides Toll. There have been reports of TRAF6 acting in JNK (Tang et al. 2013) and Notch (Mishra et al. 2014) signaling.

Most insects seem to have a single TRAF6 ortholog, although duplications have occasionally been described (Table 1).

Organism	Order	# of TRAF6 genes
Tribolium castaneum	Coleoptera	1
Drosophila melanogaster	Diptera	1
Plutella xylostella	Lepidoptera	2
Bombyx mori	Lepidoptera	1
Cimex lectularius	Hemiptera	1

Table 1. Gene counts of *TRAF6* orthologs in several insects.

Methods

Drosophila TRAF6 was used to query the predicted *D. citri* protein sets (Diacit_International_psyllid_consortium_proteins_v1 and Diacit_RefSeq_proteins_Release_100) at i5k@NAL. A locus encoding a putative TRAF6 ortholog

was identified and manually annotated in Web Apollo. The predicted protein was BLASTed against Insecta with NCBI BLAST to verify its identity. We also performed a BLAST search of the *D. citri* MCOT set at citrusgreening.org. Multiple alignments were performed using MUSCLE (http://www.ebi.ac.uk/Tools/msa/muscle/). We used MEGA7 to construct a phylogenetic tree via the neighbor-joining method. Sequences for phylogenetic analysis were obtained from NCBI, FlyBase, ImmunoDB and the Bordenstein Lab (NSF DEB-1046149).

Results

We used *Drosophila melanogaster* TRAF6 to BLAST against *Diaphorina citri* (*D. citri*) predicted protein sets. Matching proteins were encoded on two separate scaffolds, gi|645498610|ref|NW_007379755.1and gi|645508310|ref|NW_007377463.1. Multiple alignments showed that the proteins were virtually identical (data not shown), making it unlikely that they were actually separate loci. Both scaffolds had duplications of portions of the TRAF6-encoding sequence, probably due to assembly problems. The longest predicted protein on the longest scaffold (XP_008472878.1 on gi|645508310|ref|NW_007377463.1) was used to BLAST the MCOT data set. A protein encoded by a *de novo*-assembled transcript, MCOT13375.2.CT, differed by only one amino acid, suggesting that our gene model is accurate. We also BLASTed the predicted protein from our gene model against insect proteins at NCBI. The top matches were TRAF6 proteins (Table 2.) We therefore named our gene model TRAF6.

Predicted Protein	Top BLASTp hit	Bitscore	E value	Percent Identity
TRAF6	TNF receptor-associated factor 6 [Papilio xuthus]	157	3e-40	27%

Table 2. BLAST analysis of the predicted TRAF6 protein.

We constructed a phylogenetic tree using predicted TRAF6 proteins from *D. citri* and other insects (Figure 1). Traf6 groups with TRAF6 from the bed bug, *Cimex lectularius.*
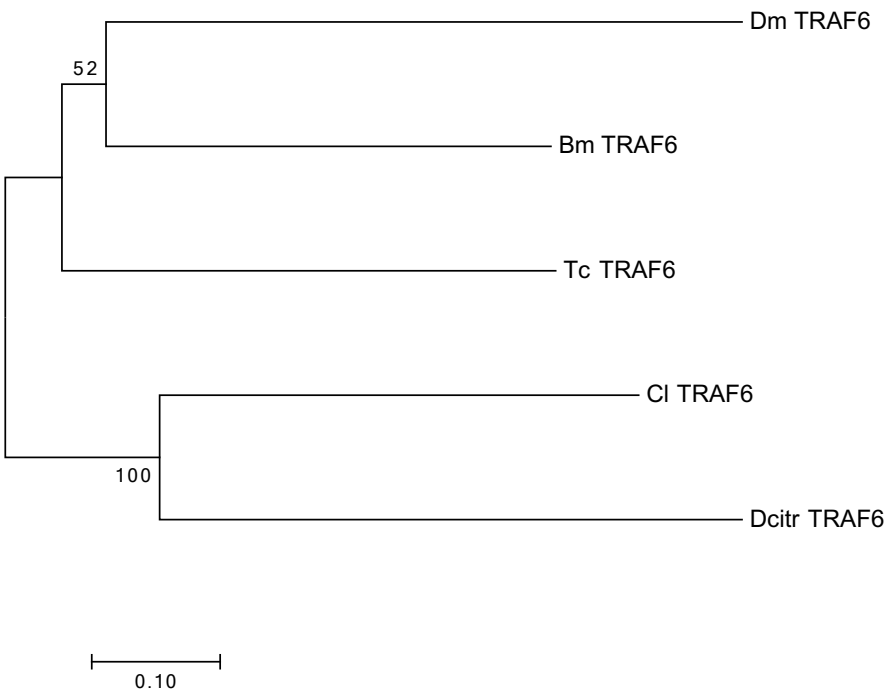
Figure 1. A phylogenetic tree constructed with TRAF6 orthologs from *Bombyx mori* (Bm), *Cimex lectularius* (Cl), *Tribolium castaneum* (Tc), *Drosophila melanogaster* (Dm), and *Diaphorina citri* (Dcitr).

References

1. Cha, G.-H., Cho, K., Lee, J., Kim, M., Kim, E., Park, J., Lee, S., and Chung, J. (2003). Discrete Functions of TRAF1 and TRAF2 in Drosophila melanogaster Mediated by c-Jun N-Terminal Kinase and NF-κB-Dependent Signaling Pathways. Mol Cell Biol*23*, 7982–7991.

2. Mishra, A., Sachan, N., Mutsuddi, M., and Mukherjee, A. (2014). TRAF6 is a novel regulator of Notch signaling in Drosophila melanogaster. Cell Signal *26*, 3016–3026.

3. Shen, B., Liu, Skolnik, E., and Manley, J. (2001). Physical and functional interactions between Drosophila TRAF2 and Pelle kinase contribute to Dorsal activation. Proc Natl Acad Sci *98*, 8596–8601.

4. Sun, H., Bristow, B., Qu, G., and Wasserman, S. (2002). A heterotrimeric death domain complex in Toll signaling. Proc Natl Acad Sci *99*, 12871–12876.

5. Tang, H.-W., Liao, H.-M., Peng, W.-H., Lin, H.-R., Chen, C.-H., and Chen, G.-C. (2013). Atg9 Interacts with dTRAF2/TRAF6 to Regulate Oxidative Stress-Induced JNK Activation and Autophagy Induction. Developmental Cell 489–503.

6. Valanne, S., Wang, J.-H., and Rämet, M. (2011). The Drosophila Toll Signaling Pathway. J Immunol *186*, 649–656.

7. Walsh, M., Lee, J., and Choi, Y. (2015). Tumor necrosis factor receptor- associated factor 6 (TRAF6) regulation of development, function, and homeostasis of the immune system. Immunological Reviews 72–92.