Supplementary Note 10: MyD88 proteins

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Introduction

MyD88 is a component of the Toll pathway in both vertbrates and invertebrates. *Drosophila* MyD88 has a Toll/Interleukin 1-R (TIR) domain that binds to the Toll TIR (Horng and Medzhitov, 2001; Tauszig-Delamasure et al 2002) and a death domain that interacts with Tube (Sun et al 2002). The formation of a trimeric complex containing MyD88, Tube and Pelle (Sun et al. 2002) allows Pelle to phosphorylate Cactus (Daigneault et al 2013). Once Cactus is phosphorylated, it is degraded, releasing its inhibition of the transcription factors Dorsal and/or Dif and allowing them to move to the nucleus to activate their target genes (Wu and Anderson, 1998).

MyD88 is found in a single copy in almost all insect genomes that have been examined (see Viljakainen 2015 and Table 1). Exceptions include the brown planthopper *Nilaparvata lugens*, which has two *MyD88* genes (Bao et al. 2015), and the diamondback moth *Plutella xylostella* where *MyD88* has not been found (Xia et al. 2015).

Organism	Order	# of MyD88 genes
Tribolium castaneum	Coleoptera	1
Anopheles gambiae	Diptera	1
Drosophila melanogaster	Diptera	1
Acyrthosiphon pisum	Hemiptera	1
Nilaparvata lugens	Hemiptera	2
Apis mellifera	Hymenoptera	1
Nasonia vitripennis	Hymenoptera	1
Linepithsma humile	Hymenoptera	1
Bombyx mori	Lepidoptera	1
Plutella xylostella	Lepidoptera	0?

Table 1. Gene counts of *MyD88* orthologs in representative insects. The question mark indicates that the absence of *MyD88* has not been proven.

Methods

Oncopeltus fasciatus MyD88 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 MyD88 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. 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 *Oncopeltus fasciatus* MyD88 to BLAST against *Diaphorina citri* (*D. citri*) predicted proteins at i5k. There were only two predicted proteins that showed significant identity to *Oncopeltus* MyD88, and both mapped to a single locus on gi|645507901|ref|NW_007377556.1. The two predicted proteins were encoded by NCBI gene model (XM_008489802.1) and maker gene model (maker-s119-augustus-gene-0.138-mRNA-1). The two models differed primarily at the 5' end, with the maker model having additional 5' exons. BLAST searches with the two predicted proteins showed that the maker model was a fusion of two genes. Comparison of the NCBI model to RNA-seq data suggested that it was missing one exon near the 3' end, so we manually merged that exon from the maker model with the NCBI model. The new model was named *MyD88*. We performed a BLAST with MyD88 against MCOT to try to obtain independent verification for our model, but the only match was to another predicted from the genome assembly.

When MyD88 is used as a query against other insect proteins, the top hit is bed bug MyD88 (Table 2). The domain analysis from NCBI BLAST indicates that, as expected, MyD88 has both a death domain and a TIR domain.

Predicted Protein	Top BLASTp hit	Bitscore	E value	Percent Identity
MyD88	PREDICTED: myeloid differentiation primary response protein MyD88 [Cimex lectularius]	117	2e-27	31%

Table 2. BLAST analysis of the predicted MyD88 protein.

We constructed a phylogenetic tree using MyD88 proteins from a variety of insects. Surprisingly, MyD88 appears to be a outgroup to all of the other proteins rather than clustering with the hemipteran MyD88 orthologs (Fig. 1). To understand this result, we compared BLAST alignments using either MyD88 or its closest match, bed bug (*Cimex lectularius*) MyD88, as the query sequence. The results suggest that MyD88 has diverged more rapidly than other MyD88 orthologs. While bed bug MyD88 is the best hit for MyD88, the converse is not true. MyD88 does not even appear on the list of top bed bug MyD88 matches. The stretches of identity shared between MyD88 and bed bug MyD88 are noticeably shorter than those shared by bed bug MyD88 with other insect orthologs. The significance of the rapid divergence of MyD88 is unclear, but it could have implications for Toll signaling in *D. citri*.
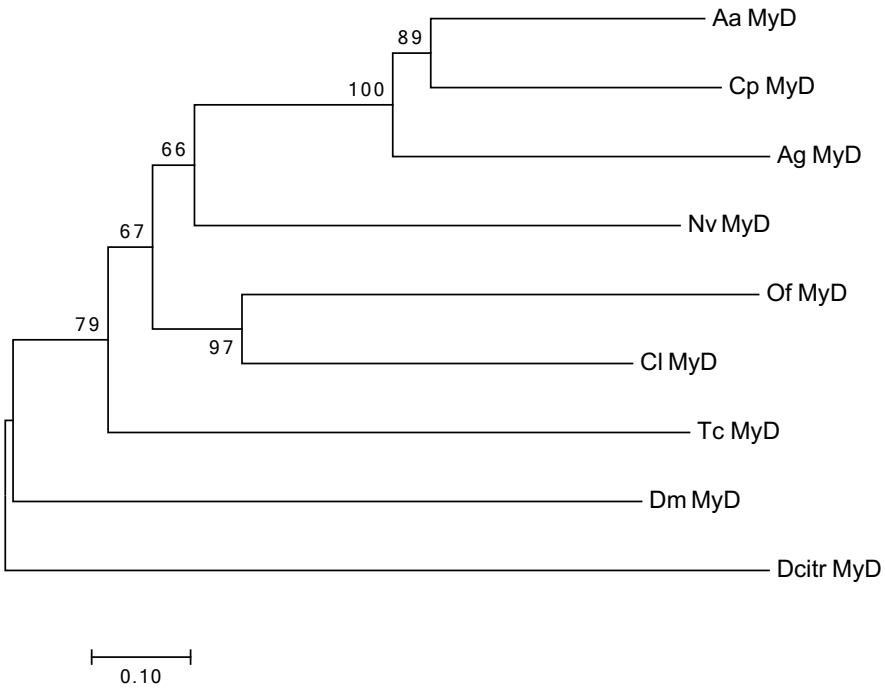
Figure 1. A phylogenetic tree constructed with MyD88 orthologs from *Aedes aegypti* (Aa), *Culex pipiens* (Cp), *Anopheles gambiae* (Ag), *Nasonia vitripennis* (Nv), *Oncopeltus fasciatus* (Of), *Cimex lectularius* (Cl), *Tribolium castaneum* (Tc), *Drosophila melanogaster* (Dm), and *Diaphorina citri* (Dcitr).

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