Supplementary Note 9: Pelle proteins

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Introduction

Pelle is a protein kinase that is a component of the Toll pathway. In *Drosophila*, it is responsible for the phosphorylation of the inhibitory protein Cactus (Daigneault et al 2013), targeting it for degradation so that the transcription factor Dorsal and/or Dif can translocate to the nucleus and activate target genes (Wu and Anderson, 1998). Pelle activity requires the formation of a heterotrimeric complex with MyD88 and Tube (Sun et al. 2002)

Tube and Pelle are paralogs, arising from an ancient duplication before the separation of the vertebrate and invertebrate lineages. They are orthologs of vertebrate IRAK4 and IRAK1, respectively (Towb et al. 2009). In Drosophila, Pelle and Tube are easily distinguished because Tube has no protein kinase domain. However, in many other insects both proteins have a death domain and a protein kinase domain (Towb et al. 2009. *pelle* is a single-copy gene in those insects that have been sequenced (see Viljakainen 2015 and Table 1).

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

Table 1. Gene counts of *pelle* orthologs in representative insects.

Methods

Pelle orthologs were used to query the predicted *D. citri* protein sets (Diacit_International_psyllid_consortium_proteins_v1 and Diacit_RefSeq_proteins_Release_100) at i5k@NAL. The best hits was BLASTed against Insecta with NCBI BLAST to check their orthology. The locus encoding Pelle was identified and manually annotated in Web Apollo. 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 BLAST searches and phylogenetic analysis were obtained from NCBI, FlyBase, ImmunoDB and the Bordenstein Lab (NSF DEB-1046149).

Results

We used *Acyrthosiphon pisum* Pelle to BLAST against the *Diaphorina citri* (*D. citri*) predicted proteins at i5K. This identified a locus on gi|645505624|ref|NW_007378073.1 that was the clear best match. The next best hit (which had a much lower bitscore) was the Tube ortholog (see *tube* gene report), suggesting that there is only one *pelle* gene in the current *D. citri* assembly.

When the NCBI-predicted Pelle protein was used to BLAST against other insect proteins, the best matches were Pelle orthologs from other hemipterans (Table 2). These proteins show identity to the predicted Pelle protein in all regions except the C-terminus of the predicted protein. However, RNA-seq data indicates that the region encoding the C-terminus appears in the majority of the transcripts. Therefore, we accepted the NCBI annotation as the gene model for *pelle*. We then used Pelle to BLAST the MCOT predicted proteins.   However, the only match was to another assembly-predicted protein, so we were unable to find independent verification of the gene model.

Predicted Protein	Top Blastp hit	Bitscore	E value	Percent Identity
Serine threonine-protein kinase pelle	PREDICTED: serine/threonine-protein kinase pelle [Acyrthosiphon pisum]	435	6e-142	48%

Table 2. BLAST analysis of the predicted Pelle protein

We constructed a phylogenetic tree containing Pelle and Tube orthologs from several insects. Pelle clearly clustered with other Pelle orthologs (Fig. 1), supporting our conclusion that the locus encodes a Pelle ortholog.
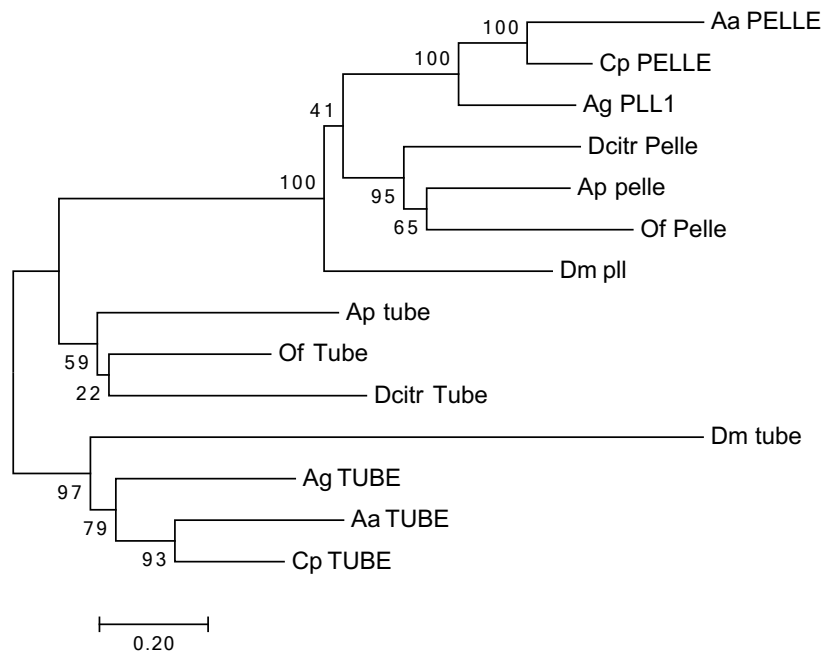
Figure 1. A phylogenetic tree constructed with Pelle and Tube orthologs from several insects.

References

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