# Supplementary Note 8: Tube proteins

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## Introduction

Tube is a component of the Toll pathway, which is important for development and innate immunity in insects. In *Drosophila*, Tube acts as the central component in a complex with MyD88 and Pelle, each of which binds to a separate site in Tube's death domain (Sun et al. 2002). This interaction, which occurs when Toll is activated, allows Pelle to phosphorylate the inhibitory protein Cactus, targeting it for degradation (Daigneault et al 2013). Degradation of Cactus allows the Nf-kappa B transcription factors Dorsal and/or Dif to enter the nucleus and activate target genes (Wu and Anderson, 1998)

The presence of a single ortholog of *tube* appears to be the general rule in insect genomes (see Viljakainen 2015 and Table 1). However, many of these orthologs differ from *Drosophila* Tube in that they have a protein kinase domain in addition to the conserved death domain (Towb et al. 2009). This discovery allowed Towb et al. (2009) to determine that Tube and its binding partner Pelle are actually paralogous genes, which arose from a very ancient duplication. They also concluded that Tube is the ortholog of vertebrate IRAK4, while Pelle is orthologous to vertebrate IRAK1.

| 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 *tube* orthologs in representative insects.

## Methods

Tube 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. A locus encoding a putative Tube 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 to compare our gene model to *de novo* assembled transcripts. Multiple

alignments were performed in Muscle (http://www.ebi.ac.uk/Tools/msa/muscle/). We used MEGA7 to construct a phylogenetic tree via the neighbor-joining method. Sequences for multiple alignment and phylogenetic analysis were obtained from NCBI, FlyBase, ImmunoDB and the Bordenstein Lab (NSF DEB-1046149).

**Results**

We used the *Tribolium castaneum* Tube ortholog (Towb et al. 2009) to perform a BLAST search of the *Diaphorina citri* (*D. citri*) predicted proteins. Several almost identical predicted proteins encoded by loci on several different scaffolds were identified. Most of these scaffolds are rather short and they appear to be artifacts of incomplete assembly rather than separate loci. We chose the locus on the longest scaffold (gi|645501093|ref|NW_007379137.1) for annotation. The NCBI model (XM_008481952.1) was a good match with the RNA-seq data, so it was renamed *tube*.

The Tube predicted protein was then used to BLAST the *D. citri* MCOT protein set. Two proteins encoded by de novo-assembled transcripts were nearly exact matches. MCOT03326.3.CO has four amino acid differences scattered throughout the protein. MCOT03326.1.CT appears to be truncated at the N-terminus and also has hree of the four amino acid differences seen in MCOT03326.3.CO. Finding only minor differences between the MCOT proteins and the gene model protein increases our confidence in the accuracy of the gene model.

We next used Tube to BLAST against other insect proteins. The top hit is annotated as a putative plant-type serine-threonine protein kinase from the body louse *Pediculus humanus corporis* (Table 2). Moving down the list were several interleukin-1 receptor-associated kinase 4-like (IRAK-4-like) proteins from other Hemipterans. Since Tube is orthologous to vertebrate IRAK4, it appears that these are Tube orthologs. However, the list also includes several good matches that are annotated as Pelle orthologs.

| **Predicted Protein** | **Top Blastp hit** | **Bitscore** | **E value** | **Percent Identity** |
|---|---|---|---|---|
| Tube | serine-threonine protein kinase, plant-type, putative [Pediculus humanus corporis] | 295 | 3e-93 | 36% |

Table 2. BLAST analysis of the predicted Tube protein.

*D. citri* has a separate *pelle* ortholog (see *pelle* gene report), but we wanted to be sure that *tube* was really *tube* and not another *pelle* gene. In most insects, Tube and Pelle both have a death domain and a protein kinase domain. However, there are a few diagnostic residues that distinguish the two groups. Tube orthologs have previously been shown to share several conserved death domain residues that are required for interaction with MyD99 and Pelle (Towb

et al. 2009). Multiple alignment with *Anopheles gambiae* Tube indicates that the three residues required for MyD99 interaction are perfectly conserved in Tube, but the residues involved in Pelle interaction are only weakly conserved. Most strikingly, a GP-A motif in the Pelle interaction site that is conserved in previously described Tube orthologs (Towb et al. 2009), is not found in Tube. The significance of these differences for interaction of Tube and Pelle in *D. citri* is not clear. Another diagnostic residue is found in the protein kinase domain. Tube orthologs have an arginine (R) preceding an aspartate (D) in sequence subdomain VI, putting them in the RD class of protein kinases (Towb et al. 2009). Pelle orthologs have a glycine (G) instead. Tube has an R in that position, as would be expected for a Tube ortholog. Taken together, these observations suggest that the Tube is, in fact, a Tube ortholog.

We constructed a phylogenetic tree to compare Tube and Pelle to Tube and Pelle orthologs from several other insects. As expected, Dcitri_Tube clustered with other Tube orthologs, while Dcitri_Pelle grouped with other Pelle orthologs (Fig. 1)

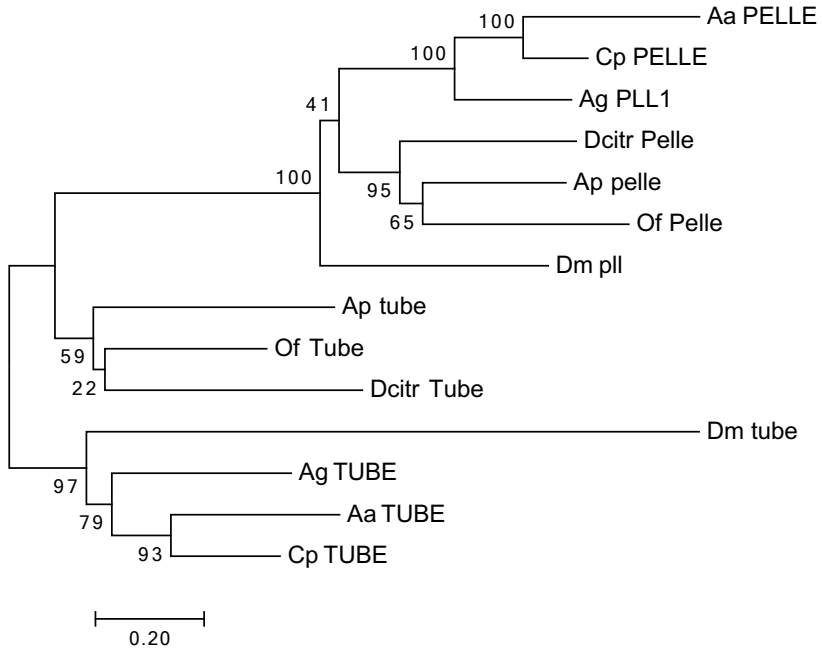


Figure 1. A phylogenetic tree constructed with Pelle and Tube orthologs from several insects.

**References**


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