Chitin Biosynthesis Genes in *Diaphorina citri*

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Abstract

The polysaccharide chitin is critical for the formation of many insect structures, including the exoskeleton, and is required for normal development. Here we report the annotation of three genes from the chitin synthesis pathway in the Asian citrus psyllid, *Diaphorina citri*. Most insects have two chitin synthase (CHS) genes but, like other hemipterans, *D. citri* has only one. In contrast, *D. citri* is unusual among insects in having two UDP-N-acetylglucosamine pyrophosphorylase (UAP) genes. One of the *D. citri* UAP genes is broadly expressed, while the other is expressed predominantly in males. Our work helps pave the way for potential utilization of these genes as pest control targets.

Introduction

Chitin is a polysaccharide essential for insect development. It plays a crucial role in the development of the insect cuticle and exoskeleton, the peritrophic membrane of the midgut of some insects, and other structures such as the trachea, wing hinges and eggshell [1]. The biosynthetic pathway for chitin involves a variety of different enzymes which act on simple sugars such as glucose, trehalose and glycogen to produce intermediates that are subsequently converted into chitin. In the penultimate step of the chitin biosynthesis pathway, N-acetylglucosamine-1-phosphate is converted into UDP-N-acetylglucosamine. This reaction is catalyzed by the enzyme UDP-N-acetylglucosamine pyrophosphorylase (UAP) [1]. In the final step of the pathway, UDP-N-acetylglucosamine is converted to chitin by enzymes known as chitin synthases (CHS) [1]. Because chitin is essential for insect development, but is not found in mammals, the enzymes involved in its synthesis are considered attractive targets for pest control. Here we report the annotation of one CHS gene and two UAP genes in the Asian citrus psyllid, *Diaphorina citri*. Although most insects have two CHS genes, the presence of a single CHS gene is consistent with reports from other hemipteran genomes [2]. In contrast, *D. citri* seems to be unusual in having two UAP genes. RNA-Seq data indicates that one of the *D. citri* UAP genes is broadly expressed, while the other is expressed predominantly in males. Our manual annotation of these chitin biosynthesis genes provides improved gene targets for future experiments.

Chitin Synthase and UAP orthologs Identified in Insects									
	Drosophila melanogaster	*Anopheles gambiae*	*Aedes aegypti*	*Tribolium castaneum*	*Apis. mellifera*	*Nasonia vitripennis*	*Acyrthosiphon pisum*	*Bemisia tabaci*	*Diaphorina citri*
CHS1/A	1	1	1	1	1*	1*	1	1*	1
CHS2/B	1	1	1	1	1*	1*	0	0*	0
UAP	1	1	1	2	1	1	1	1*	2

Table 1: Chitin Synthase and UAP Orthologs in Insects from Representative Taxa. *D. citri* numbers were determined based on annotation of D. citri genome v 3.0. An asterisk (*) indicates the number was determined by BLAST analysis. All other numbers have been previously reported.

Results and Discussion

Chitin Synthases

Most insects have two *CHS* genes [1] (Table 1). Functional studies suggest that *CHS1*, also referred to as *CHSA*, produces the chitin essential for proper cuticle development. *CHS2*, also referred to as *CHSB*, is not required for cuticle development but is instead essential for proper development of the gut peritrophic membrane. Chitin synthases are the only enzymes in the chitin biosynthetic pathway that act specifically in the synthesis of chitin, and thus they are the most insect-specific targets for an RNA interference (RNAi) based insecticide. RNAi knockdown of either *CHS* gene is lethal in holometabolous insects [3].

Previous searches of three hemipteran genomes (*Acyrthosiphon pisum, Nilaparvata lugens* and *Rhodnius prolixus)* identified *CHS1* but not *CHS2*, suggesting that *CHS2* may have been lost in the hemipteran lineage [2]. This apparent loss of the chitin synthase gene required for peritrophic membrane development is correlated with the reported lack of peritrophic membranes in hemipterans [2,4]. Some functional and expression data are already available for *CHS* in *D. citri*. Lu et al [5] reported that *CHS* was expressed at high levels in most adult body tissues, but at low levels in midgut, as would be expected for a *CHS1* gene.. Two groups have reported increased lethality with RNAi-based knockdown of *CHS* in *D. citri* [5,6], supporting the claim that this gene is a good target for pest control.

As expected, we found only a single *CHS* gene in the *D. citri* v3 genome (Table 1). Sequences from several transcriptomes support the existence of two isoforms (Supplementary Table 1) that differ only in the use of one alternative exon and produce proteins with slightly different C-termini. Similar isoforms of *CHS1/A* have also been described in many other insects [1]. We found that both isoforms of *D. citri* CHS cluster in a monophyletic clade with CHS1 proteins from other insects (Supplemental Fig. 1), so we have named this gene *CHS1*.

Our manual annotation of *CHS1* corrects several errors that were present in the previous computationally-predicted annotation for *D. citri* CHS (XP_017303059). Changes to the model

include the addition of previously missing sequence and the removal of artifactually duplicated regions. Domain analysis with TMHMM Server, v. 2.0 indicates that the corrected CHS1-RA and CHS1-RB proteins have 15 transmembrane helices (data not shown) as expected for insect CHS proteins, rather than the 14 that were reported for the earlier version of the protein [5].

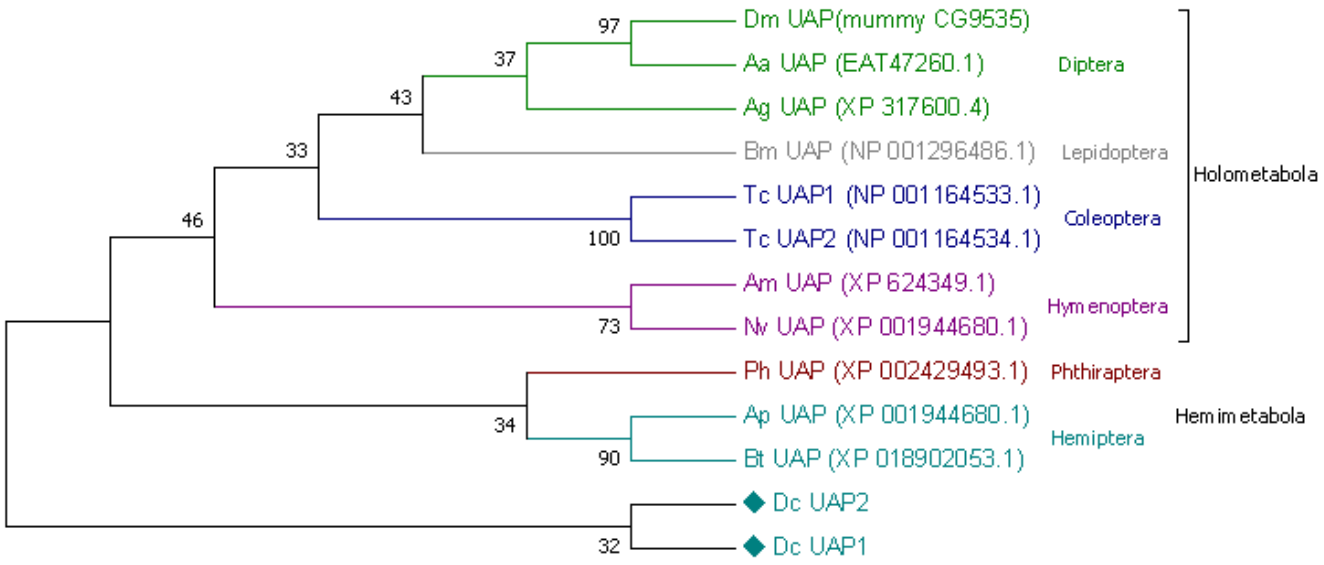

Fig. 1. Phylogenetic analysis of representative insect UAP orthologs in *Drosophila melanogaster* (Dm), *Anopheles gambiae* (Ag), *Aedes aegypti* (Aa), *Bombxy mori* (Bm), *Tribolium castaneum* (Tc), *Apis mellifera* (Am), *Nasonia vitripennis* (Nv), *Pediculus humanus* (Ph), *Acyrthosiphon pisum* (Ap), *Bemisia tabaci* (Bt) and *Diaphorina citri* (Dc). ClustalW software was used to perform multiple sequence alignments of full-length protein sequences and the tree was constructed with MEGA 7.0 software using the neighbor-joining with bootstrap consensus method. Colors represent insect orders and the *Diaphorina citri* proteins are marked with diamonds.

UDP-N-acetylglucosamine pyrophosphorylase (UAP)

In *Drosophila* there is a single gene encoding UAP. Mutants of *UAP* (also called *mummy*, *cabrio* and *cystic*) have defects in tracheal development, dorsal closure, eye development and nervous system function. Some of these developmental defects are due to UAP's role in chitin synthesis while others are due to the role UAP plays in glycosylation of other proteins [7]. Most insects appear to have a single *UAP* gene, although *Tribolium* has two (*UAP1* & *UAP2*) [7]. RNAi experiments showed that, in *Tribolium,* UAP1 is involved in the biosynthesis of chitin both in the cuticle and the peritrophic membrane, while UAP2 has roles in the modification of other macromolecules [7].

Interestingly, BLAST analysis of the *D. citri* MCOT transcriptome and further analysis of the *D. citri* v3 genome identified two *UAP* genes located on different chromosome-length scaffolds

(Table 1 and Supplementary Table 1), which we named *UAP1* and *UAP2*. The proteins encoded by these apparent paralogs share 50 percent identity distributed throughout the length of the proteins (Supplemental Fig. 2), which is very similar to the level of identity shared with UAP orthologs from closely related insect species (data not shown). Amino acid residues known to be important for substrate binding in the human UAP ortholog and conserved in the Tribolium UAP proteins [7] are also well conserved in the *D. citri* UAP proteins (Supplemental Fig. 2). Phylogenetic analysis (Fig. 1) suggests these two genes are not one to one orthologs of *Tribolium UAP1* and *UAP2*, but instead represent a lineage specific duplication.

We compared available expression data from the two *D. citri UAP* genes using the Psyllid Expression Network [8]. *D. citri UAP1* is expressed at moderate levels in all tissues and stages examined (Supplemental Fig. 3 and data not shown). *D. citri UAP2*, however, appears to be expressed at a low level in most male tissues, but shows little or no expression in the same tissues from females (Supplemental Fig. 3 and 4). In contrast, both Tribolium *UAP* genes are expressed in ovaries and testes. More detailed analysis of *UAP2* expression and function in males and females will be necessary to understand the role of this gene in *D. citri*.

Materials and Methods

D. citri genes in genome v3 [9] were identified by BLAST analysis of *D. citri* sequences with insect CHS and UAP orthologs. Reciprocal BLAST of the NCBI non-redundant protein database was used to confirm orthology. Manual annotation of genes was performed in Apollo using RNA-Seq reads, IsoSeq transcripts and *de novo*-assembled transcripts as evidence. Multiple alignments of the predicted *D. citri* proteins and their insect homologs were performed using Muscle (http://www.ebi.ac.uk/ Tools/msa/muscle/). Phylogenetic trees were constructed using full-length protein sequences in MEGA7.

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