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Comparative transcriptome analysis reveals networks of genes activated in the whitefly, *Bemisia tabaci* when fed on tomato plants infected with *Tomato yellow leaf curl virus*

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ABSTRACT

The whitefly *Bemisia tabaci* can transmit hundreds of viruses to numerous agricultural crops in the world. Five genera of viruses, including *Begomovirus* and *Crinivirus*, are transmitted by *B. tabaci*. There is little knowledge about the genes involved in virus acquisition and transmission by whiteflies. Using a comparative transcriptomics approach, we evaluated the gene expression profiles of whiteflies (*B. tabaci* MEAM1) after feeding on tomato infected by a begomovirus, *Tomato yellow leaf curl virus* (TYLCV), in comparison to a recent study, in which whiteflies were fed on tomato infected by the crinivirus, *Tomato chlorosis virus* (ToCV). The data revealed similar temporal trends in gene expression, but large differences in the number of whitefly genes when fed on TYLCV or ToCV-infected tomato. Transcription factors, cathepsins, receptors, and a hemocyanin gene, which is implicated in mediating antiviral immune responses in other insects and possibly virus transmission, were some of the genes identified.

1. Introduction

Begomoviruses (Family: *Geminiviridae*) are efficiently transmitted by the sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), resulting in crop losses estimated to exceed billions of U.S. dollars annually and threatening global food security (Stansly and Naranjo, 2010; Cock, 1993). Begomoviruses affect the production of a wide range of important crops, including beans, cassava, cotton, squash, sweet potato, and tomato. Two of the most devastating diseases caused by begomoviruses include tomato yellow leaf curl disease, which is widely distributed throughout the world (Lefeuvre et al., 2010), and cassava mosaic disease, which has reached pandemic levels in African countries (Legg et al., 2014).

Begomoviruses possess single-stranded DNA genomes of ~2700 nt and are encapsidated by viral coat proteins in an incomplete icosahedral structure (Navot et al., 1991). Many begomoviruses have bipartite genomes, termed DNA-A and DNA-B, while others, including *Tomato yellow leaf curl virus* (TYLCV) are monopartite and possess a single DNA-A-like genome component, containing six genes (Basak, 2016).

Begomoviruses and *B. tabaci* have been co-evolving for millions of years (Czosnek and Ghanim, 2012; Ghanim, 2014). These plant viruses are transmitted by *B. tabaci* in a persistent, circulative manner (Czosnek and Ghanim, 2012). Once ingested by an adult whitefly, begomovirus virions pass to the midgut, where they move across the midgut membrane to the hemolymph, possibly via receptor-mediated endocytosis (Rosen et al., 2015; Kollenberg et al., 2014), and circulate back into the primary salivary glands, where they are egested with saliva during insect feeding (Czosnek and Ghanim, 2012; Hunter et al., 1998). Several proteins have been implicated in the circulation of TYLCV in the whitefly, including two heat shock proteins (Ohnesorge and Bejarano, 2009; Gotz et al., 2012), and GroEL, a protein secreted by the secondary bacterial endosymbiont *Hamiltonella,* that is thought to stabilize virion passage by interacting with the viral coat protein in the hemolymph (Morin et al., 2000; Kliot and Ghanim, 2013). Cyclophilin B was shown

Abbreviations: MEAM1, Middle East-Asia Minor 1; TYLCV, *Tomato yellow leaf curl virus*; TYLCCNV, *Tomato yellow leaf curl China virus*; AAP, acquisition access period; ANP, atrial natriuretic peptide; LDLR, low-density lipoprotein receptor; LpR, lipophorin receptor; ToCV, *Tomato chlorosis virus*

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to interact with the coat protein of TYLCV in the whitefly midgut, eggs, and salivary glands (Kanakala and Ghanim, 2016), and knottin-1 has been shown to act as a suppressor of TYLCV by restricting the number of virions that can be acquired and transmitted by the whitefly (Hariton Shalev et al., 2016).

Although the majority of plant viruses transmitted by *B. tabaci* are begomoviruses, viruses in several other genera, including *Carlavirus, Crinivirus, Ipomovirus and Torradovirus* are also transmitted by whiteflies (Navas-Castillo et al., 2011). *B. tabaci* is a complex of cryptic species formerly referred to as, "biotypes" that can colonize over 1000 plant species (Abd-Rabou and Simmons, 2010), and exhibits a range of biological diversity, including host preference, reproductive incompatibility, insecticide resistance, secondary bacterial endosymbiont composition, and virus transmission potential (Zang et al., 2006; Houndété et al., 2010; Bedford et al., 1994). One of the most broadly distributed and damaging *B. tabaci* populations is the Middle-East Asia Minor 1 population (MEAM1; formerly known as the B biotype and *B. argentifolii*) (Rosen et al., 2015; Bellows et al., 1994).

TYLCV can affect the fitness of *B. tabaci* depending on the cryptic species (Jiu et al., 2007; Rubinstein and Czosnek, 1997), and even though evidence of TYLCV replication in the whitefly host was shown (Sinisterra et al., 2005; Pakkianathan et al., 2015), conflicting reports continue to emerge (Sanchez-Campos et al., 2016). Transovarial transmission of TYLCV was recently shown to occur in *B. tabaci*, with virus entry into the whitefly ovary mediated by interactions between vitellogenin and the viral coat protein (Wei et al., 2017). TYLCV is also capable of manipulating the settling, probing, and feeding behavior of the whitefly in a way that would facilitate virus transmission (Moreno-Delafuente et al., 2013; Liu et al., 2013; He et al., 2015; Jahan et al., 2014).

An earlier transcriptome study analyzed the whitefly's response to acquisition of another begomovirus, *Tomato yellow leaf curl China virus* (TYLCCNV), where 1606 genes were differentially expressed and involved in regulating cell cycle, primary metabolism, and cellular and humoral immunity (Luan et al., 2011). Although the TYLCCNV study and earlier expressed sequence tag (EST) libraries (Leshkowitz et al., 2006; Li et al., 2011) have contributed to understanding whitefly-begomovirus relationships, technology for generating gene expression data has significantly improved and two whitefly reference genomes are now available (Chen et al., 2016; Xie et al., 2017), which provide greatly improved annotation of gene expression data.

In this study, we were interested in understanding how the whitefly responds to feeding on tomato that is infected with TYLCV, arguably one of the ten most economically important plant viruses in the world (Scholthof et al., 2011). Using RNA-Seq analysis and three biological replicates per treatment, whitefly gene expression was analyzed after feeding on TYLCV-infected or uninfected tomato for acquisition access periods of 24 h, 48 h, and 72 h. The data were also compared to a published study that analyzed the response of *B. tabaci* MEAM1 to a semi-persistently transmitted crinivirus, *Tomato chlorosis virus* (ToCV), using the same experimental procedures (Kaur et al., 2017). Together, these data provide: 1) insight into how the whitefly responds to feeding on TYLCV-infected tomato plants during acquisition and early transmission periods, and 2) a comparison of similarities and differences between whiteflies fed on tomato plants infected by either of two distinct types of viruses (TYLCV and ToCV) with different modes of transmission. These data contribute to broader knowledge on the whitefly's molecular response to feeding on a virus-infected host and vector-virus relationships, which can serve as a basis for devising new strategies to control whiteflies and whitefly-transmitted viruses.

2. Materials and methods

2.1. Insect rearing, feeding assays, and RNA isolation

An isogenic whitefly colony was established from a single female *B. tabaci* MEAM1 and reared as previously described (Chen et al., 2015). A subset of the population was transferred to broccoli (*Brassica oleracea* L. var. botrytis), a non-host for TYLCV, and maintained in a greenhouse (26 ± 5 °C). The MEAM1 population was confirmed by PCR using established primers against the mitochondrial cytochrome oxidase 1 gene (Shatters et al., 2009).

The feeding conditions for whiteflies have been previously described in (Chen et al., 2016; Kaur et al., 2017). Briefly, ~1500 adult whiteflies of mixed ages per treatment were collected from the isogenic colony reared on broccoli plants, transferred to either TYLCV-infected or uninfected tomato (cv. Moneymaker) plants (cuttings) in two separate cages, and allowed to feed. The TYLCV-infected tomato plants were generated through whitefly transmission and the virus-infection status confirmed by PCR. Test plants were maintained in a greenhouse (25–30 °C and 14 h natural sunlight). Small cuttings (3–4 leaves) were taken from test plants that were at the 10–12 leaf stage and placed in a flask of water that was sealed with a sheet of parafilm to prevent whiteflies from entering. At the end of each pre-determined acquisition access period (AAP) of 24, 48, or 72 h, 200–500 live whiteflies were collected and immediately stored at −80 °C until processing. To confirm virus acquisition, RNA was isolated from 50 whiteflies fed on either healthy or TYLCV-infected tomato from each AAP prior to RNA isolation. To determine the percent of whiteflies that had acquired TYLCV, PCR analysis was conducted using DNA preparations from each of 10 individual whiteflies per treatment feeding on TYLCV-infected plants for each AAP. Results showed that 60% (6 of 10) of whiteflies were positive for TYLCV at 24 h, and 100% (10/10) were positive for either 48 h or 72 h. as expected, individual whiteflies fed on uninfected tomato plants tested negative for TYLCV (Supplementary Fig. S1). Therefore, the genes identified as differentially expressed in whiteflies feeding on TYLCV-infected versus uninfected tomato plants were most likely exhibiting differential expression due to their responses to the acquired TYLCV or the physiological changes in the TYLCV-infected plants.

Whitefly DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, USA) and used for PCR reactions containing 2X GoTaq Green Master Mix (Promega, USA) and primers specific to the V1 coat protein of a TYLCV, South Carolina, USA isolate (GenBank: DQ139329) (Supplementary Table S1). Three biological replicates were performed per treatment at each AAP. Total RNA was processed as previously described (Chen et al., 2016). For each sample, an aliquot of the RNA was used for validation by reverse transcription quantitative PCR (RT-qPCR) (see below) and the remainder was stored using RNAstable (Sigma-Aldrich, USA).

2.2. Transcriptome sequencing and analysis

RNA-Seq libraries were constructed, sequenced, and processed as previously described (Chen et al., 2016). Briefly, 18 strand-specific barcoded RNA-Seq libraries were pooled and sequenced using an Illumina HiSeq. 2500 system with the paired-end mode. Adaptor and low quality sequences were removed from the RNA-Seq reads. The cleaned reads were aligned to the ribosomal RNA database (Gurevich et al., 2013), the assembled *B. tabaci* mitochondrion genome, and the three bacterial endosymbiont genomes of *Candidatus* Portiera aleyrodidarum, *Hamiltonella*, and *Rickettsia* (Chen et al., 2016, http://www.whiteflygenomics.org). The aligned reads were filtered. The remaining high-quality cleaned paired-end reads were aligned to the reference *B. tabaci* genome (Chen et al., 2016). Raw counts for each predicted gene were normalized to fragments per kilobase of exon model per million mapped fragments (FPKM). Differentially expressed genes between the whiteflies fed on TYLCV and uninfected tomato plants were identified using edgeR (Robinson et al., 2010). The resulting raw *p* values were adjusted for multiple testing using the false discovery rate (FDR) (Benjamini and Hochberg, 1995). For each comparison, genes with an adjusted *p* value (FDR) less than 0.05 and fold

change greater than or equal to 1.5 were considered as significantly differentially expressed genes. Volcano plots were generated in R, and differentially expressed genes were assigned to gene ontology classes using Blast2GO (Conesa et al., 2005). Annotations of *B. tabaci* genes were all provided by alignment to the genome (Chen et al., 2016). Furthermore, differentially expressed genes were compared to those published in (Kaur et al., 2017) which reported the *B. tabaci* transcriptome response to feeding on plants infected with the semi-persistent, non-circulative crinivirus, *Tomato chlorosis virus* (ToCV).

2.3. RT-qPCR validation

Five genes differentially expressed at 24 h and four genes at 72 h were randomly selected for RT-qPCR analysis. Primer sequences used for these nine genes are listed in Supplementary Table S1. Approximately 2 µg of total RNA from each sample was reverse transcribed into cDNA using the SuperScript III First-Strand Synthesis for RT-PCR kit (Invitrogen, USA) and random hexamer primers according to the manufacturer's instructions. Quantitative PCR reactions were assembled in 20 µl triplicate reactions using 2X Brilliant II SYBR Green QPCR Low ROX Master Mix (Agilent Technologies, USA) according to the manufacturer's instructions. Reactions were performed using a Stratagene Mx3000p system (Stratagene, USA) and relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Two endogenous whitefly genes, *α-tubulin* and *70 kDa heat shock protein,* were used to normalize gene expression and no template controls were included for each reaction. Normalizer genes did not differ in expression between the treatments. Dissociation and standard curves were analyzed at the end of each run to verify the absence of primer dimers and to assess amplification specificity and efficacy.

2.4. Characterization of the whitefly hemocyanin

The coding sequence (CDS) of the hemocyanin, *Bta12158* was obtained from the Whitefly Genome Database (http://www.whiteflygenomics.org) and the sequence was validated using primers listed in Supplementary Table S1. Multiple sequence alignments were performed in MegAlignPro®, v13.0 (DNASTAR, USA) using Clustal Omega. A phylogenetic tree was also generated in MegAlignPro® and visualized in FigTree (v1.4.3) (http://tree.bio.ed.ac.uk/software/). All sequences used in the analysis are listed in Supplementary Table S2. A protein structure model was predicted using RaptorX (Kallberg et al., 2012) and visualized in Protean 3D®, v13.0 (DNASTAR, USA). The GenBank accession number for this sequence is KY368388.

3. Results

3.1. Summary of the RNA-Seq dataset

To assess the global transcriptome profile of whiteflies in response to feeding on TYLCV-infected tomato, we performed RNA-Seq analysis on adult whiteflies exposed to TYLCV-infected or uninfected tomato plants for AAP of 24, 48, and 72 h. Three biological replicates were performed per exposure time and treatment, with a total of 18 libraries constructed. Overall, ~142 million read pairs were obtained from the 18 libraries, with ~5–13 million read pairs per library. After trimming and removing the reads that aligned to the ribosomal RNA database, the *B. tabaci* mitochondrion genome, and to the three bacterial endosymbiont genomes of *Candidatus* Portiera aleyrodidarum, *Hamiltonella*, and *Rickettsia*, ~4–10 million cleaned read pairs were generated per library with an average of ~91% of the reads mapped, in proper pair, to the *B. tabaci* MEAM1 genome (http://www.whiteflygenomics.org, (Chen et al., 2016); Table 1). Pearson's correlation coefficients were near 1 for all biological replicates (Supplementary Table S3), suggesting the data were highly reproducible.

3.2. Overview of differentially expressed genes at 24, 48, and 72 h

Of the 15,664 genes annotated in the genome of whitefly *B. tabaci* MEAM1 (Chen et al., 2016), a total of 79 genes were differentially expressed when whiteflies were introduced to TYLCV-infected tomato for AAP of 24, 48, and 72 h, compared to whiteflies on uninfected tomato. At 24 h, 38 genes were differentially expressed (20 upregulated, 18 downregulated), whereas only 7 genes (all downregulated) were differentially expressed at 48 h, and 37 genes (16 upregulated, 21 downregulated) were differentially expressed at 72 h (Fig. 1). Among the 79 genes, only 3 were differentially expressed at more than one exposure time.

To validate the RNA-Seq expression results, we performed RT-qPCR on nine randomly selected differentially expressed genes using two endogenous whitefly genes as internal standards (Supplementary Table S4). The results showed that the expression trends (up-regulation and down-regulation) obtained from RNA-Seq and RT-qPCR were highly consistent (Supplementary Table S4).

3.3. 24 h: regulation of metabolism, antiviral immunity and genes with implications for feeding behavior

A total of 38 genes were differentially expressed in the whitefly after 24 h on TYLCV-infected tomato (20 upregulated, 18 downregulated), many of which were associated with nucleic acid binding (Fig. 2, Table 2). Further analysis revealed several upregulated nucleic acid binding proteins, a LIM homeobox 9 (LHX9) transcription factor, the Hox gene, *ultrabithorax (ubx),* and an RNA-binding protein, MEX3 C. Additional genes with binding properties that were upregulated included a hemocyanin, a type 2 orexin receptor, and a guanylate cyclase. Interestingly, the gene *Bta10514*, which encodes a member of the tryptophan repeat gene family was not expressed in non-viruliferous whiteflies at 24 h, but was expressed in whiteflies that fed on TYLCV-infected tomato, although at a relatively low level (FPKM = 1.9) (Table 2).

A BLASTP search to the NCBI database for the upregulated guanylate cyclase, *Bta05793,* showed that seven out of 10 proteins with the highest homologies were described as atrial natriuretic peptide (ANP) receptors, including one from pea aphid, *Acyrthosiphon pisum*, which had 75% identity, 99% coverage, and an e-value of 0 (Supplementary Table S5), suggesting that *Bta05793* is likely to be an ANP receptor. Several genes coding for transporters and receptors were also identified, including three sugar transporters (one upregulated, two downregulated), one upregulated thiamine transporter, and one upregulated low-density lipoprotein receptor (Table 2).

Twenty-one genes were predicted to contribute to various metabolic processes (Fig. 2). Five downregulated genes involved in fatty acid metabolism were identified, several of them being critical catalysts prior to *β*-oxidation, which supplies the Krebs cycle with acetyl CoA, and ultimately yields energy in the form of ATP (Table 2). We also identified two downregulated genes involved in amino acid metabolism, *pyrroline-5-carboxylate dehydrogenase* and *4-aminobutyrate aminotransferase* (Table 2). These data suggest the whitefly may undergo a reduction in fatty acid and amino acid metabolism at 24 h.

3.4. 48 h

After 48 h of feeding on TYLCV-infected tomatoes, only seven genes were differentially expressed in whiteflies, all of which were downregulated. Although none of these were differentially expressed at 24 h, we identified separate genes coding for a fatty acid synthase, a serine protease, a chemosensory protein, and two genes associated with muscle: *myofilin isoform B* and *sarcalumenin* (Table 3).

Table 1
Summary of RNA-Seq datasets generated from whiteflies (*Bemisia tabaci*) fed for 24, 48 or 72 h on *Tomato yellow leaf curl virus*-infected or virus-free tomato plants.

Sample description	No. raw read pairs	No. final cleaned read pairs	Mapped to *B. tabaci* MEAM1 genome	
			No. mapped	% mapped
24 h Uninfected rep 1	10,347,207	8825,201	8141,128	92.25
24 h Uninfected rep 2	6337,292	5093,830	4660,566	91.49
24 h Uninfected rep 3	6359,470	5302,093	4843,411	91.35
24 h TYLCV rep 1	6879,187	5921,916	5407,482	91.31
24 h TYLCV rep 2	9550,976	7664,498	6968,925	90.92
24 h TYLCV rep 3	6965,218	5943,368	5348,585	89.99
48 h Uninfected rep 1	13,225,828	10,473,792	9471,551	90.43
48 h Uninfected rep 2	7996,841	6463,669	5870,073	90.82
48 h Uninfected rep 3	6275,704	5039,395	4580,438	90.89
48 h TYLCV rep 1	7013,542	5608,633	5104,925	91.02
48 h TYLCV rep 2	5314,056	4191,558	3711,752	88.55
48 h TYLCV rep 3	7065,343	5845,199	5312,702	90.89
72 h Uninfected rep 1	8851,871	7188,797	6576,131	91.48
72 h Uninfected rep 2	8402,636	6738,909	6200,365	92.01
72 h Uninfected rep 3	7352,094	6267,234	5623,758	89.73
72 h TYLCV rep 1	9597,258	7930,231	7147,401	90.13
72 h TYLCV rep 2	6765,522	5517,745	5054,227	91.6
72 h TYLCV rep 3	7769,834	6282,451	5695,565	90.66
Total	142,069,879	116,298,519	105,718,985	90.86

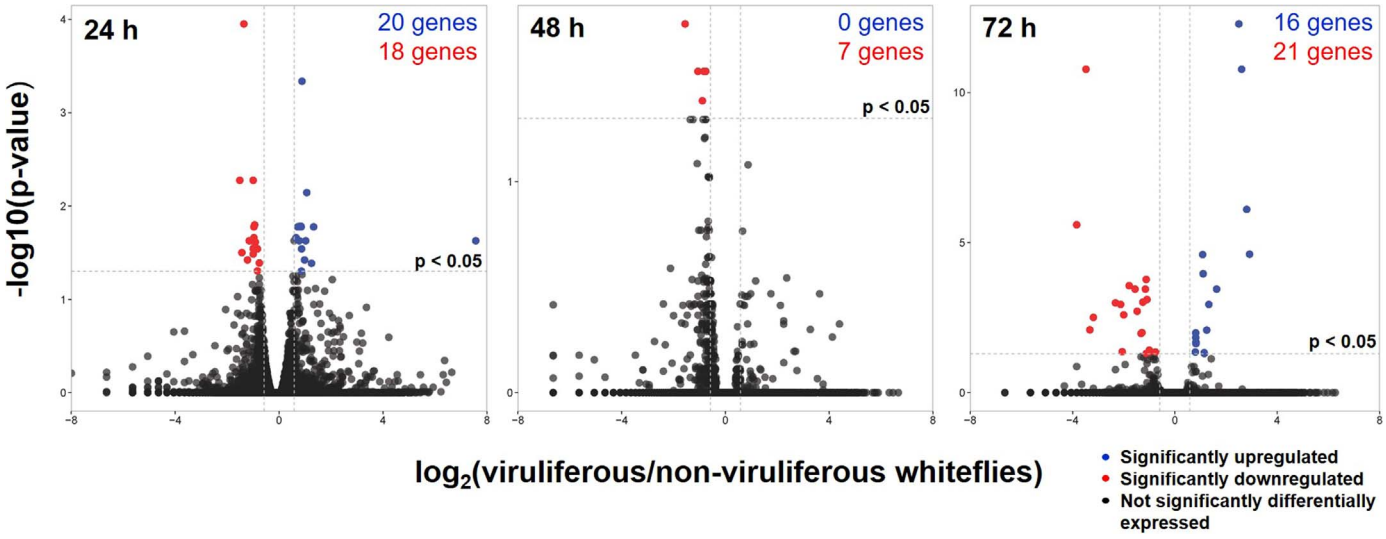

Fig. 1. Volcano plots of differentially expressed genes in whitefly (*Bemisia tabaci*) after 24, 48, and 72 h of feeding on TYLCV-infected vs. virus-free tomato plants. Each point represents a single gene plotted with their p-value (-log10(p-value)) as a function of fold change in expression (log2(viruliferous/non-viruliferous)). Points above the horizontal line represent genes that had p-values less than 0.05; points to the left and right of the two vertical lines had fold changes greater than or equal to 1.5. Blue points represent significantly upregulated genes, red points represent significantly downregulated genes, and black points represent non-differentially expressed genes. Genes with a p-value < 0.05 and fold change ≥ 1.5 were considered as differentially expressed genes.

3.5. 72 h: Regulation of lysosomal activity, carbohydrate metabolism, hemocyanin, and genes with unknown functions

After 72 h on TYLCV-infected tomatoes, 37 genes were differentially expressed in whiteflies (16 upregulated, 21 downregulated). We identified five transporters, including the same downregulated *sugar transporter ERD6-like 6* that was differentially expressed at 24 h, and an upregulated proton-coupled amino acid transporter (Table 4). We also identified four genes associated with the lysosome, three of which belonged to the cathepsin B family, as well as an acid phosphatase-1 gene.

Four genes were identified as having a role in carbohydrate metabolism, which included two α-glucosidases, a glycogen branching enzyme, and a sucrase. All four genes were downregulated except one α-glucosidase, suggesting that carbohydrate metabolism might be altered in whiteflies after 72 h on TYLCV-infected tomatoes. In addition, we also identified three upregulated aldo-keto reductase genes, which collectively with the α-glucosidases, have been implicated in the survival and transmission of viruses at the mosquito midgut interface (Tchankouo-Nguetcheu et al., 2010).

Of the 37 genes identified, 7 had unknown functions, while three were similarly differentially expressed at 24 h: downregulated *sugar transporter ERD6-like 6*, downregulated *inhibin beta B chain*, and upregulated *hemocyanin* (Table 4). Together, these data suggests that whiteflies experience a shift in gene regulation, with those genes associated with lysosome, carbohydrate metabolism, and numerous unknown proteins being altered in expression after 72 h feeding on TYLCV-infected tomatoes.

3.6. Characterization of the whitefly hemocyanin, Bta12158

The whitefly hemocyanin was the only gene that was upregulated across more than one time period (24 h and 72 h), suggesting its potential association in TYLCV acquisition, trafficking, transmission, or response to feeding on the infected tomato plants. Sanger sequencing of

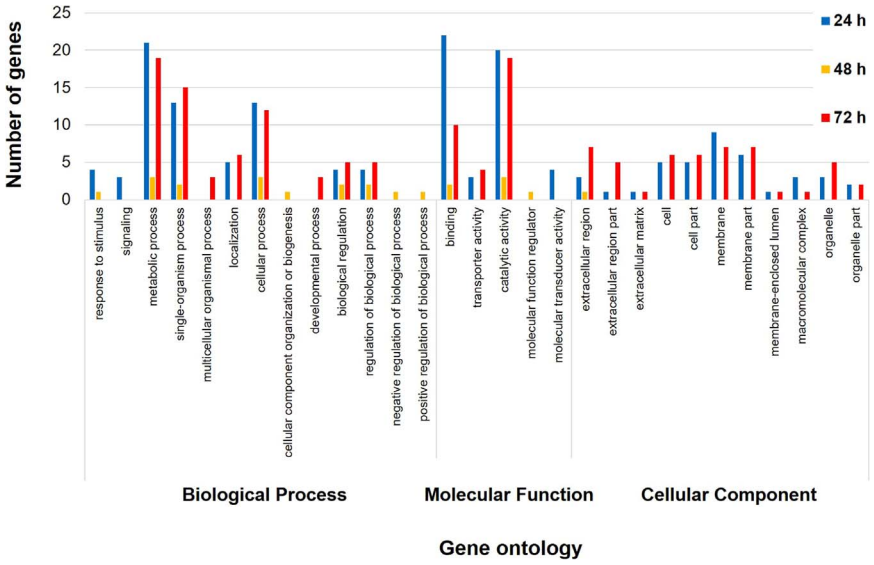

Fig. 2. Functional classification of differentially expressed whitefly genes. Number of differentially expressed whitefly genes for each ontology at 24 h (blue), 48 h (yellow), and 72 h (red). Gene ontology terms of level 2 are shown.

Bta12158 cDNA from our *B. tabaci* MEAM1 colony confirmed a single transcript with identical sequence as predicted in the Whitefly Genome Database (http://www.whiteflygenomics.org; (Chen et al., 2016)). The complete gene had a CDS of 2442 nt and a deduced protein sequence of 813 amino acid residues. Hemocyanins are present in crustaceans and arthropods, with the exception of some primitive species, have evolved into hexamerins within the insect lineage (Burmester, 2002). Phylogenetic analysis of *Bta12158* with other insect hemocyanins and hexamerins revealed that the sequence is unique in whitefly (*B. tabaci*), due to its distal placement from other hemipteran sequences identified from the firebug (*Pyrrhocoris apterus*), brown marmorated stink bug (*Halyomorpha halys*), and Asian citrus psyllid (*Diaphorina citri*) (Fig. 3). Interestingly, hemocyanins or hexamerins have not been identified in the pea aphid (*Acyrthosiphon pisum*) (Ishikawa et al., 2012), which is a

Table 2
Differentially expressed genes in whiteflies (*Bemisia tabaci*) fed on TYLCV-infected tomato versus whiteflies fed on virus-free tomato for 24 h.

Class	Gene ID	Annotation	Fold change	P-value
Transport	Bta13035	Facilitated glucose transporter protein 1	2.03	0.0236
	Bta03497	Thiamine transporter 2	1.79	0.0165
	Bta00261	Low-density lipoprotein receptor, putative	1.57	0.0219
	Bta02871	Facilitated glucose transporter protein 1	−2.01	0.0053
	Bta01591	Sugar transporter ERD6-like 6	−2.91	0.0053
Nucleic acid binding	Bta09274	Zinc finger protein, putative	2.37	0.0411
	Bta01511	RNA-binding protein MEX3C	1.97	0.0379
	Bta14318	LIM homeobox 9	1.79	0.0167
	Bta05187	Homeobox protein ultrabithorax	1.76	0.0167
	Bta14183	Zinc finger protein basonuclin − 2	1.75	0.0167
Other binding	Bta04072	Elicitin-like protein 6	1.82	0.0288
	Bta12158	Hemocyanin subunit, putative	1.81	0.0499
	Bta00579	Orexin receptor type 2	1.80	0.0167
	Bta05793	Guanylate cyclase	1.77	0.0167
	Bta11771	F-box only protein 5	1.71	0.0236
	Bta03443	SEC. 23-interacting protein	−2.10	0.0236
	Bta02187	Inhibin beta B chain	−2.20	0.0236
Fatty acid metabolism	Bta09442	Pancreatic lipase-related protein 2	−1.71	0.0408
	Bta00049	3-ketoacyl-CoA thiolase	−1.96	0.0236
	Bta03074	Fatty acid synthase	−2.33	0.0379
	Bta08821	Glycerol − 3-phosphate dehydrogenase	−2.53	0.0001
	Bta11116	Acyl-coenzyme A thioesterase 9	−2.72	0.0316
Amino acid metabolism	Bta11812	Pyrroline − 5-carboxylate dehydrogenase	−1.79	0.0288
	Bta15543	4-aminobutyrate aminotransferase	−1.91	0.0286
Other metabolism	Bta06365	Carboxylesterase 3, putative	2.09	0.0072
	Bta07544	Alkaline phosphatase	1.73	0.0165
	Bta05168	Inositol − 3-phosphate synthase 1-B	−1.93	0.0159
	Bta08426	Alpha-glucosidase	−1.95	0.0219
	Bta14886	Purine nucleoside phosphorylase	−1.96	0.0167
	Bta03563	Carboxylesterase	−1.99	0.0286
	Bta04870	Cathepsin F	−2.02	0.0327
Other	Bta10514	Tryptophan repeat gene family	190.00	0.0236
	Bta00243	Muscle-specific protein 300 kDa, isoform I	2.51	0.0167
	Bta13518	Dachshund, putative	1.84	0.0005
	Bta02890	Headcase protein-like protein	1.75	0.0167
	Bta14634	Unknown protein	1.64	0.0167
	Bta10597	Transmembrane 9 superfamily member 4	−1.80	0.0496
	Bta15277	CG13868, isoform A	−1.88	0.0244

Table 3
Differentially expressed genes in whiteflies (*Bemisia tabaci*) fed on TYLCV-infected tomato versus whiteflies fed on virus-free tomato for 48 h.

Gene ID	Annotation	Fold change	P-value
Bta06169	Myofilin isoform B	−1.69	0.0300
Bta07645	Fatty acid synthase	−1.71	0.0300
Bta07747	Sarcalumenin	−1.75	0.0300
Bta04260	CG8740, isoform A	−1.78	0.0300
Bta00817	Unknown protein	−1.84	0.0414
Bta03265	Serine protease 7, isoform A	−2.07	0.0300
Bta13640	Chemosensory protein	−2.93	0.0179

Table 4
Differentially expressed genes in whiteflies (*Bemisia tabaci*) fed on TYLCV-infected tomato versus whiteflies fed on virus-free tomato for 72 h.

Class	Gene ID	Annotation	Fold change	P-value
Transport	Bta01723	Proton-coupled amino acid transporter 1	2.51	0.0011
	Bta09674	Transporter, putative	−2.92	0.0004
	Bta01591	Sugar transporter ERD6-like 6	−3.50	0.0003
	Bta04879	Dipeptide and tripeptide permease A	−5.00	0.0010
	Bta04677	Solute carrier family 15 member 1	−9.68	0.0080
Lysosome	Bta09313	Cathepsin B	1.77	0.0205
	Bta03880	Cathepsin B	−2.01	0.0382
	Bta14751	Cathepsin B, partial	−2.39	0.0010
	Bta10829	Acid phosphatase −1	−11.33	0.0000
Binding	Bta12158	Hemocyanin subunit, putative	3.10	0.0004
	Bta14821	Wnt inhibitory factor 1	−2.12	0.0008
	Bta02187	Inhibin beta B chain	−2.76	0.0019
	Bta09273	Lin29, isoform B	−4.05	0.0431
Carbohydrate metabolism	Bta12680	Alpha-glucosidase family 31	2.37	0.0082
	Bta14422	Alpha-glucosidase	−4.39	0.0011
	Bta14313	1,4-alpha-glucan branching enzyme GlgB	−8.87	0.0031
	Bta14312	Sucrase	−13.96	0.0000
Other metabolism	Bta10339	Aldo-keto reductase	2.15	0.0001
	Bta02817	Methyltransferase-like protein 13	2.12	0.0000
	Bta14861	D-alanine–poly (phosphoribitol) ligase subunit 1	1.76	0.0102
	Bta10337	Aldo-keto reductase	1.76	0.0146
	Bta10341	Aldo-keto reductase	1.74	0.0441
	Bta07792	Thymus-specific serine protease	−2.00	0.0441
	Bta09676	Cathepsin F	−2.20	0.0004
	Bta07789	Thymus-specific serine protease	−2.52	0.0106
	Bta07602	UDP-glucuronosyltransferase 1–1	−4.05	0.0025
Unknown proteins	Bta06882	Unknown protein	7.56	0.0000
	Bta06885	Unknown protein	7.00	0.0000
	Bta09407	Unknown protein	6.10	0.0000
	Bta03870	Unknown protein	5.66	0.0000
	Bta13457	Unknown protein	2.22	0.0496
	Bta03954	Unknown protein	−1.69	0.0441
	Bta03952	Unknown protein	−2.19	0.0002
Other	Bta02276	Ubiquitin carboxyl-terminal hydrolase	2.21	0.0465
	Bta20014	methyltransferase	1.77	0.0233
	Bta03575	Juvenile hormone-inducible protein	−2.16	0.0496
	Bta00622	Galectin	−2.46	0.0100

sister taxon to the whitefly, *B. tabaci* (Chen et al., 2016).

Further analysis revealed that the whitefly hemocyanin protein shares only 33% sequence identity (96% coverage, e value = 3e-155) with the closest BLASTP match, which is a hexamerin from the stonefly, *Perla marginata* (Supplementary Table S5). Multiple protein sequence alignment with hexamerins and a homologous hemocyanin from nine insects representing seven different orders revealed that the whitefly hemocyanin is enriched with multiple asparagine repeat domains, similar to that seen in the Asian citrus psyllid (Fig. 4). Interestingly, recent proteomics analysis by Ramsey et al. (2017) identified that a hemocyanin from Asian citrus psyllid is associated with the transmission of *Candidatus* Liberibacter asiaticus, the causal agent of citrus greening disease, which is also transmitted by psyllids in a persistent circulative manner (Ramsey et al., 2017). Together, the upregulation of the hemocyanin after whitefly feeding on TYLCV-infected tomato at two time periods, and its sequence novelty, suggests a unique function for this gene, possibly in association with TYLCV acquisition and transmission.

3.7. Comparative analysis of whiteflies fed on TYLCV-infected versus ToCV-infected tomato

The current study was designed in coordination with (Kaur et al., 2017) so that comparative analysis could be performed between whiteflies that fed on TYLCV-infected tomato and those that fed on ToCV-infected tomato. Unlike TYLCV, which is transmitted by whiteflies in a persistent, circulative manner, ToCV is transmitted by whiteflies in a semi-persistent, non-circulative manner (Wisler et al., 1998; Wintermantel and Wisler, 2006). Previous studies have shown that ToCV virus particles are retained in the whitefly mouthparts and foregut for several hours to days (Kaur et al., 2016; Whitfield et al., 2015). In contrast to the 79 genes identified as differentially expressed in whiteflies that fed on TYLCV-infected Moneymaker tomato plants compared to those that fed on virus-free tomato, 1155 genes were differentially expressed when whiteflies fed on ToCV-infected Moneymaker tomato plants over the same AAPs. The largest difference between the two virus treatments was observed with the 24 h AAP, in which a 26-fold difference in differentially expressed genes was seen between whiteflies feeding on TYLCV-infected (38 genes) and ToCV-infected (989 genes) plants (Fig. 5). Although there were large differences in the number of genes expressed between the two virus treatments, the trend across the three time periods was very similar, with very few genes differentially expressed at 48 h (7 genes, TYLCV; 11 genes, ToCV), but an increasing number of genes at 72 h (37 genes, TYLCV; 210 genes, ToCV) (Fig. 5) (Kaur et al., 2017).

A total of 28 differentially expressed genes were common in whiteflies fed on TYLCV and ToCV-infected tomato compared to whiteflies fed on virus-free tomato (Fig. 6). Sixteen common genes were downregulated in whiteflies that fed on TYLCV- and ToCV-infected tomato, and 12 of these genes were downregulated at the same time points (5 genes at 24 h and 7 genes at 72 h). Most of these genes are involved in metabolism, including those encoding a glycerol-3-phosphate dehydrogenase, fatty acid synthase, purine nucleoside phosphorylase, pyrroline-5-carboxylate dehydrogenase, sucrase, 1,4-alpha-glucan branching enzyme GlgB, and alpha-glucosidase.

The 12 remaining differentially expressed genes that were common between whiteflies fed on TYLCV and ToCV-infected tomato showed opposite regulation between the virus treatments, with 9 of these genes upregulated in whiteflies fed on TYLCV-infected tomato at 24 h, while the same genes were downregulated in whiteflies fed on ToCV-infected tomato at 72 h (Fig. 6). Some of these genes included those encoding a low-density lipoprotein receptor, alkaline phosphatase, homeobox protein ultrabithorax, LIM homeobox 9, and thiamine transporter 2. Additional genes that were identified include cathepsin F and cathepsin B, chemosensory protein, and facilitated glucose transporter 1. No common genes were upregulated between TYLCV and ToCV treatments.

4. Discussion

The differences in gene expression observed between whiteflies fed on TYLCV-infected tomato and ToCV-infected tomato (Kaur et al.,

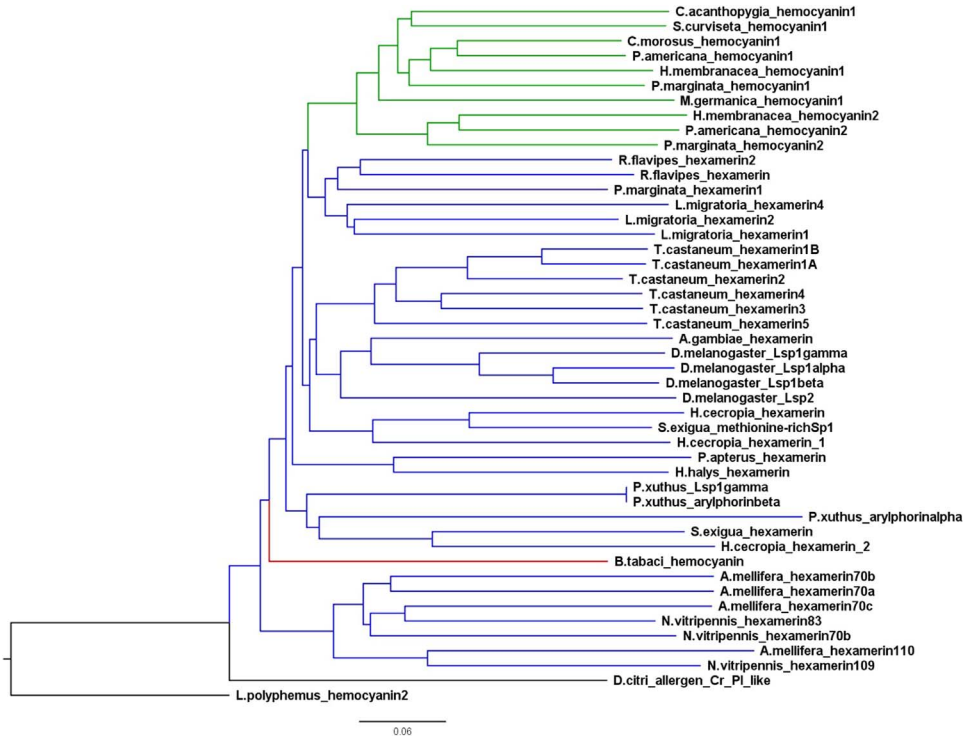

Fig. 3. Phylogenetic relationship of the whitefly, *Bta12158* with other insect hexamerins and hemocyanins. Hexamerin sequences representing nine orders of insects and hemocyanins representing seven orders of insects were included in the analysis. Sequences were aligned using Clustal Omega and the neighbor-joining tree was visualized in FigTree. The hemocyanin sequence from the horseshoe crab, *Limulus polyphemus* (Phylum: Arthropoda; Subphylum: Chelicerata) was used as an outgroup. Blue represents the hexamerin clade; green, hemocyanin clade; red, *B. tabaci* hemocyanin. Information about the sequences used in the analysis can be found in Supplementary Table S2.

2017) suggests that the whitefly's molecular response to virus acquisition and transmission is dependent on the nature of a virus, the mode of vector transmission, and secondary physiological effects caused by virus infection of the source plant. Differences in the time associated with virus acquisition, retention and transmission by the whitefly (*B. tabaci*) between the persistently transmitted begomovirus, TYLCV and the semi-persistently transmitted crinivirus, ToCV may have contributed to the widely different numbers of differentially expressed genes observed between whiteflies fed on tomato plants infected with the two viruses with distinct modes of transmission. ToCV is acquired quickly with a feeding period lasting as little as a few hours and can be transmitted immediately to plants without an obvious latent period; ToCV also has a relatively short retention time within *B. tabaci* MEAM1 (Wintermantel, 2016; Wintermantel and Wisler, 2006). In contrast, TYLCV has a latent period of several hours following virus acquisition that is required for effective virus transmission to occur. Ingested TYLCV virions must pass from the filter chamber and/or midgut into the hemolymph, and accumulate in the primary salivary glands before transmission. Relatively slow acquisition of TYLCV by *B. tabaci* MEAM1 was reflected with only approximately 60% of the whitefly population testing positive for TYLCV after a 24 h AAP, although all whiteflies tested positive after 48 h and 72 h AAP. Similar virus acquisition information was not presented in (Kaur et al., 2017), thus preventing us from making a direct comparison. Nevertheless, the differences in the timing of virion acquisition, translocation, inoculation, and retention of TYLCV and ToCV are likely to be strong influences on the gene expression patterns observed in the current study.

However, the subtle changes observed at the 48 h AAP for both viruses suggests a common temporal phenomenon in whiteflies, possibly associated with feeding on tomato regardless of the infection status of the host. The results from the comparative analysis showed that several metabolic genes were similarly affected in whiteflies feeding either on TYLCV-infected or on ToCV-infected tomato plants (Fig. 6). For some genes, including those encoding a low-density lipoprotein receptor, alkaline phosphatase, homeobox protein ultrabithorax, LIM homeobox 9, and thiamine transporter 2, the opposite effects were observed (Fig. 6), suggesting differential regulation of gene expression during virus acquisition and early virus transmission between whiteflies fed on plants infected by two viruses with distinct modes of transmission. The data presented here provides new knowledge on how the whitefly responds biologically to feeding on and acquiring different viruses from infected tomato hosts. This comparative analysis of differential gene profiling in a common whitefly vector fed on plants infected with two types of economically important viruses will help researchers identify common or unique gene targets useful for genetic manipulation, including RNA interference (RNAi) technologies for whitefly and virus management (Ghanim et al., 2007; Zhang et al., 2017).

Whiteflies that fed on TYLCV-infected tomato only exhibited differential expression of 79 genes across all three time points. The only gene that was upregulated at more than one time point was the whitefly hemocyanin (24 h and 72 h). Hemocyanins are large copper-binding glycoproteins in the hemolymph that bind oxygen and perform antiviral functions in arthropods and crustaceans (Dolashka and Voelter, 2014; Markl, 2013). However, with the exception of some primitive species, insect hemocyanins are thought to have lost the ability to bind oxygen as tracheal systems evolved during the occupancy of terrestrial niches. Hexamerins evolve from hemocyanins and serve as amino acid storage proteins in the circulating hemolymph. They have been implicated in other biological processes, including the regulation of juvenile hormone production and immune responses, as demonstrated in fruit fly (Wang et al., 2010), mosquito (Lombardo and Christophides, 2016), and leafhopper (Eliautout et al., 2016). Specifically, *Drosophila* hexamerins participate in humoral immunity by directly interacting with microbial surfaces during an immune challenge (Wang et al., 2010). In Asian citrus psyllid, a hemocyanin was highly upregulated in response to the bacterial citrus pathogen, *Candidatus* Liberibacter asiaticus, and was shown to interact with a bacterial protein involved in metabolism (Ramsey et al., 2017).

The whitefly hemocyanin possesses the conserved N, M, and C hemocyanin domains that are present across all arthropod hemocyanins and hexamerins, but lacks the hallmark copper-binding histidine residues that bind oxygen (Beintema et al., 1994; Hagner-Holler et al., 2004), such as those present in the American cockroach, *Periplaneta*

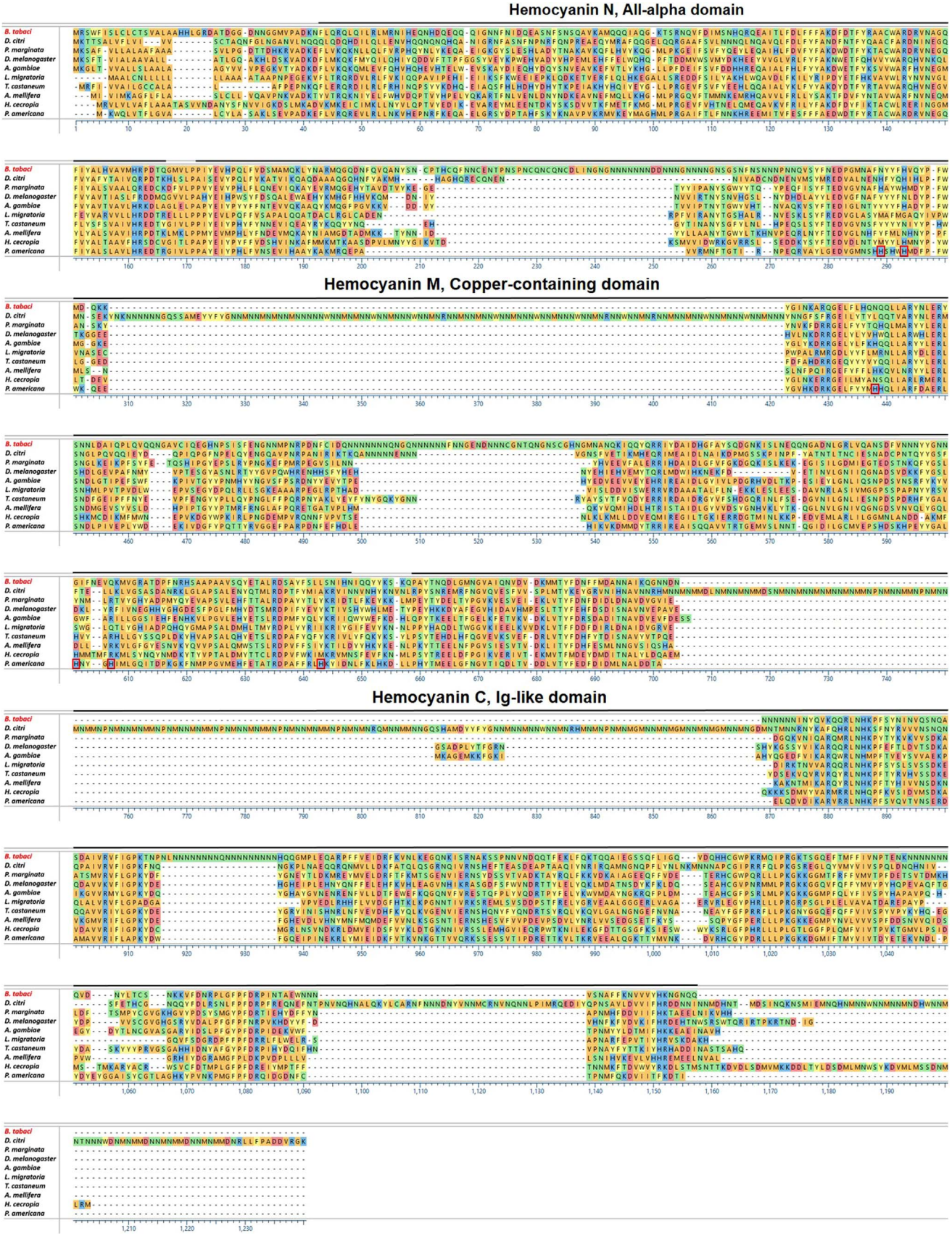

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Fig. 4. Whitefly hemocyanin amino acid sequence alignment with other hexamerins and a homologous hemocyanin from nine insects representing seven different orders. Deduced amino acid sequence for the whitefly hemocyanin aligned with hexamerins from asian citrus psyllid, *Diaphorina citri* (Order: Hemiptera; Accession no. XP_008477906.1), stonefly, *Perla marginata* (Plecoptera; CAM84196.1), fruit fly, *Drosophila melanogaster* and mosquito, *Anopheles gambiae* (Diptera; CAA66371.1 and XP_321436.5), migratory locust, *Locusta migratoria* (Orthoptera; AAC47391.1), red flour beetle, *Tribolium castaneum* (Coleoptera; XP_008197349.1), western honeybee, *Apis mellifera* (Hymenoptera; NP_001011600.1), cecropia moth, *Hyalophora cecropia* (Lepidoptera; AAB86647.1), and a hemocyanin from the American cockroach, *Periplaneta americana* (Blattodea; CAR85701.1). Sequences were aligned using Clustal Omega. Black bars above the sequences indicate the conserved domains, hemocyanin N, all-alpha domain, hemocyanin M, copper-containing domain, and hemocyanin C, Ig-like domain. Red boxes indicate the six conserved copper-binding histidine residues found in hemocyanins (oxygen-transport proteins).

americana (Fig. 4). The protein comprises several repeated regions of asparagine (N) and glutamine (Q) residues, which make up 21% and 10%, respectively, of the entire amino acid sequence, and this is similar to that seen in the Asian citrus psyllid (Fig. 4 and Table 5). Asparagine and glutamine are the most common amino acids to occur in eukaryotic repeat sequences, with Q-repeats frequently seen in vertebrates (Lu and Murphy, 2015). Despite similar chemistries between N and Q residues, little is known about the significance of N-repeat sequences, although they are commonly seen in invertebrates and have a propensity to aggregate, and thus are less likely to form a single stable structure (Lu and Murphy, 2015). Despite the presence of asparagine repeat domains seen in both hemocyanins from whiteflies and psyllids, the two sequences only share 35% identity (56% coverage, e value = 2e-36). The predicted three-dimensional structure of the whitefly hemocyanin suggests there are unique structures at the N/Q repeat sites, in addition to maintaining similar conformation at the conserved N, M, and C hemocyanin domains, when compared to the stonefly hexamerin (Fig. 7). Overall, the identification of a hemocyanin, and its increased expression in whiteflies fed on TYLCV-infected tomato for 24 h and 72 h suggests it may fulfill a specific physiological role. Future studies are needed to understand the function of the hemocyanin during whitefly feeding on TYLCV-infected tomato.

Cathepsins are proteases involved in protein degradation, apoptosis, and signaling, and they regulate viral infection and transmission (Kubo et al., 2012; Sim et al., 2012; Pinheiro et al., 2016). In the green peach aphid, a lysosomal cathepsin B is upregulated following acquisition of the circulative-transmitted virus, *Potato leafroll virus* (PLRV), and together, the protein and virus colocalize at the cell membranes of midgut cells (Pinheiro et al., 2016). Regulation of multiple cathepsins in our study, as well as identification of a downregulated acid phosphatase gene, suggests that lysosome regulation may also be involved during whitefly acquisition and transmission of TYLCV. Because we only identified genes associated with the lysosome at 72 h, lysosome regulation may be a temporal phenomenon, in which such changes occur later rather than earlier during the feeding and circulation of the virus in the whitefly. We hypothesize that like other viruses, regulation of genes associated with the endosome/lysosome pathway, including the cathepsins, might be critical for TYLCV circulation and transmission.

Previous studies suggest that whiteflies transmitting TYLCV exhibit reduced velocity and duration of movement, but increased probing and feeding behavior (Moreno-Delafuente et al., 2013; Jahan et al., 2014; Liu et al., 2013; He et al., 2015). The shift to an arrested behavior and longer periods of feeding is thought to increase the transmission efficiency of the virus, a phenomenon that similarly occurs in other vector-borne pathogen systems, including thrips and *Tomato spotted wilt virus,* aphids and *Barley yellow dwarf virus,* and mosquitos transmitting La Crosse encephalitis virus (Moreno-Delafuente et al., 2013; Jahan et al., 2014; Ingwell et al., 2012; Grimstad et al., 1980). Although it is thought that plant viruses may stimulate neuronal processes to control the vector's feeding-related behavior, the underlying molecular mechanisms are largely unknown (Jahan et al., 2014). We identified an upregulated whitefly orexin 2 receptor after 24 h of feeding on TYLCV-infected tomato. Orexin neuropeptides regulate wakefulness through activation of numerous mechanisms, including the stimulation of feeding behavior (Sakurai, 2014). In insects, the allatotropin (AT) receptors are orthologous to the orexin receptors, and are known to contribute to a variety of functions, including induction of myostimulatory and cardioacceleratory activities and an increase in the frequency of contractions in the aorta and gut (Alzugaray et al., 2013; Villalobos-Sambucaro et al., 2015). In addition, we identified an upregulated ANP receptor, which exhibits diuretic, natriuretic, and vasodilating activities in vertebrates (Saito, 2010). ANP receptors are highly immunoreactive in the midgut of some insects (Chen, 1989). They have been demonstrated to possess similar properties in mosquito and *Drosophila*, and appear to be highly active in the midgut of the stable fly (*Stomoxys calcitrans*) after feeding (Coast et al., 2005; Chang et al., 1989). These two genes have implications for regulating whitefly feeding behavior and hemolymph circulation in a way that could favor virus acquisition and transmission, but future studies are required to test this hypothesis.

During virus circulation, there are two major physical barriers a begomovirus must cross in the whitefly vector: the midgut/filter chamber and primary salivary glands. Receptor-mediated endocytosis is thought to facilitate this process, in which a virion binds to an

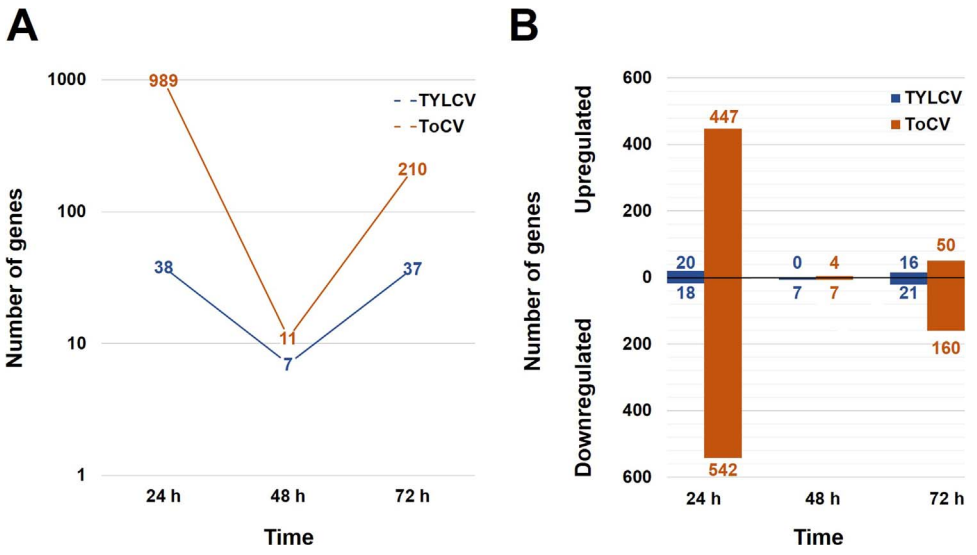

Fig. 5. Differentially expressed genes in whiteflies fed on TYLCV-infected and ToCV-infected tomato compared to whiteflies fed on virus-free tomato. (A) Total number of genes differentially expressed at acquisition access periods of 24 h, 48 h, and 72 h. (B) Number of upregulated and downregulated genes differentially expressed at acquisition access periods of 24 h, 48 h, and 72 h. Genes with a p-value < 0.05 and fold change ≥ 1.5 were considered as differentially expressed genes.

Gene ID	Annotation	Virus	24 h	48 h	72 h
Bta08821	Glycerol-3-phosphate dehydrogenase	TYLCV	-2.53		
		ToCV	-4.62		
Bta03074	Fatty acid synthase	TYLCV	-2.33		
		ToCV	-2.08		
Bta03443	SEC23-interacting protein	TYLCV	-2.10		
		ToCV	-2.08		
Bta04870	Cathepsin F	TYLCV	-2.02		
		ToCV			-2.53
Bta02871	Facilitated glucose transporter protein 1	TYLCV	-2.01		
		ToCV	1.85		
Bta14886	Purine nucleoside phosphorylase	TYLCV	-1.96		
		ToCV	-2.36		
Bta11812	Pyrroline-5-carboxylate dehydrogenase	TYLCV	-1.79		
		ToCV	-2.06		
Bta00261	Low-density lipoprotein receptor, putative	TYLCV	1.57		
		ToCV			-1.87
Bta07544	Alkaline phosphatase	TYLCV	1.73		
		ToCV			-2.47
Bta14183	Zinc finger protein basonuclin-2	TYLCV	1.75		
		ToCV			-2.43
Bta02890	Headcase protein-like protein	TYLCV	1.75		
		ToCV			-2.57
Bta05187	Homeobox protein ultrabithorax	TYLCV	1.76		
		ToCV			-2.57
Bta05793	Guanylate cyclase	TYLCV	1.77		
		ToCV			-2.36
Bta03497	Thiamine transporter 2	TYLCV	1.79		
		ToCV			-2.65
Bta14318	LIM homeobox 9	TYLCV	1.79		
		ToCV			-2.64
Bta13518	Dachshund, putative	TYLCV	1.84		
		ToCV			-1.70
Bta13640	Chemosensory protein	TYLCV		-2.93	
		ToCV	-5.85		
Bta04260	CG8740, isoform A	TYLCV		-1.78	
		ToCV	-1.95		
Bta07747	Sarcalumenin	TYLCV		-1.75	
		ToCV	-2.16		
Bta10829	Acid phosphatase-1	TYLCV			-11.30
		ToCV		-2.17	-3.47
Bta14312	Sucrase	TYLCV			-13.96
		ToCV		-3.61	-5.75
Bta14313	1,4-alpha-glucan branching enzyme GlgB	TYLCV			-8.87
		ToCV		-4.01	-3.40
Bta14422	Alpha-glucosidase	TYLCV			-4.39
		ToCV	-2.22	-2.04	-3.10
Bta07602	UDP-glucuronosyltransferase 1-1	TYLCV			-4.05
		ToCV			-2.64
Bta07789	Thymus-specific serine protease	TYLCV			-2.52
		ToCV			-1.71
Bta03880	Cathepsin B	TYLCV			-2.01
		ToCV			-2.78
Bta20014	Methyltransferase	TYLCV			1.77
		ToCV	-1.81		
Bta13457	Unknown protein	TYLCV			2.22
		ToCV	-3.93		

Fig. 6. Common differentially expressed genes in whiteflies fed on TYLCV-infected and ToCV-infected tomato plants compared to whiteflies (*Bemisia tabaci*) fed on virus-free tomato. Values represent Fold change > 1.5 and P-value < 0.05.

Table 5
Comparative analysis of asparagine/glutamine content in the whitefly hemocyanin compared to hexamerins and hemocyanins from other insect species.

Insect Species	GenBank Accession	A.a. length	No. Asn (N)	% Asn (N)	No. Gln (Q)	% Gln (Q)
B. tabaci	KY368388	813	168	21%	83	10%
D. citri	XP_008477906.1	1114	289	25%	76	6%
P. marginata	CAM84196.1	702	25	4%	27	4%
D. melanogaster	CAA66371.1	718	33	5%	28	4%
A. gambiae	XP_321436.5	712	24	3%	27	4%
L. *migratoria*	AAC47391.1	668	21	3%	19	3%
T. castaneum	XP_008197349.1	698	42	6%	42	6%
A. mellifera	NP_001011600.1	683	50	7%	26	4%
H. cecropia	AAB86647.1	753	37	5%	12	2%
P. americana	CAR85701.1	674	28	4%	21	3%

extracellular receptor of the midgut and/or primary salivary glands and is shuttled intracellularly to the endocytic pathway (Rosen et al., 2015). However, the receptors involved in virion binding have not been identified. This transcriptome study revealed the upregulation of a low-density lipoprotein receptor (LDLR). Members of the LDLR family mediate lipid uptake from the hemolymph, and have been implicated in endocytosis of nutrients in the midgut (Dantuma et al., 1999). LDLR is actively involved in receptor-mediated endocytosis of viruses, including *Feline leukemia virus, Vesicular stomatitis virus* and *Hepatitis C virus* (Finkelshtein et al., 2013; Agnello et al., 1999). The insect LDLR homolog, lipophorin receptor (LpR), mediates the uptake of lipophorin, and are involved in endocytic uptake of molecules in various insect tissues, including lipophorins in the fat body and albumin in the midgut (Dantuma et al., 1999; Casartelli et al., 2008). Furthermore, a thiamine transporter and several sugar and amino acid transporters were also upregulated, all of which have been implicated as viral receptors (Mendoza et al., 2006; Manel et al., 2005; Ito et al., 2008). In silkworm (*Bombyx mori*), a gene coding for a gut-specific member of the amino acid transporter family was implicated in virus resistance (Ito et al., 2008). Although it is possible that these genes are regulated for other reasons, including altered nutrient provisioning in the whitefly, whether as a response to acquiring TYLCV, or as a response to nutritional imbalances in infected host plants, our data provides a network of candidate genes to explore at the interface of TYLCV-whitefly interactions.

Interestingly, we did not identify any whitefly genes related to autophagy, whereas an earlier transcriptome study conducted by (Luan et al., 2011) using a related begomovirus species, *Tomato yellow leaf curl China virus* (TYLCCNV), did. This was not unexpected considering our study addressed transcriptional changes following 24, 48, and 72 h of feeding on TYLCV-infected tomatoes, whereas (Luan et al., 2011) focused on transcriptional changes occurring five days after a single day of acquisition from TYLCCNV-infected tobacco. In total, Luan and colleagues found ~1600 *B. tabaci* genes were differentially expressed based on a single biological replicate using digital gene expression analysis, and suggested that TYLCCNV can induce changes in the host's cell cycle, primary metabolism, and cellular and humoral immunity (Luan et al., 2011). Some commonalities between the two studies include the expression of genes involved in fatty acid metabolism, amino acid metabolism and lysosome function. The identification of common genes between the different studies may reflect reoccurring or overlapping physiological changes in whitefly vector. It is also likely that the whitefly responds differently to the two begomoviruses and/or the host plants on which they feed, and may explain the differences found between the studies.

5. Conclusions

Viruses transmitted by the whitefly *B. tabaci*, cause some of the most devastating plant diseases in crop production, and thus, threaten global food security. Here, we conducted gene expression analysis of *B. tabaci* in response to tomato infected with a globally-distributed begomovirus, TYLCV, and identified genes that may regulate the whitefly's ability to acquire and transmit begomoviruses. These results fill a gap of knowledge in understanding how two different types of viruses with distinct modes of transmission; the persistent, circulative begomovirus, TYLCV, and the semi-persistent, non-circulative crinivirus, ToCV, influences global gene expression in the whitefly *B. tabaci* vector, when acquired from the relevant and naturally occurring tomato host. This study combined with the associated publication (Kaur et al., 2017), enhance the fundamental knowledge of whitefly-virus relationships, particularly in relation to viruses with distinct modes of transmission, which will serve as a reference for devising novel strategies including RNA interference (RNAi) to control whitefly-transmitted viruses that impact crop production throughout the world.

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Author contributions

K.S.L conceived the experiment. D.K.H, N.K, W.M.W, A.S, Z.F, K.S.L designed the experiments. DKH conducted the experiments. D.K.H, W.C, Y.Z analyzed the data. D.K.H drafted the manuscript. All authors revised and approved the manuscript for submission.

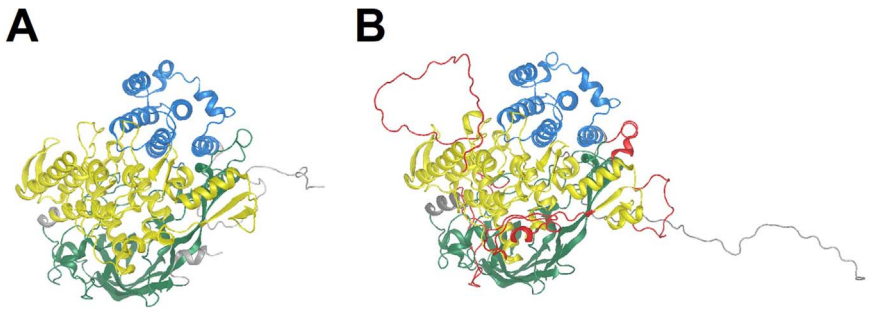

Fig. 7. Predicted protein structure of the whitefly hemocyanin. Protein structures for (A) hexamerin from stonefly, *Perla marginata* and (B) hemocyanin from whitefly, *Bemisia tabaci*. Structures were predicted using RaptorX and visualized in Protean 3D. Conserved hemocyanin N, all-alpha domain (blue); hemocyanin M, copper-containing domain (yellow); hemocyanin C, Ig-like domain (green); asparagine (N) and glutamine (Q)-rich regions in the whitefly hemocyanin (red).

Additional Information

Accession codes: Whitefly hemocyanin (hexamerin) *Bta12158,* KY368388; transcriptome sequence reads have been deposited in the SRA as BioProject PRJNA312467.

Competing financial interests

The authors declare that they have no competing interests.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.virol.2017.10.008.

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