Revisiting dengue virus-mosquito interactions: molecular insights into viral fitness

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Abstract

Dengue virus (DENV), like other viruses, closely interacts with the host cell machinery to complete its life cycle. Over the course of infection, DENV interacts with several host factors with pro-viral activities to support its infection. Meanwhile, it has to evade or counteract host factors with anti-viral activities which inhibit its infection. These molecular virus-host interactions play a crucial role in determining the success of DENV infection. Deciphering such interactions is thus paramount to understanding viral fitness in its natural hosts. While DENV-mammalian host interactions have been extensively studied, not much has been done to characterize DENV-mosquito host interactions despite its importance in controlling DENV transmission. Here, to provide a snapshot of our current understanding of DENV-mosquito interactions, we review the literature that identified host factors and cellular processes related to DENV infection in its mosquito vectors, Aedes aegypti and Aedes albopictus, with a particular focus on DENV-mosquito omics studies. This knowledge provides fundamental insights into the DENV life cycle, and could contribute to the development of antiviral strategies.

Dengue is arguably the most important mosquito-borne viral infectious disease in humans. This acute disease is caused by four genetically related but antigenically distinct dengue viruses (DENV-1 to -4). All four DENVs are transmitted by Aedes (Ae.) mosquitoes, principally Ae. aegypti. As the global footprint of Ae. aegypti continues to expand from the tropics to the sub tropics [1], well over half of the world’s population lives in areas at risk of DENV infection [2]. An estimated 100 million people develop dengue and thousands die from this disease each year [3]. Presently, the only licensed dengue vaccine, Dengvaxia, comes with some limitations prior to usage and there are no specific therapeutics to treat dengue [4]. Dengue prevention has and will continue to rely on vector population suppression. To strengthen conventional mosquito control strategies, the development of novel insecticide-free strategies for vector control has been a major focus of research in recent years. The use of natural bacterium Wolbachia infection to reduce the vectorial capacity of Ae. aegypti has shown promising results in a recently completed randomized controlled trial [5]. Other promising approaches include the use of genetic engineering technology to develop vector populations which are refractory to DENV infection [6]. Advances in our understanding of Ae. aegypti at the molecular level will allow us to improve these strategies and use them in combination to effectively reduce the global disease burden of dengue.

The DENV genome is composed of a positive-sense, single-stranded RNA (+ssRNA). The genome is approximately 10.7 kb...
in length and constitutes a single open reading frame (ORF) flanked with 5′ and 3′ untranslated regions (UTRs). The ORF of the genome encodes three structural proteins (capsid (C), pre-membrane/membrane (prM/M) and envelope (E)) and seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5). Given that the DENV genome encodes only 10 proteins, the virus is inevitably reliant on its host dependency (sometimes also referred to as pro-viral) factors (HDFs) to complete its life cycle. At the same time, it has to evade or suppress host restriction (or anti-viral) factors (HRFs) that would otherwise suppress or inhibit infection. The availability of genomic and proteomic tools has enabled the identification of HDFs and HRFs of DENV in mammalian cells [7–12]. These studies have collectively revealed a complex network of DENV-mammalian host interactions as well, summarized in previous reviews [13–17]. While the understanding between DENV and its mammalian host has made remarkable progress, the interaction of DENV with the mosquito host is less well defined.

The life cycle of DENV in a mosquito begins when it ingests a blood meal from a viremic person, whereupon DENV must successfully infect the mosquito midgut before it can disseminate systemically in the mosquito, including to the salivary glands. Once the salivary glands are infected, the mosquito is then capable of transmitting DENV to other humans via its saliva during subsequent blood meals. The virus-mosquito interactions that underpin the DENV life cycle in the mosquito vectors, however, are not well understood even though such critical interactions could shape viral fitness in epidemiological and clinical settings [18, 19]. This knowledge could pave the way for future mechanistic studies, and would be crucial to identify novel targets that may disrupt DENV transmission from the mosquito vectors to humans. Here, we review the literature to establish a snapshot of our current understanding of DENV-mosquito interactions, with a focus on DENV-mosquito omics studies.

Due to the lack of resources to perform functional genomics in both Ae. aegypti and Ae. albopictus, the secondary vector of DENVs, the first high-throughput screen to identify host factors that are required for DENV infection in insects was conducted in Drosophila melanogaster cells [20]. A genome-wide loss of function screen based on RNA interference (RNAi) targeting 22632 genes identified 116 HDFs. These include genes involved in endocytosis, vesicular transport, the unfolded protein response, and RNA-binding proteins potentially involved in viral genome replication, transcription, translation or packaging. This study provided the first comprehensive list of DENV HDFs in insects, and identified remarkable conservation of host factors required for DENV infection between human and insect hosts.

**DENV-MOSQUITO TRANSCRIPTOMIC STUDIES**

**Innate immune response**

With the advent of publicly available complete genome sequences of Ae. aegypti [21] and more recently Ae. albopictus [22], omics studies for DENV infection in its natural vectors became possible. Using microarray and RNA sequencing (RNAseq), various groups have identified differentially expressed genes (DEGs) in DENV-infected mosquito cells and mosquitoes related to innate immunity, metabolism, and stress response. Among the immunity-related genes, the more prominently represented ones include those associated with the Toll pathway and to a lesser extent the JAK-STAT pathway.

The midgut, salivary glands and carcass of Ae. aegypti infected with DENV consistently showed upregulation of genes in the Toll pathway, a major component of the mosquito innate immune system [23–25]. In the follow-up studies, targeted RNAi-based gene knockdown identified the signal transduction adaptor protein MyD88 of the Toll pathway as a HRF whose knockdown increased viral load in DENV-infected Ae. aegypti midgut [26, 27]. Meanwhile, knockdown of its negative regulator Cactus reduced viral load [25]. The importance of the Toll pathway in controlling DENV infection has also been observed across different DENV serotypes and Ae. aegypti strains [26, 27].

On the other hand, DENV is able to inhibit the mosquito innate immune response to favour its infection. Downregulation of several genes related to the Toll pathway was observed at different time-points during DENV infection in Ae. aegypti-derived Aag2 cells [28, 29], Ae. albopictus-derived C6/36 cells [30], and Ae. aegypti midguts and fat bodies [26]. The Toll pathway was also shown to be preferentially inhibited in the salivary glands of mosquitoes infected with DENVs containing the 3′UTR substitutions associated with high subgenomic flavivirus RNA (sfRNA) quantity and high epidemiological fitness [19]. Altogether, these data emphasize the ability of DENV to counteract the mosquito innate immune response against DENV infection.

Apart from the Toll pathway, transcriptional profiling of DENV-infected Ae. aegypti midgut, salivary glands and carcass also revealed upregulation of genes linked to the JAK-STAT pathway as well as the IMD pathway, another two major components in the mosquito innate immune system [23, 25, 31]. Targeted RNAi-mediated gene silencing specifically identified the receptor Dome and the signal transduction adaptor protein Hop/JAK of the JAK-STAT pathway as HRFs whose silencing increased viral load in Ae. aegypti midgut, and the negative regulator PIAS, together with two uncharacterized effectors, as HDFs whose silencing resulted in the opposite effect [32]. The involvement of the JAK-STAT pathway in anti-DENV defence was also observed when experiments were carried out using different Ae. aegypti strains [32]. Similar to the Toll pathway, DENV seems to be able to counteract the JAK-STAT pathway. The presence of DENV secreted NS1 (sNS1) (expressed abundantly in dengue patients) in the mosquito blood meal has been shown to be able to inhibit the JAK-STAT pathway in Ae. aegypti midgut, thus augmenting DENV infection in Ae. aegypti [33].

In contrast to the Toll and JAK-STAT pathways, the roles of the IMD pathway in controlling DENV infection remain elusive. The IMD pathway was shown to be inhibited upon DENV infection in some studies, suggesting its involvement...
in anti-DENV defence [28, 30]. In contrast, another study showed that while knockdown of the signal transduction adaptor protein IMD of the IMD pathway increased viral load in the midgut of DENV-infected Ae. aegypti, knockdown of its negative regulator Caspar did not reduce viral load in this organ as expected [27]. Further investigations are thus required to elucidate the significance of this innate immune pathway in anti-DENV defence.

There also appears to be crosstalk between the IMD pathway and the small interfering RNA (siRNA) pathway, a part of the RNAi machinery which forms another major component in the mosquito innate immune system [34]. Currently, there are three main RNAi-related pathways characterized in insects [35]: (i) siRNA pathway in which siRNAs are generated from double-stranded RNA (dsRNA) derived from either exogenous sources (such as viruses) or endogenous sources within cells, (ii) microRNA (miRNA) pathway in which miRNAs are generated from cell-encoded transcripts, and (iii) PIWI-interacting RNA (piRNA) pathway in which piRNAs are transcribed from the cell genome. Among the RNAi-related pathways, this review will focus on the exogeneous siRNA pathway which is the most well-characterized pathway for antiviral properties in insects [36]. It was first found that DENV-specific siRNAs were detected in Ae. aegypti midguts during DENV infection, suggesting its involvement in anti-DENV response in the mosquito [37, 38]. Indeed, the inhibition of the siRNA pathway by silencing either of its components, Dcr2, R2D2 or Ago2, increased viral load in Ae. aegypti, shortened viral incubation period, as well as enhanced viral transmissibility [37]. Furthermore, transgenic Ae. aegypti overexpressing DENV-specific dsRNAs showed significant reduction in DENV infection in the midgut, salivary glands as well as saliva, and the interruption of the siRNA pathway eliminated this reduction [39, 40]. Collectively, these findings support the involvement of the siRNA pathway in anti-DENV defence in mosquitoes.

Taken together, while the innate immune pathways are activated during DENV infection, the ability of the virus to evade these innate immune responses will determine whether DENV can successfully complete its life cycle in the mosquito vectors.

**Other cellular processes**

Besides mosquito innate immunity, a comparative transcriptomic study using DENV-susceptible and -refractory strains of Ae. aegypti revealed that the midgut of the susceptible strain specifically showed upregulation of genes involved in mRNA surveillance, protein processing in the endoplasmic reticulum (ER) and the proteasome [41]. In contrast, the midgut of the refractory strain showed upregulation of genes involved in metabolic processes including the Wnt signalling pathway, the glycolysis pathway and glycan biosynthesis. It would be interesting to further examine if these factors influence vector
| Study                                                                 | Methods                          | Findings                                                                 | References |
|----------------------------------------------------------------------|----------------------------------|---------------------------------------------------------------------------|------------|
| **Transcriptomic studies**                                           |                                  |                                                                           |            |
| Identification of the mosquito innate immune system as a part of anti-DENV defences | Microarray                        | Upregulation of genes related to the Toll, JAK-STAT and IMD pathways.    | [23–25, 31]|
|                                                                     | Digital gene expression          | The signalling components of the Toll (MyD88) and JAK-STAT (Dome and JAK/Hop) pathways are HREs while the negative regulators of the Toll (Cactus) and JAK-STAT (PIAS) pathways are HDFs. | [25–27, 32]|
|                                                                     | RNAi-based gene knockdown        | A conserved function in anti-DENV defence of the Toll and JAK-STAT pathways across different Ae. aegypti strains. | [19, 33] |
|                                                                     | Microarray                       | Downregulation of genes related to the Toll and IMD pathways following DENV infection. | [37–40]    |
|                                                                     | RNA sequencing                   | The ability of DENV vRNA and sNS1 to inhibit the Toll and JAK-STAT pathways, respectively. |            |
|                                                                     | RT-PCR                           | The presence of the siRNA pathway and its importance in anti-DENV defence in Ae. aegypti. | [41]       |
| Identification of other relevant cellular processes during virus infection through comparative transcriptomic studies | Microarray                        | A potential role of differentially transcriptomic changes underlying vector competence of Ae. aegypti. | [42]       |
|                                                                     | Microarray                       | - DENV-susceptible Ae. aegypti strain: upregulation of genes involved in the proteasome, mRNA surveillance and protein processing in the ER. | [30, 28]  |
|                                                                     | RNA-sequencing                   | DENV-refractory Ae. aegypti strain: upregulation of genes involved in the Wnt signalling pathway, the glycolysis pathway and glycan biosynthesis. |            |
|                                                                     |                                  | A potentially conserved transcriptomic signature of flavivirus infection. |            |
|                                                                     |                                  | - Alteration in the expression of genes involved in metabolic processes, peptidase activity, ion binding and transport during DENV, WNV and YFV infection. |            |
|                                                                     |                                  | A consistent upregulation of genes involved in starch and sucrose metabolism, pyrimidine metabolism and drug metabolism, and downregulation of genes involved in RNA transport, purine metabolism, drug metabolism, folate biosynthesis, and valine, leucine and isoleucine degradation in DENV infected Aa22 and C6/36 cells. |            |
| **Proteomic studies**                                               |                                  |                                                                           |            |
| Proteomic changes during DENV infection                             | 2D-DIGE                          | Upregulation of proteins involved in the glycolysis pathway and the cellular stress response in C6/36 cells. | [43]       |
|                                                                     | MALDI-TOF MS                     | Increased production of proteins involved in carbohydrate and lipid metabolism as well as the production of reactive oxygen species (ROSs) in Ae. aegypti midguts. | [44]       |
|                                                                     | MALDI-TOF MS/MS                  | Alteration in the expression of proteins; in particular proteins with anti-hemostatic and pain inhibitory properties in Ae. aegypti salivary glands. |            |
| Identification of potential cellular receptors                      | VOPBA                            | With the use of Ae. aegypti midgut homogenates and C6/36 cell lysates, cadherin, enolase, beta-adrenergic receptor kinase (beta-AR) and translation elongation factor EF-1 alpha/Tu were identified. | [50]       |
|                                                                     | Mass spectrometry                | With the use of the membrane fractions of A27 cells, C6/36 cells and Ae. aegypti midguts, actin, oris, van-1, prohibitin, ATP synthase beta subunit, tubulin beta chain, and 70-kDa heat shock cognate protein (HSC70) were identified. | [51]       |
|                                                                     | VOPBA                            | With the use of C6/36 cell lysates, HSC70, 78kDa glucose-regulated protein (GRP78 or BiP), 70kDa heat shock protein (HSP70) and 40kDa protein with homology to protein disulide isomerase (PDI) were identified. | [52]       |
|Identification of DENV-mosquito protein interactions                | Tandem affinity purification      | 18 mosquito proteins as potential interacting partners of DENV and WNV proteins. | [53]       |
|                                                                     | LC-MS/MS                         | Actin, myosin, myosin light chain kinase and PI3-kinase are DENV and WNV HDFs. | [11]       |
|                                                                     | RNAi-based gene knockdown        | 28 host proteins both humans and mosquitoes as interacting partners of DENV and ZIKV proteins. |            |
|                                                                     | Affinity purification            | SEC61 is a shared DENV and ZIKV HDF in both mammalian and mosquito cells. | [42, 43]  |
|                                                                     | Pharmacological inhibition       |                                                                           |            |
| **Metabolomic studies**                                            | LC-MS                            | Upregulation of lipid anabolism and catabolism. Enrichment of lipids that can modify the physical properties of membranes. | [54]       |
| Lipidomic changes during DENV infection                            | LC-HRMS                          | Increased production of membrane phospholipids which are required to modify mosquito membrane structures to support DENV replication. | [56, 57]  |
|                                                                     | RNAi-based gene knockdown        |                                                                           |            |
| Potential importance of lipid modulation during DENV infection      | LC-MS                            | Identification of lipid modulation as a molecular mechanism underlying Wolbachia-mediated DENV blocking in Ae. aegypti. | [58]       |
|                                                                     | RNAi-based gene knockdown        | Uptregulation of genes encoding lipid-binding proteins - the myeloid differentiation 2-related lipid recognition protein (MLR) and the Niemann Pick-type C1 (NPC1) family members in Ae. aegypti, and silencing of those gene family members restricted DENV infection in Ae. aegypti midgut. | [24, 25]  |

Continued
Table 1. Continued

| Study                                      | Methods                                      | Findings                                                                 | References |
|--------------------------------------------|----------------------------------------------|--------------------------------------------------------------------------|------------|
| Computational studies                      |                                              |                                                                          |            |
| Establishment of DENV-mosquito interaction networks | Computational approach based on available data from genome-wide RNAi screens, transcriptomic studies and physical protein-protein interactions | 714 DENV-Ae. aegypti interactions with Ae. aegypti proteins involved in transport, immunity, metabolism and replication/transcription/translation being the most enriched. | [60]       |
|                                            | Computational approach based on structural similarity of DENV and Ae. aegypti proteins | 176 DENV-Ae. aegypti interactions with Ae. aegypti proteins involved in RNA processing and regulation of stress response being the most enriched. | [61]       |

2D-DIGE, two-dimensional differential in-gel electrophoresis; 2DE, two-dimensional gel electrophoresis; LC-HRMS, liquid chromatography-high resolution mass spectrometry; LC-MS, liquid chromatography-mass spectrometry; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MALDI-TOF MS, matrix assisted laser desorption ionisation time-of-flight mass spectrometry; MALDI-TOF/TOF-MS, matrix assisted laser desorption ionisation time-of-flight/time-of-flight mass spectrometry; RNAi, RNA interference; RT-PCR, real-time polymerase chain reaction; VOPBA, virus overlay protein binding assay.

Competence of Ae. aegypti. Meanwhile, another comparative transcriptional profiles of Ae. aegypti infected with DENV and other two flaviviruses including West Nile virus (WNV) and yellow fever virus (YFV) revealed a potentially conserved transcriptomic signature of flavivirus infection [42]. This includes alterations in the expression of genes involved in transport, metabolic processes, ion binding and peptide activity. Similarly, in DENV-infected Aag2 and C6/36 cells, upregulation of genes involved in starch and sucrose metabolism, pyrimidine metabolism and drug metabolism, and downregulation of genes involved in RNA transport, purine metabolism, drug metabolism, folate biosynthesis, and valine, leucine and isoleucine degradation were observed [28, 30].

Taken together, physiological pathways related to innate immunity, metabolism, energy production and redox activity were major pathways modulated during DENV infection in the mosquito vectors. However, only a limited number of specific genes were concordant across different high-throughput studies. Depending on the species or strains of mosquitoes and infecting DENV, it is important to note that the mosquito transcriptome may also differ accordingly during infection.

### DENV-MOSQUITO PROTEOMIC STUDIES

In addition to the transcriptomic studies, proteomic studies have been performed. DENV-infected C6/36 cells significantly increased the expression of proteins involved in the glycolysis pathway and cellular stress response [43]. In another study, proteomic analysis of DENV-infected Ae. aegypti midguts showed an increased production of midgut proteins involved in carbohydrate and lipid metabolism, as well as the production of reactive oxygen species (ROSs) when compared to uninfected midguts [44]. These proteins potentially serve as HDFs that support energy production for viral infection and help DENV to cope with the stress induced by its infection [45]. DENV infection has also been shown to alter the expression of several salivary proteins; in particular proteins with anti-hemostatic and pain inhibitory properties [46]. The changes in the proteome of the mosquito saliva could potentially enhance successful transmission of DENV from an infected mosquito to humans during blood feeding [47].

Apart from the studies focusing on proteomic changes upon DENV infection, the virus overlay protein binding assay (VOPBA) has been used to identify cellular receptors for DENV [48–51]. This approach has identified enolase, cadherin, beta-adrenergic receptor kinase (beta-ARK) and translation elongation factor EF-1 alpha/Tu as potential DENV binding receptors in Ae. aegypti midgut homogenates and C6/36 cell lysates [50]. Other proteins such as actin, orisis, vav-1, prohibitin, ATP synthase beta subunit, tubulin beta chain and 70 kD heat shock cognate protein (HSC70) were also shown to bind to the membrane fractions of Ae. aegypti-derived A7 cells and C6/36 cells, and the midgut brush border membrane fraction of Ae. aegypti [51]. Independently, another group has also identified HSC70 interacting with DENV in C6/36 cells, together with 78kDa glucose-regulated protein (GRP78 or BiP), 70kDa heat shock protein (HSP70) and 40kDa protein with homology to protein disulfide isomerase (PDI) [52]. However, future work is required to determine if these proteins are indeed required for DENV entry and transport into mosquito cells.

Next, the use of affinity purification assays coupled with proteomic analyses has also expanded the list of DENV-mosquito protein interactions [11, 53]. A tandem affinity purification (TAP) assay followed by proteomic analysis in DENV- or WNV-infected C6/36 cells identified 18 mosquito proteins as potential interacting partners of viral proteins [53]. Using a targeted RNAi-based knockdown approach, actin, myosin, myosin light chain kinase and PI3-kinase were identified as HDFs of DENV and WNV although their roles in the virus life cycle remain unknown. Using a similar method, a more recent study identified 28 conserved host proteins in both humans and mosquitoes as interacting partners of DENV and Zika virus (ZIKV) proteins, of which pharmacological inhibition of SEC61 inhibited both DENV and ZIKV infection in mammalian and mosquito cells [11]. Taken together, these studies showed that comparative proteomic analyses could be utilized to not only identify DENV HDFs, but also HDFs that are shared among flaviviruses in their hosts. These shared HDFs may ultimately serve as targets for broad-acting antivirals, and knowledge of these interactions may prove useful in the design of novel vector control strategies.
DENV-MOSQUITO METABOLOMIC STUDIES

Using a metabolomic analysis, DENV-infected C6/36 cells were shown to induce the production of sphingomyelins, phosphatidylycholine and ceramide which are lipids that can modify the membrane physical properties such as permeability and curvature [54]. While the changes on membrane permeability have been proposed to support the exchange of required components between the cytosol and the ER lumen during DENV replication [54], the changes on membrane curvature could potentially be exploited by DENV, to use the host membranes to form virions or/and to prevent viral RNA detection by the mosquito antiviral defence [55]. This study also found that DENV-infected C6/36 cells increased both lipid anabolism and catabolism. Although their importance on DENV infection remains unclear, lipid anabolism may be required for virus-induced membrane changes, while lipid catabolism might be essential for energy supply for efficient virus production. Recently, studies have further shown that increased production of membrane phospholipids are required to modify mosquito membrane structures to support DENV replication in mosquitoes [56, 57]. Interestingly, another recent study has shown that one of the molecular mechanisms underlying Wolbachia-mediated DENV blocking in Ae. aegypti is lipid modulation [58]. Besides, DENV infection has also been shown to induce the expression of genes encoding lipid-binding proteins – the myeloid differentiation 2-related lipid recognition protein (ML) and the Niemann Pick-type C1 (NPC1) family members – in Ae. aegypti, suggesting a role of these lipid-binding proteins as DENV host factors [24, 25]. Indeed, targeted RNAi-mediated gene silencing of a ML (AaaegML33) and a NPC1 (AaegNPC1b) gene family member restricted DENV infection in Ae. aegypti midguts [59]. These intricate metabolic interactions between DENV and the mosquito vectors are essential in the viral life cycle and potentially represent targets to control disease transmission. Further work to study these interactions should be explored.

DENV-MOSQUITO INTERACTION NETWORKS

With the advent of computational approaches, the knowledge on DENV-mosquito interactions has been greatly expanded in terms of both prediction of novel virus-host interactions, as well as integration of known virus-host interactions into a network. This aids in the identification of significant host factors and cellular processes required during DENV infection. By using a computational method, 714 interactions between DENV and Ae. aegypti were predicted based on three high-throughput screens including genome-wide RNAi screens, transcriptomic studies and physical protein-protein interactions with the most enriched Ae. aegypti proteins involved in transport, immunity, replication/transcription/translation and metabolism [60]. Another computational study also predicted 176 DENV-Ae. aegypti interactions based on their protein structural similarities, with proteins involved in RNA processing and regulation of stress response being the most enriched [61].

PROSPECTIVE DIRECTIONS FOR RESEARCH

As reviewed above and summarized in Fig. 1, Table 1, while the knowledge on DENV-mosquito interactions is expanding, the interactions critical for DENV fitness remain poorly defined. One major challenge lies in the lack of functional validation performed on the HDFs and HRFs identified in most of these studies. Also, these studies have studied DENV-mosquito interactions using laboratory-passaged DENVs; mutations that accumulate through serial passaging could thus confound interpretation of virus-host interactions necessary for DENV replication and transmission by the mosquito vectors.

To date, only a limited number of studies have dissected DENV-mosquito interactions by comparing virulent and clinically proven attenuated DENVs. Studies have shown that genetic differences in the DENV genomes play a critical role in determining viral fitness both epidemiologically and clinically [18, 19, 62–66]. In mosquitoes, using a clinically tested vaccine strain DENV-2 PDK53, which differs from the wild-type clinical isolate DENV-2 16681 genetically by only nine nucleotides, our laboratory has recently showed that the change of glycine to aspartic acid at position 53 of the NS1 protein (NS1 G53D) attenuated 16681 infection in Ae. aegypti; NS1 G53D infection in the mosquito midgut is confined by the early induction of the mosquito immune response similar to PDK53 [18]. Besides the midgut, this early innate immune response also restricts viral dissemination to the other parts of Ae. aegypti including the salivary glands, impeding productive infection and subsequently successful DENV transmission by the mosquito.

Another study in mosquitoes compared DENV-2 strains with different epidemic potential in Puerto Rico. Nucleotide substitutions in the 3′UTR of an epidemiologically fitter strain of DENV-2 that had caused an outbreak in Puerto Rico in 1994 resulted in increased sRNA production in Ae. aegypti salivary glands and thus increased virus transmission, at least in part, due to innate immune suppression in this organ [19], when compared to DENV-2 isolated before the outbreak. Altogether, these studies highlight that the use of DENVs with different clinical or epidemiological fitness with known genetic differences could enable a more targeted approach to efficiently identify critical DENV-mosquito interactions that govern viral fitness.

In addition, DENV exists as a collection of closely related genomes, or quasispecies due to the error prone nature of their replication machinery, the RNA dependent RNA polymerase (RdRp) enzyme. Genome diversity has previously been shown to contribute to changes in viral fitness and pathogenesis [67]. Recently, ultra-sequencing has been used to identify beneficial mutations that may facilitate host-specific DENV adaption, and enabled the assessment of the role of these mutants in viral fitness [68]. Elucidating how these mutants influence virus-mosquito interactions could also shed light into HDFs that will be the key to conceive new antiviral approaches.

To conclude, this review provides a brief summary of DENV-mosquito interactions with a focus on omics studies. It is
likely that this body of information represents only the tip of the iceberg of the total DENV-mosquito interaactome. Studies that are on-going and planned for the future will likely shed more light on the complexity of the DENV-mosquito interplay in the coming years. The use of DENVs with known genetic differences would significantly progress the identification of critical virus-mosquito interactions required for viral fitness and successful virus transmission, which will be useful for the identification of antiviral targets as well as the development of transgenic mosquitoes that are resistant to DENV infection.

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Conflicts of interest
E.E.O. served as a dengue vaccine advisory board member for Takeda Vaccines, which uses DENV2 PDK53 strain as a component of their dengue vaccine candidate, TAK-003.

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