Arbovirus lifecycle in mosquito: acquisition, propagation and transmission

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Abstract

Mosquitoes are haematophagous vectors for hundreds of pathogenic viruses that are aetiological agents of human diseases. In nature, mosquito-borne viruses maintain a lifecycle between mosquitoes and vertebrate animals. Viruses are acquired by a naive mosquito from an infected host by blood meals and then propagate extensively in the mosquito's tissues. This mosquito then becomes a virus reservoir and is competent to transmit the viruses to a naive vertebrate host through the next blood meal. To survive in and efficiently cycle between two distinct host environments, mosquito-borne viruses have evolved delicate and smart strategies to comprehensively exploit host and vector factors. Here, we provide an update on recent studies of the mechanisms of virus survival in, acquisition and transmission by mosquitoes.

Mosquitoes, which belong to the *Culicidae* family, are a group of haematophagous vectors that carry and transmit many human viruses in nature. Most mosquito-borne human viruses, belonging to the *Flaviviridae*, *Togaviridae* and *Bunyaviridae* families (Ref. 1), are aetiological agents of severe human diseases such as haemorrhagic fever, biphasic fever, encephalitis, and meningitis (Ref. 2). Hundreds of millions of infections are caused by mosquito-borne viruses annually (Refs 3–5). Dengue virus (DENV, *Flavivirus*, *Flaviviridae* family), the most prevalent mosquito-borne virus worldwide transmitted by the *Aedes aegypti* and *A. albopictus* mosquitoes, is estimated to cause 390 million infections every year, of which approximately 500 000 are severe cases that require hospitalisation, with more than 20 000 cases lead to death, mostly in tropical countries (Ref. 6). Recent Zika virus (ZIKV, *Flavivirus*, *Flaviviridae* family) outbreaks in the Pacific islands and the Americas have resulted in more than 223 000 confirmed cases by the end of 2017 (Refs 7, 8). Several neurological complications, such as Guillain–Barré syndrome in adults and microcephaly in neonates, are associated with ZIKV infection (Ref. 9). In addition, both West Nile virus (WNV, *Flavivirus*, *Flaviviridae* family) and chikungunya virus (CHIKV, *Alphavirus*, *Togaviridae* family) caused millions of infections in Americas in the past two decades. Although arboviruses have propagated to a vast region of the globe and posed major public health concerns (Fig. 1), there are no vaccines or therapeutics available against most mosquito-borne viruses. The lifecycle of mosquito-borne viruses involves survival in and transmission between two distinct host environments. The strategies employed by viruses at each stage are thus different. Understanding the triangular interplay between host, virus and mosquito in the arboviral lifecycle may provide novel strategies for limiting viral transmission and their prevalence in nature.

Mosquito-borne viruses naturally cycle between mosquitoes and vertebrate animals. As a natural vector, a mosquito incidentally feeds on a virus-infected host to acquire viruses circulating in the host blood. The viruses subsequently establish an infection in the mosquito's epithelial cells by overcoming the physical and immune barrier of the epithelia and then spread into the mosquito haemocoel. The viruses subsequently infect other tissues, such as the salivary glands, ovaries, and neural system. Thus, the infected mosquito becomes competent to transmit the viruses to naive hosts through blood feeding (Ref. 1) (Fig. 2). During their lifecycle, the viruses exploit many strategies enabling mosquitoes to carry and transmit them efficiently. Here, we provide an overview of recent progress on the underlying mechanisms by which the arboviruses complete their life journey.

From host to mosquito: arboviral acquisition by mosquitoes feeding on viraemic hosts

Arboviral acquisition, which is the process of viral transmission from an infected host to a mosquito vector, is an essential step in the viral lifecycle. The circulating viral particles in the infected hosts' blood are taken into the midgut by blood meal, thereby initiating an infection in the mosquitoes. The viruses must conquer the gut epithelial barrier to establish their infection in the epithelial cells, prior to systemic dissemination. The outcomes of arboviral acquisition are determined by multiple factors, such as the gut immunity, the components in the host blood and the gut resident commensal microbes.
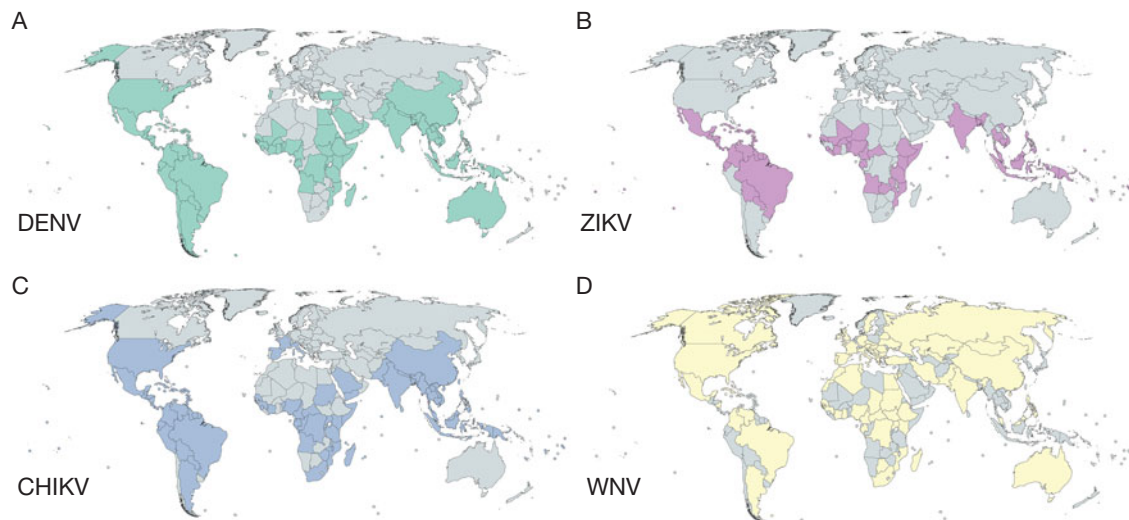
Fig. 1. Global distribution of arboviruses. The map depicts countries or territories with previous or current transmission of Dengue virus (a), Zika virus (b), chikungunya virus (c) and West Nile virus (d). The map was generated using a free online tool Mapchart with public data from WHO, CDC, PAHO and a review of literature (Refs 10–13).

As a haematophagous insect, mosquitoes take a large amount of blood from the infected host by blood meal. Therefore, the host blood components may modulate the immune and physiological status of the mosquito gut, thus regulating the mosquito's permissiveness to viral infection. Based on current knowledge, several host blood factors may modulate viral replication in the mosquito gut epithelium. For example, variations in the host immunological responses may correspond with the capacity for DENV acquisition from viraemic patients to *A. aegypti* mosquitoes. Increasing titres of immunoglobulin M and immunoglobulin G against DENV envelope proteins may contribute to a reduction in DENV infection in mosquitoes (Ref. 14). Besides, low-density lipopolyprotein (LDL) is a highly abundant blood component whose expression is decreased during severe DENV infection. Indeed, LDL acquired from host blood can be endocytosed by the mosquito midgut epithelial cells and accumulate at the luminal epithelium during blood digestion, therefore hindering flavivirus acquisition in *A. aegypti* by blood meal (Ref. 15). In addition, a haemoglobin metabolite called haem can induce the expression of genes related to redox metabolism, thereby triggering large, variable expression of energy metabolism and immune genes in the mosquito gut to modulate the vectorial susceptibility to arboviruses (Ref. 16). We recently found that the digestion of blood proteins generally facilitates replication of arboviruses through activation of GABAergic signalling in mosquitoes. Activation of the GABAergic system enhances the infection of arboviruses of the *Flavivirus*, *Alphavirus* and *Orthobunyavirus* genera by modulating the gut antiviral immunity. Gamma-aminobutyric acid (GABA) can be generated via decarboxylation of the amino acid glutamic acid. The ingestion of blood in the mosquito guts results in robust GABA production from glutamic acid derived from blood protein digestion, thereby facilitating viral replication of the mosquito vectors (Ref. 17). Altogether, the haematophagous nature of mosquitoes regulates arboviral acquisition by blood meal. In addition to the host blood components, the virus also utilises its own non-structural proteins secreted in host blood to efficiently overcome the hostile gut environment, thereby enabling viral acquisition. Flavivirus nonstructural protein-1 (NS1) is a virus-encoded nonstructural protein that is secreted into the blood of infected hosts. Circulating NS1 can be acquired by mosquitoes together with virions when they feed on a viraemic host. Indeed, flavivirus NS1 helps the viruses conquer the gut immune barrier by inhibiting gut immunity, such as reactive oxygen species production and the Janus kinase (JAK)-signal transduction and activators of transcription (STAT) pathway, thereby enabling efficient viral infection in mosquitoes (Refs 18, 19).

The mosquito gut is a pivotal natural entry site for arboviruses. The intricate ecological gut environment, including the gut immunity and resident gut commensal microbes, plays a role in modulating arbovirus infection in the gut epithelial cells. The gut antiviral mechanisms in mosquitoes have been well summarised in previous reviews (Refs 1, 20, 21). Briefly, RNA interference (RNAi) is deemed as a predominant antiviral mechanism in invertebrates. Recent studies have demonstrated that the RNAi system responds to many arboviral infections in mosquitoes. Suppression of the RNAi components resulted in higher viral replication in the mosquito guts (Refs 21–23). In addition, several evolutionarily conserved innate immune pathways, such as the Toll pathway, the immune deficiency (Imd) pathway and the JAK-STAT pathways, also play resistant roles against arbovirus replication in the gut epithelium. O'nyong'nyong virus (*Alphavirus*, *Togaviridae* family) infection in *Anopheles gambiae* was enhanced by knockdown of the Imd components in the mosquito midguts (Ref. 24). Silencing of key components in the Toll (*Myd88*) and JAK-STAT (*Dome* and *Hop*) pathways significantly increased DENV replication (Ref. 25), while silencing PIAS (a negative regulator of the JAK-STAT pathway) showed an opposite role (Ref. 26) in DENV infection by blood meal. These pieces of evidence indicate the crucial roles of these mosquito antiviral pathways in controlling arboviral acquisition. In addition, the gut inhabitant commensal microbiome can modulate viral replication in the mosquito gut epithelia. During arboviral acquisition by blood meal, the viruses are taken into the mosquito gut, where an abundant commensal microbiome resides. Indeed, colonisation by a *Chromobacterium* species, a commensal gut bacterium isolated from field-caught *A. aegypti*, compromised mosquito vector competence to DENV infection (Ref. 27). A gut commensal bacterium, *Chromobacterium* sp. Panama, presents its anti-DENV activity by secreting an aminopeptidase to degrade the DENV envelope protein, thus preventing DENV attachment and infection of the mosquito cells (Ref. 28). In addition, oral introduction of a *Proteus* sp. strain, which was derived from the mosquito gut, led to an increase of resistance against DENV infection through up-regulation of the expression of antimicrobial peptide (AMP) genes in the gut epithelial cells (Ref. 29). An
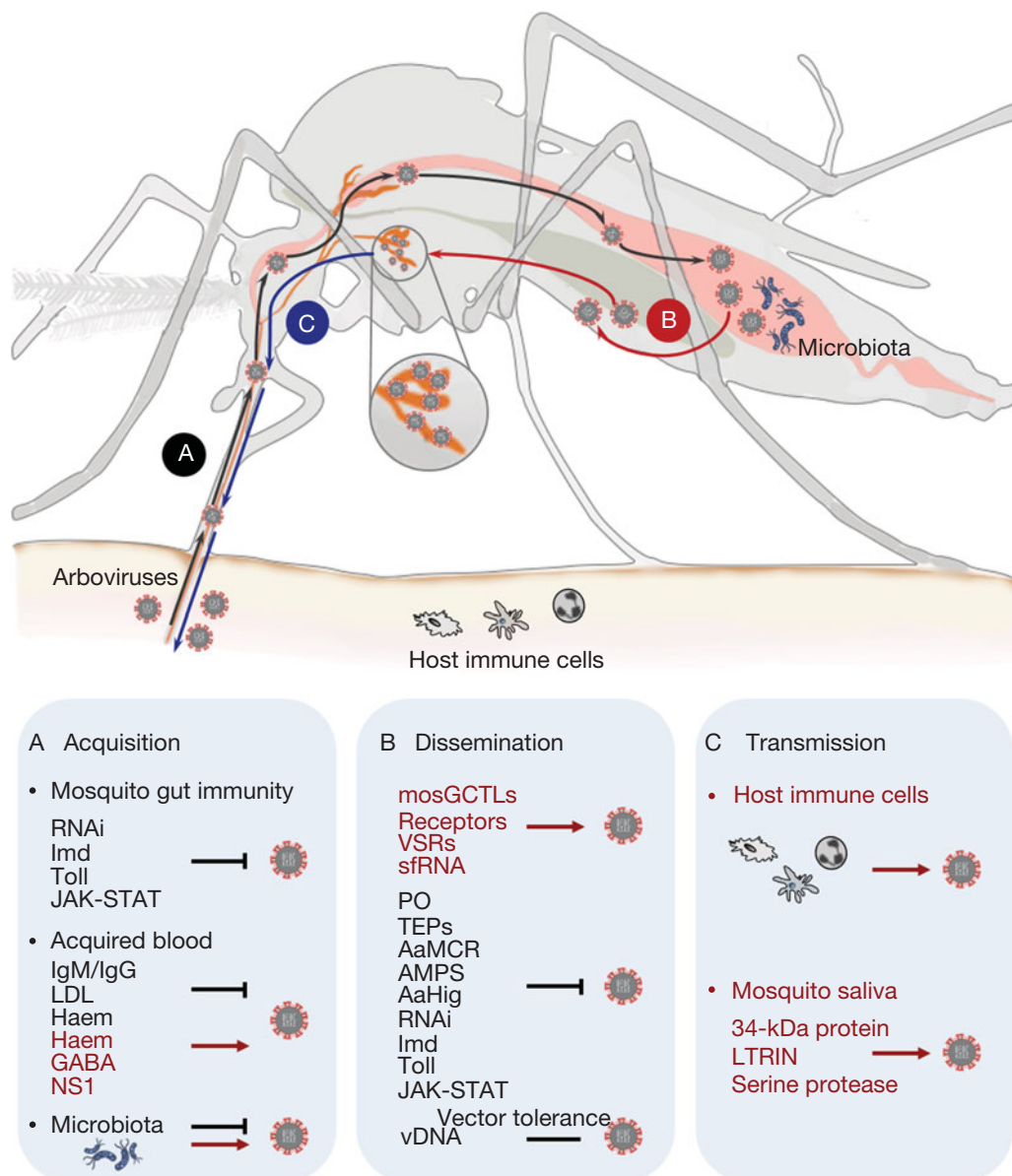
Fig. 2. Schematic overview of the arbovirus lifecycle in mosquito. (a) After acquisition of arbovirus from an infectious blood meal, the mosquito gut immunity, components of the host blood and the mosquito gut commensal microbiome regulate mosquito permissiveness to viral infection. Several evolutionarily conserved innate immune pathways, such as immune deficiency, Toll and Janus kinase-signal transduction and activators of transcription, as well as RNA interference, are key factors in inhibiting arbovirus replication in the mosquito gut epithelium. Host blood components either hinder the infection capacity (immunoglobulin M, immunoglobulin G or low-density lipopolyprotein), or enhance arbovirus infection by suppressing mosquito gut antiviral immunity (gamma-aminobutyric acid, flavivirus nonstructural protein-1). The commensal microbiome residing in the mosquito gut lumen also plays an important role in modulating vector susceptibility to arboviruses. (b) Arboviruses exploit vectorial or viral factors to facilitate systemic infection in mosquito tissues. Vectorial factors, such as mosquito galactose-specific C-type lectin-1, or other putative receptors and viral factors, such as viral suppressor against RNA silencing and subgenomic flaviviral RNA, can contribute to the spreading of arboviruses from the midgut epithelium into the haemolymph circulation. Mosquitoes utilise different strategies, such as phenoloxidase, thioester-containing proteins, *A. aegypti* macroglobulin complement-related factor, antimicrobial peptides, *A. aegypti* Hikaru genki and innate immune pathways, to interfere with arbovirus infection. Virus-derived DNA plays a role in tolerance of viral replication in mosquitoes, thus enabling effective transmission of arbovirus by mosquitoes in nature. (c) Arboviruses are transmitted by mosquito bites into human skin along with mosquito saliva. The intradermal immune cells, such as dendritic cell subsets, monocytes and macrophages, are considered primary permissive targets during the initial infection in hosts. Mosquito saliva has also been proven to facilitate arbovirus transmission by exploiting saliva factors, including the 34-kDa protein LTRIN and a serine protease, to enhance the infection of host cells.

entopathogenic fungus, *Beauveria bassiana*, can activate the Toll and JAK-STAT pathway-controlled effector genes and shows a resistance role in DENV infection of *A. aegypti* (Ref. 30). Conversely, oral introduction of *Serratia odorifera*, a gut bacterium isolated from field *A. aegypti* mosquitoes, rendered mosquitoes highly susceptible to DENV infection (Ref. 31). A *Talaromyces* fungus identified in the *A. aegypti* gut promotes more permissive of mosquitoes to DENV infection, a phenotype that is attributed to the modulation of digestive enzymes and trypsin activity in the mosquito gut (Ref. 32). Altogether, the complicated interplay among these aforementioned factors,

including the gut immunity, the blood components and the gut commensal microbes, determines the outcomes of infection in mosquitoes after arboviral acquisition by blood meal.

Viral propagation in mosquito tissues

After their initial replication in the epithelia of the mosquito mid-gut, arboviruses spread into the haemolymph in the mosquito haemocoel. Haemolymph circulation facilitates systemic viral invasion into tissues such as the fat body, ovaries, salivary glands and neural system (Ref. 33). Indeed, arboviruses utilise multiple

strategies to complete their systemic infection in mosquito tissues, thereby enabling mosquitoes to carry and transmit the viruses efficiently (Ref. 34). Recent studies reported that multiple mosquito C-type lectins play susceptibility roles in dengue and WNV infection (Refs 35, 36). An *A. aegypti* C-type lectin, mosquito galactose-specific C-type lectin-1 (*mosGCTL-1*), interacts with WNV in a calcium-dependent manner. This 'mosGCTL-virus' complex consequently interacts with the mosquito protein tyrosine phosphatase-1 (*mosPTP-1*), a mosquito homologue of human CD45 in *A. aegypti*, thus enabling viral attachment to the plasma membrane for viral entry (Ref. 35). In addition, another nine *mosGCTL* paralogues facilitate DENV infection in *A. aegypti*. These mosGCTLs interact with DENV envelope proteins to serve as susceptibility factors (Ref. 36). Furthermore, several mosquito proteins, such as prohibitin (Ref. 37), heat-shock related protein (Ref. 38), and laminin-binding protein (Ref. 39), may act as putative receptors for DENV infection in mosquitoes. A putative cysteine-rich venom protein (CRVP379) can bind to mosquito prohibitin, thereby facilitating DENV replication in mosquitoes (Ref. 40). In addition, an evolutionarily conserved protein in both insects and mammalian hosts, natural resistance-associated macrophage protein (NRAMP), serves as a host cell surface molecule required for Sindbis virus (SINV, *Alphavirus*, *Togaviridae* family) binding and entry into *Drosophila* cells (Ref. 41), suggesting a potential role for NRAMP in alphavirus infection of mosquitoes. Recently, mounting evidence suggested that arthropod-borne viruses encode antagonists of antiviral immune pathways in mosquitoes. A non-structural protein encoded by the S-segment (NSs) of Bunyamwera virus has been proposed to act as a viral suppressor against RNA silencing (Ref. 42). NS4B protein of DENV interferes with processing of siRNAs by modulating host RNAi factors to favour virus replication (Ref. 43). Analysis of CHIKV encoding proteins reveals that the non-structural proteins, nsP2 and nsP3, exhibit the suppressor activity against RNAi (Ref. 44). Besides the protein factors, non-coding subgenomic viral RNA also contributes to viral replication in mosquitoes. Flaviviruses can produce an abundant subgenomic flaviviral RNAs (sfRNAs) during infection in the host cells. sfRNA determines the infection and transmission rates of WNV in the *Culex* mosquitoes by overcoming the mosquito midgut barrier (Ref. 45). Besides, the flavivirus sfRNA directly interacts with the RNAi component to suppress RNAi in cultured cells and mosquitoes (Ref. 46). In addition, DENV subgenomic RNA suppresses Toll-mediated immunity in the mosquito salivary glands to increase DENV replication, thereby promoting virus transmission by mosquitoes (Ref. 47).

Although the tissues in the mosquito haemocoel generally show permissiveness for arbovirus infection (Refs 48, 49), mosquitoes must equip many cellular and humoral immune strategies to prevent the dramatic pathological sequelae caused by robust viral replication (Ref. 1). One of the humoral antiviral mechanisms in the haemolymph is the phenoloxidase (PO) cascade (Ref. 50). In this humoral immune cascades, the *Prophenoloxidase* (*PPO*) gene, which encodes a prototype of PO, is secreted into the mosquito haemolymph (Ref. 51). The formation of melanin mediated by the PO cascades can be activated by Semliki Forest virus (SFV, *Alphavirus*) infection in *A. albopictus* U4.4 cells. Inhibition of PO activity facilitated both SFV replication in U4.4 cells and viral burden and mortality in *A. aegypti* (Ref. 52). In addition to the PO cascade, a recent study reported extracellular pattern recognition mechanisms mediated by complement-like proteins suppress flavivirus infection of *A. aegypti* (Refs 53, 54). A group of thioester-containing proteins (TEPs) in mosquitoes shares evolutionary conservation with the mammalian C3 protein (Refs 55, 56). In the mosquito haemolymph, a member of the TEP family, named as macroglobulin complement-related

factor (AaMCR), shows antiviral immune activity against DENV infection (Refs 35, 54). However, AaMCR is incapable of directly interacting with the viral surface; instead, AaMCR employs a scavenger receptor with complement control protein domains to bind DENV virions, subsequently suppressing viral infection by inducing multiple AMPs. In the mosquito nervous system, a neuron-specific complement-related factor, designated *Hikaru genki* (*Hig*), controls both DENV and Japanese encephalitis virus replication by interfering with viral entry (Ref. 53). In addition to the humoral antiviral systems, immune signalling cascades in tissues (i.e. the RNAi, Toll, Imd and JAK-STAT pathways) may also limit viral replication by inducing AMPs or other antiviral effectors (Ref. 57). Overall, as a result of the delicate balance between viral action and mosquito counteraction, the viruses can efficiently replicate and spread in the mosquito tissues without eliciting significant adverse effects. In nature, this kind of mutual adaptation makes mosquitoes an ideal reservoir for many human viruses.

Mosquitoes bear long-lasting viral infections without substantial deleterious effects. The previous studies found that the virus-derived DNA (vDNA) plays an essential role of mosquito vector tolerance to arboviral infection, thereby facilitating arbovirus dissemination and transmission (Ref. 49). Indeed, insects have a sophisticated systemic RNAi-based immunity mediated by macrophage-like haemocyte**s**. Haemocytes take up dsRNA from infected cells, and then produce virus-derived complementary DNAs (vDNA) through endogenous transposon reverse transcriptases. These vDNAs template *de novo* synthesis of secondary viral siRNAs, which are secreted in exosome-like vesicles RNA viruses produce vDNAs through the activity of endogenous retrotransposons. These vDNAs boost the RNAi-mediated antiviral immune response and are indispensable for establishing persistent viral infections in *Drosophila melanogaster* (Refs 58, 59).

From mosquito to host: arbovirus transmission by the mosquito blood meal

After robust replication in the mosquito salivary glands, viruses are now ready for transmission via the next mosquito blood meal. Mosquitoes transmit the viruses by intra-dermal injection of the infectious particles into hosts together with some mosquito saliva proteins. The intradermal immune cells, such as dendritic cell subsets (Ref. 60), monocytes (Ref. 61) and macrophages (Refs 60, 62, 63), are regarded as the predominant permissive targets for the initial arbovirus infection in hosts. After the primary replication in these cells, the viruses spread into the host blood circulation for further systemic dissemination. Indeed, mosquito saliva can facilitate arbovirus transmission to hosts and contributes to the subsequent disease sequelae. For example, inoculation of WNV with mosquito salivary gland extract led to a higher viraemia and faster neuroinvasion compared with that of WNV inoculation by needles (Ref. 64). In addition, mice bitten by DENV-infected mosquitoes showed higher and sustained viraemia compared with that of animals with direct DENV injection (Ref. 65). Indeed, the discovery of mosquito saliva factors in arboviral transmission has received great attention due to their potential exploitation for disease transmission control. A 34-kDa protein identified from *A. aegypti* saliva can enhance DENV replication in human keratinocytes by suppressing interferon signalling (Ref. 66). Mosquito salivary gland extracts modulate antiviral activity and cytokine responses to enhance SINV infection (Ref. 67). Recently, a study found that an *A. aegypti* salivary factor, LTRIN, facilitates ZIKV transmission by interfering with nuclear factor-κB signalling and the downstream inflammatory cytokine production (Ref. 68). In addition to regulating immune responses, salivary proteins can also influence viral entry into host cells. A serine protease in *A. aegypti* saliva augmented

DENV infection by proteolysing extracellular matrix proteins, thereby increasing viral attachment to heparan sulphate proteoglycans and inducing cell migration (Ref. 69). Interestingly, the oedema caused by mosquito saliva may promote viral transmission by enhancing the infection in the host's virus-permissive neutrophils and myeloid cells (Ref. 70). In addition, mosquito saliva may dysregulate antiviral signalling of antigen-presenting cells to reduce T lymphocyte and antiviral activities at the inoculation site, therefore supporting WNV replication (Ref. 71). Taken together, these observations show that arboviruses comprehensively utilise mosquito salivary proteins to enhance their transmission from infected vectors to naive hosts.

Concluding remarks

Mosquito-borne viruses maintain their lifecycle between permissive hosts and mosquito vectors. The viruses have evolved sophisticated strategies to sequentially accomplish their complicated life journey in mosquitoes, such as overcoming the barriers in the mosquito midguts, robustly disseminating and replicating in the tissues, and finally moving to a permissive host by blood feeding. Mosquitoes have multiple immune systems to limit invasion of these viral pathogens, although the viruses still successfully exploit either host or vectorial factors to achieve their efficient replication and transmission. Although our understanding of the interactions among mosquitoes, viruses and hosts has rapidly expanded, there are numerous puzzling questions that must be studied further. The major remaining challenge is to understand the mechanisms that regulate the permissiveness of mosquitoes to viral infection. The mechanisms that control viral infection in mosquitoes, including vectorial genetic status, host blood components, gut microbiota and environmental variations, are still poorly understood. Additionally, additional mosquito salivary factors that facilitate viral transmission must be further identified. Interplay between host immunity and mosquito salivary proteins should also be a target for future investigation. Furthermore, an interesting scientific question is how mosquitoes successfully control persistent arbovirus replication without dramatic pathological sequelae in the mosquito tissues. Altogether, an understanding of the interplay between mosquito-borne viruses and their mosquito vectors will provide us with a better understanding of the basis of arboviral survival and may provide novel strategies for limiting arboviral transmission in nature.

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References

1. **Cheng G et al.** (2016) Mosquito defense strategies against viral infection. *Trends in Parasitology* **32**, 177–186.
2. **Gaunt MW et al.** (2001) Phylogenetic relationships of flaviviruses correlate with their epidemiology, disease association and biogeography. *The Journal of General Virology* **82**, 1867–1876.
3. **Weaver SC and Reisen WK** (2010) Present and future arboviral threats. *Antiviral Research* **85**, 328.
4. **Gubler DJ** (1998) Resurgent vector-borne diseases as a global health problem. *Emerging Infectious Diseases* **4**, 442–450.
5. **Leta S et al.** (2018) Global risk mapping for major diseases transmitted by *Aedes aegypti* and *Aedes albopictus*. *International Journal of Infectious Diseases* **67**, 25–35.
6. **Bhatt S et al.** (2013) The global distribution and burden of dengue. *Nature* **496**, 504–507.
7. **The Pan American Health Organization [PAHO]/World Health Organization [WHO]** (2017) Zika cases and congenital syndrome associated with Zika virus reported by countries and territories in the Americas, 2015–2017 Cumulative cases.
8. **Gao GF** (2018) From 'A'IV to 'Z'IKV: attacks from emerging and re-emerging pathogens. *Cell* **172**, 1157–1159.
9. **Parra B et al.** (2016) Guillain-Barre syndrome associated with Zika virus infection in Colombia. *New England Journal of Medicine* **375**, 1513–1523.
10. **Iranpour M et al.** (2016) Apoptosis, autophagy and unfolded protein response pathways in arbovirus replication and pathogenesis. *Expert Reviews in Molecular Medicine* **18**, e1.
11. **Lessler J et al.** (2016) Assessing the global threat from Zika virus. *Science* **353**, aaf8160.
12. **Grinev A et al.** (2015) The global ecology and epidemiology of West Nile virus. *BioMed Research International* **2015**, 376230.
13. **Ruckert C et al.** (2017) Impact of simultaneous exposure to arboviruses on infection and transmission by *Aedes aegypti* mosquitoes. *Nature Communications* **8**, 15412.
14. **Nguyen NM et al.** (2013) Host and viral features of human dengue cases shape the population of infected and infectious *Aedes aegypti* mosquitoes. *Proceedings of the National Academy of Sciences, USA* **110**, 9072–9077.
15. **Wagar ZL et al.** (2017) Low density lipoprotein inhibits flavivirus acquisition in *Aedes aegypti*. *Insect Molecular Biology* **26**, 734–742.
16. **Bottino-Rojas V et al.** (2015) Heme signaling impacts global gene expression, immunity and dengue virus infectivity in *Aedes aegypti*. *PLoS One* **10**, e0135985.
17. **Zhu Y et al.** (2017) Blood meal acquisition enhances arbovirus replication in mosquitoes through activation of the GABAergic system. *Nature Communications* **8**, 1262.
18. **Liu J et al.** (2016) Flavivirus NS1 protein in infected host sera enhances viral acquisition by mosquitoes. *Nature Microbiology* **1**, 16087.
19. **Liu Y et al.** (2017) Evolutionary enhancement of Zika virus infectivity in *Aedes aegypti* mosquitoes. *Nature* **545**, 482–486.
20. **Merkling SH and van Rij RP** (2013) Beyond RNAi: antiviral defense strategies in Drosophila and mosquito. *Journal of Insect Physiology* **59**, 159–170.
21. **Blair CD and Olson KE** (2015) The role of RNA interference (RNAi) in arbovirus-vector interactions. *Viruses* **7**, 820–843.
22. **Franz AW et al.** (2014) Fitness impact and stability of a transgene conferring resistance to dengue-2 virus following introgression into a genetically diverse *Aedes aegypti* strain. *PLoS Neglected Tropical Diseases* **8**, e2833.
23. **Sanchez-Vargas I et al.** (2009) Dengue virus type 2 infections of *Aedes aegypti* are modulated by the mosquito's RNA interference pathway. *PLoS Pathogens* **5**, e1000299.
24. **Carissimo G et al.** (2015) Antiviral immunity of *Anopheles gambiae* is highly compartmentalized, with distinct roles for RNA interference and gut microbiota. *Proceedings of the National Academy of Sciences of the United States of America* **112**, E176–E185.
25. **Xi Z et al.** (2008) The *Aedes aegypti* toll pathway controls dengue virus infection. *PLoS Pathogens* **4**, e1000098.
26. **Souza-Neto JA et al.** (2009) An evolutionary conserved function of the JAK-STAT pathway in anti-dengue defense. *Proceedings of the National Academy of Sciences, USA* **106**, 17841–17846.
27. **Ramirez JL et al.** (2014) Chromobacterium Csp_P reduces malaria and dengue infection in vector mosquitoes and has entomopathogenic and in vitro anti-pathogen activities. *PLoS Pathogens* **10**, e1004398.
28. **Saraiva RG et al.** (2018) Aminopeptidase secreted by Chromobacterium sp. Panama inhibits dengue virus infection by degrading the E protein. *PLoS Neglected Tropical Diseases* **12**, e0006443.
29. **Ramirez JL et al.** (2012) Reciprocal tripartite interactions between the *Aedes aegypti* midgut microbiota, innate immune system and dengue virus influences vector competence. *PLoS Neglected Tropical Diseases* **6**, e1561.
30. **Dong Y et al.** (2012) The entomopathogenic fungus *Beauveria bassiana* activate toll and JAK-STAT pathway-controlled effector genes and anti-dengue activity in *Aedes aegypti*. *Insect Biochemistry and Molecular Biology* **42**, 126–132.
31. **Apte-Deshpande A et al.** (2012) *Serratia odorifera* a midgut inhabitant of *Aedes aegypti* mosquito enhances its susceptibility to dengue-2 virus. *PLoS One* **7**, e40401.

32. **Anglero-Rodriguez YI** *et al.* (2017) An *Aedes aegypti*-associated fungus increases susceptibility to dengue virus by modulating gut trypsin activity. *Elife* **6**, e28844.

33. **Franz AW** *et al.* (2015) Tissue barriers to arbovirus infection in mosquitoes. *Viruses* **7**, 3741–3767.

34. **Barrows NJ** *et al.* (2018) Biochemistry and molecular biology of Flaviviruses. *Chemical Reviews* **118**, 4448–4482.

35. **Cheng G** *et al.* (2010) A C-type lectin collaborates with a CD45 phosphatase homolog to facilitate West Nile virus infection of mosquitoes. *Cell* **142**, 714–725.

36. **Liu Y** *et al.* (2014) Transmission-blocking antibodies against mosquito C-type lectins for dengue prevention. *PLoS Pathogens* **10**, e1003931.

37. **Kuadkitkan A** *et al.* (2010) Identification and characterization of prohibitin as a receptor protein mediating DENV-2 entry into insect cells. *Virology* **406**, 149–161.

38. **Salas-Benito J** *et al.* (2007) Evidence that the 45-kD glycoprotein, part of a putative dengue virus receptor complex in the mosquito cell line C6/36, is a heat-shock related protein. *The American Journal of Tropical Medicine and Hygiene* **77**, 283–290.

39. **Sakoonwatanyoo P** *et al.* (2006) Growth and production of the dengue virus in C6/36 cells and identification of a laminin-binding protein as a candidate serotype 3 and 4 receptor protein. *Intervirology* **49**, 161–172.

40. **Londono-Renteria B** *et al.* (2015) Dengue virus infection of *Aedes aegypti* requires a putative cysteine rich venom protein. *PLoS Pathogens* **11**, e1005202.

41. **Rose PP** *et al.* (2011) Natural resistance-associated macrophage protein (NRAMP) is a cellular receptor for Sindbis virus in both insect and mammalian hosts. *Cell Host & Microbe* **10**, 97–104.

42. **Samuel GH** *et al.* (2018) Antiviral immunity and virus-mediated antagonism in disease vector mosquitoes. *Trends in Microbiology* **26**, 447–461.

43. **Kakumani PK** *et al.* (2013) Role of RNA interference (RNAi) in dengue virus replication and identification of NS4B as an RNAi suppressor. *Journal of Virology* **87**, 8870–8883.

44. **Mathur K** *et al.* (2016) Analysis of chikungunya virus proteins reveals that non-structural proteins nsP2 and nsP3 exhibit RNA interference (RNAi) suppressor activity. *Scientific Reports* **6**, 38065.

45. **Göertz GP** *et al.* (2016) Noncoding subgenomic flavivirus RNA is processed by the mosquito RNA interference machinery and determines west Nile virus transmission by *Culex pipiens* mosquitoes. *Journal of Virology* **90**, 10145–10159.

46. **Moon SL** *et al.* (2015) Flavivirus sfRNA suppresses antiviral RNA interference in cultured cells and mosquitoes and directly interacts with the RNAi machinery. *Virology* **485**, 322–329.

47. **Pompon J** *et al.* (2017) Dengue subgenomic flaviviral RNA disrupts immunity in mosquito salivary glands to increase virus transmission. *PLoS Pathogens* **13**, e1006535.

48. **Parikh GR** *et al.* (2009) A haemocyte tropism for an arbovirus. *The Journal of General Virology* **90**, 292–296.

49. **Goic B** *et al.* (2016) Virus-derived DNA drives mosquito vector tolerance to arboviral infection. *Nature Communications* **7**, 12410.

50. **Christensen BM** *et al.* (2005) Melanization immune responses in mosquito vectors. *Trends in Parasitology* **21**, 192–199.

51. **Cerenius L and Soderhall K** (2004) The prophenoloxidase-activating system in invertebrates. *Immunological Reviews* **198**, 116–126.

52. **Rodriguez-Andres J** *et al.* (2012) Phenoloxidase activity acts as a mosquito innate immune response against infection with Semliki Forest virus. *PLoS Pathogens* **8**, e1002977.

53. **Xiao X** *et al.* (2015) A neuron-specific antiviral mechanism prevents lethal flaviviral infection of mosquitoes. *PLoS Pathogens* **11**, e1004848.

54. **Xiao X** *et al.* (2014) Complement-related proteins control the flavivirus infection of *Aedes aegypti* by inducing antimicrobial peptides. *PLoS Pathogens* **10**, e1004027.

55. **Blandin S** *et al.* (2004) Complement-like protein TEP1 is a determinant of vectorial capacity in the malaria vector *Anopheles gambiae*. *Cell* **116**, 661–670.

56. **Shokal U and Eleftherianos I** (2017) Evolution and function of thioester-containing proteins and the complement system in the innate immune response. *Frontiers in Immunology* **8**, 759.

57. **Kingsolver MB** *et al.* (2013) Insect antiviral innate immunity: pathways, effectors, and connections. *Journal of Molecular Biology* **425**, 4921–4936.

58. **Tassetto M** *et al.* (2017) Circulating immune cells mediate a systemic RNAi-based adaptive antiviral response in drosophila. *Cell* **169**, 314–325, e313.

59. **Goic B** *et al.* (2013) RNA-mediated interference and reverse transcription control the persistence of RNA viruses in the insect model Drosophila. *Nature Immunology* **14**, 396–403.

60. **Cerny D** *et al.* (2014) Selective susceptibility of human skin antigen presenting cells to productive dengue virus infection. *PLoS Pathogens* **10**, e1004548.

61. **Schmid MA and Harris E** (2014) Monocyte recruitment to the dermis and differentiation to dendritic cells increases the targets for dengue virus replication. *PLoS Pathogens* **10**, e1004541.

62. **Hamel R** *et al.* (2015) Biology of Zika virus infection in human skin cells. *Journal of Virology* **89**, 8880–8896.

63. **Blackley S** *et al.* (2007) Primary human splenic macrophages, but not T or B cells, are the principal target cells for dengue virus infection in vitro. *Journal of Virology* **81**, 13325–13334.

64. **Styer LM** *et al.* (2011) Mosquito saliva causes enhancement of West Nile virus infection in mice. *Journal of Virology* **85**, 1517–1527.

65. **Cox J** *et al.* (2012) Mosquito bite delivery of dengue virus enhances immunogenicity and pathogenesis in humanized mice. *Journal of Virology* **86**, 7637–7649.

66. **Surasombatpattana P** *et al.* (2014) *Aedes aegypti* saliva contains a prominent 34-kDa protein that strongly enhances dengue virus replication in human keratinocytes. *The Journal of Investigative Dermatology* **134**, 281–284.

67. **Schneider BS** *et al.* (2004) *Aedes aegypti* salivary gland extracts modulate anti-viral and TH1/TH2 cytokine responses to sindbis virus infection. *Viral Immunology* **17**, 565–573.

68. **Jin L** *et al.* (2018) Salivary factor LTRIN from *Aedes aegypti* facilitates the transmission of Zika virus by interfering with the lymphotoxin-beta receptor. *Nature Immunology* **19**, 342–353.

69. **Conway MJ** *et al.* (2014) Mosquito saliva serine protease enhances dissemination of dengue virus into the mammalian host. *Journal of Virology* **88**, 164–175.

70. **Pingen M** *et al.* (2016) Host inflammatory response to mosquito bites enhances the severity of arbovirus infection. *Immunity* **44**, 1455–1469.

71. **Schneider BS** *et al.* (2010) *Aedes aegypti* saliva alters leukocyte recruitment and cytokine signaling by antigen-presenting cells during west Nile virus infection. *PLoS One* **5**, e11704.