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Review

Progress towards Understanding the Mosquito-Borne Virus Life Cycle

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Mosquito-borne arboviruses are a group of heterogeneous viruses that are mainly transmitted to vertebrate hosts and are the aetiological agents of many human diseases. These viruses naturally maintain a life cycle between distinct hosts by transmission from an infected mosquito to a naive host, and acquisition from a viraemic host back to a fed mosquito. To survive in and maintain a cycle between different host environments, mosquito-borne arboviruses exploit sophisticated approaches, including subverting the immune system, hijacking host factors, and taking advantage of gut microbes. We summarize the recent progress towards understanding the mechanisms of arboviral transmission and acquisition by mosquitoes. This knowledge offers an insight into the emergence and re-emergence of arboviruses in nature and an avenue for disease prevention in the future.

Highlights

Mosquito-borne arboviruses have evolved sophisticated strategies to efficiently maintain their life cycles, which consist of cross-kingdom interplays including both viral transmission and acquisition between mosquitoes and hosts.

Mosquito saliva proteins, which are indispensable for a successful arboviral transmission, contribute to efficient arboviral replication and tissue dissemination by immune modulation, induction of the migration of permissive host cells, enhancement of viral attachment, and regulation of vascular permeability.

Arboviral acquisition is regulated by multiple factors, such as gut antiviral immunity, resident gut microbial flora, host blood components, and the gut physical barrier, thus leading to modulation of vector competence for arboviruses.

Mosquito-Borne Arboviruses: Increasing Threats to Human Health

Arboviruses (see Glossary), a heterogeneous group of numerous RNA viruses – mainly in the Togaviridae, Flaviviridae, Reoviridae, and multiple families in the order Bunyavirales – are transmitted to vertebrate hosts by haematophagous arthropod vectors in nature [1]. Arboviruses are the aetiological agents of severe human diseases, including haemorrhagic fever, biphasic fever, arthritis, encephalitis, and meningitis, and they cause hundreds of millions of infections and a large number of deaths every year [2–4]. The culicine mosquitoes, such as *Aedes* and *Culex* spp., are spread throughout the world and are the dominant arthropod vectors for numerous arboviruses. Many mosquito-borne arboviruses have spread widely and raised major public health concerns throughout the world. Dengue virus (DENV, *Flavivirus*, Flaviviridae family), dominantly transmitted by *Aedes aegypti* and *Aedes albopictus*, is the most prevalent arbovirus emerging or re-emerging in more than 100 countries worldwide [5]. Both Chikungunya virus (CHIKV, *Alphavirus*, Togaviridae family) and Zika virus (ZIKV, *Flavivirus*, Flaviviridae family) are other *Aedes*-transmitted arboviruses posing a recent global threat to public health [6–9]. In addition, many neurotropic viruses, such as West Nile virus (WNV, *Flavivirus*, Flaviviridae family), Japanese encephalitis virus (JEV, *Flavivirus*, Flaviviridae family) and Sindbis virus (SINV, *Alphavirus*, Togaviridae family), transmitted by *Culex* mosquitoes, are disseminated in many areas of the world and have caused high mortality and morbidity in past decades [10–12]. There are effective vaccines targeting a few of the mosquito-borne arboviruses, such as yellow fever virus (YFV, *Flavivirus*, Flaviviridae family) and JEV; nonetheless, antiviral therapeutics against most mosquito-borne arboviruses are unavailable [13]. Therefore, a comprehensive understanding of the mechanisms of the arboviral life cycle between vertebrates and mosquitoes may provide novel targets for antiviral intervention and offer a solution to control viral transmission and prevalence in nature.

Mosquito-borne arboviruses naturally survive between vertebrate hosts and mosquitoes. A blood meal is essential for transmission of mosquito-borne viruses across native hosts [14]. As a haematophagous arthropod, the mosquito incidentally feeds on a virus-infected host to acquire viruses circulating in the host blood. Subsequently, the viruses infect the epithelial cells in the mosquito gut and then spread into the **mosquito haemocoel**. Generally, doses of at least 10^4 plaque-forming units (PFU) of viruses are required to achieve productive mosquito infection using artificial membrane blood feeding [15]. However, a blood meal from viraemic mice is more infectious than an artificial blood meal containing comparable doses [16]. Indeed, the mosquito midgut acts as a major barrier against arboviral invasion, in which the antiviral effects may be attributed to the local gut immunity, resident gut microbial flora, and physical gut barriers [17]. In contrast, the tissues in the mosquito haemocoel, such as haemolymph, salivary glands, and fat body, are highly permissive to arboviral

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propagation [18]. The viruses subsequently disseminate into the salivary glands, thus enabling viral transmission by the infected mosquito to naive hosts through blood feeding [19–21]. During the WNV transmission, the *Culex* mosquitoes inoculate viral doses around <5 PFU to $10^{6.6}$ PFU extravascularly and $10^{0.7}$–$10^{3.9}$ PFU intravascularly while probing and feeding on a live host [22]. Generally, *Culex pipiens* salivates an average of 4.7 nl, while *Aedes aegypti* salivates an average of 6.8 nl per mosquito bite. However, virus titres in mosquito salivary glands showed no correlations with the saliva volume [23]. Although mosquitoes acquire arboviruses from, and transmit arboviruses to, hosts primarily through blood feeding, a recent piece of evidence from laboratory settings demonstrated that mosquitoes may acquire ZIKV from human urinal discharge during breeding, suggesting that an alternative blood-meal-free life cycle might exist for some particular arboviruses [24]. Given the complex life cycle of these viruses, it is reasonable to assume that they employ many strategies to enable mosquitoes to efficiently acquire and transmit them between hosts and mosquitoes. Here, we summarize recent progress on the underlying mechanisms by which mosquito-borne arboviruses survive across different host species.

Arboviral Transmission from Infected Mosquitoes to Naive Hosts

To obtain a blood meal, a mosquito needs to frequently probe for blood vessels within the dermal layer of host skin by the proboscis, which leads to intradermal injection of the infectious particles into the host [25]. Intradermal fibroblasts [26], and immune cells, such as dendritic cell subsets [27], monocytes [28], and macrophages [29,30], are regarded as the predominant cells highly permissive for the initial arboviral replication. Different virus families have their own tropisms for distinct types of host cell [27,28,30–35]. After the initial round of replication, the infected dendritic cells migrate to draining lymph nodes, which is followed by a second round of viral replication in the secondary lymphoid tissues [36]. Subsequently, the progeny viruses generated from these cells spread into the host blood circulation for further systemic dissemination. Indeed, mosquitoes do not merely serve as suppliers to passively transfer numerous viruses from one individual to another. Instead, mosquito salivary proteins, which are inoculated together with viruses into the host dermis, aid arboviral replication and tissue dissemination, thereby enabling a more rapid **viraemia**, higher viral burden, and more severe subsequent disease sequelae [37] (Figure 1A, Key Figure). For example, inoculation of WNV with the extract of mosquito salivary glands led to a higher viraemia, more severe neuron invasion, and a higher mortality, compared with those of WNV inoculation by needles alone [37,38]. Humanized mice presented a higher and more sustained viraemia, and exhibited exacerbated disease, including fever and thrombocytopenia, after being bitten by DENV-infected mosquitoes compared with animals which had had direct viral injection [39]. In addition, mosquito probing resulted in a significantly higher DENV viraemia in mice compared to those that were not probed by *A. aegypti* [40]. Mosquito saliva-mediated transmission of Semliki Forest virus (SFV, *Alphavirus*, Togaviridae family) and Rift Valley fever virus (RVFV, *Phlebovirus*, Phenuiviridae family) largely enhanced virus replication, dissemination, and mortality in hosts [41,42]. Overall, the accumulating evidence indicates that a mosquito bite is critical to establish effective infection and systemic dissemination of arboviruses within the hosts. Indeed, uncovering the roles of mosquito salivary factors in arboviral transmission has received great attention due to their potential exploitation for the prevention of disease transmission. Multiple salivary factors have been identified to regulate arboviral infection within the hosts.

Mosquito Saliva Regulates Host Immune Responses to Arboviral Transmission

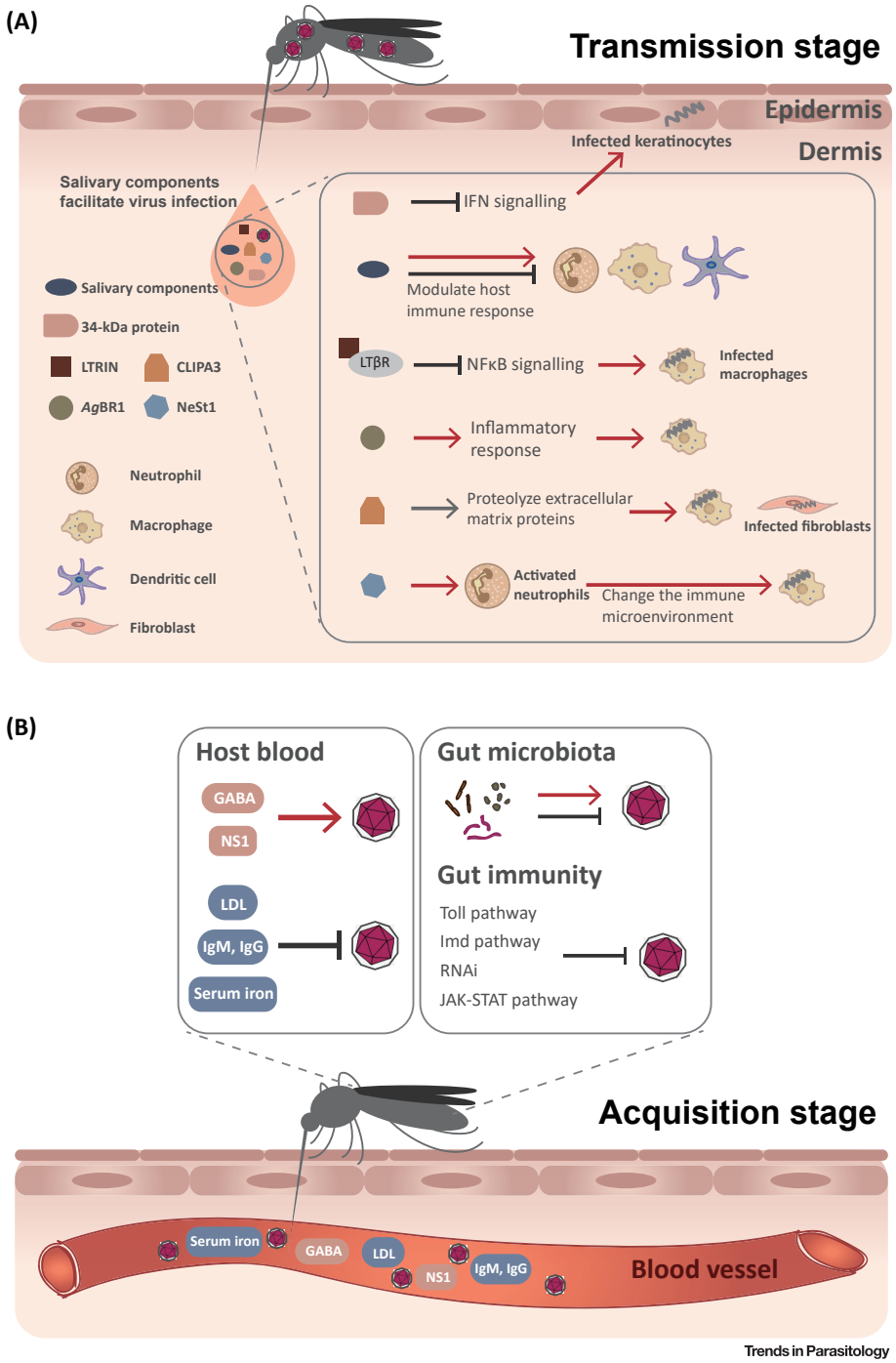
Salivary components facilitate effective acquisition of host blood by mosquitoes [43] and also contribute to optimal arboviral transmission by regulating host immune responses [43,44] (Figure 1A). Inoculation of SFV, together with mosquito saliva, into the human skin triggers an inflammatory response and the recruitment of neutrophils to the inoculation site. The influx of neutrophils promotes the infiltration of myeloid cells highly permissive to arboviral infection and may support viral replication and dissemination [41]. Similarly, a mosquito salivary protein, neutrophil stimulating factor 1 (NeSt1), stimulates neutrophils at the mosquito bite site in mice [45]. Passive immunization against NeSt1 in susceptible mice prevented macrophages from infiltrating the bite site, thus leading to protection from early ZIKV replication and reduced pathogenesis [45]. Recently, a study found that an

Glossary

Antimicrobial peptides (AMPs): also called host defence peptides (HDPs), AMPs are part of the innate immune response found in insects as well as in other classes of life. These peptides are potent, broad-spectrum antibiotics.
Arbovirus: any virus that is transmitted by arthropod vectors. Arboviruses maintain their life cycles in nature between a host, an organism that carries the virus, and a vector, an organism that carries and transmits the virus to other organisms.
Immune deficiency (Imd) pathway: an evolutionarily conserved signalling cascade that activates NF-κB and controls the expression of most of the antimicrobial peptides in insects.
Insect Janus kinase (JAK) – signal transduction and activators of transcription (STAT) pathway: an innate immune pathway with evolutionary conservation to the JAK-STAT pathway in mammals.
Mosquito haemocoel: the haemocoel is the main body cavity of invertebrates. Insects have a cavity that is full of insect blood (known as haemolymph) instead of an arrangement of blood vessels, and the organs of the insect are suspended in this cavity.
Reactive oxygen species (ROS): ROS are formed as a natural by-product of the normal metabolism of oxygen and they play important roles in cell signalling and homeostasis. ROS levels can increase dramatically during times of environmental stress and lead to damage in cell structures, which is called oxidative stress.
Toll pathway: an innate immune pathway that can be induced by Gram-positive bacteria, fungi, or viruses in insects, and leads to the activation of cellular immunity as well as the systemic production of certain antimicrobial peptides.
Vector competence: the physiological ability of a blood-sucking arthropod to acquire, maintain, and transmit microbial agents.
Viraemia: a form of bloodstream infection when viruses enter the bloodstream and have access to the rest of the body.

Key Figure

Arboviral Transmission and Acquisition between Mosquitoes and Hosts



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A. aegypti salivary protein, *A. aegypti* bacteria-responsive protein 1 (*Ag*BR1), induces early inflammatory responses in the skin of bitten mice, thereby facilitating ZIKV transmission. The antiserum against *Ag*BR1 partially protected mice from lethal ZIKV infection [46]. Passive immunization with *Ag*BR1 antiserum also suppressed the early local host responses in mice bitten by WNV-infected mosquitoes, reduced WNV viraemia at an early stage of infection, delayed virally induced weight loss, and prolonged median survival time of mice [47].

During early stages of infection, innate immune responses play crucial roles in restricting viral replication and pathogenesis. Several salivary factors in mosquitoes have been identified to modulate early viral replication in human host cells. Mosquito salivary factors may affect the expression of antiviral genes through targeting components of the type I interferon (IFN) pathway, promoting viral replication in infected cells. Indeed, an *in vitro* study demonstrated that an *A. aegypti* saliva 34-kDa protein can enhance DENV replication in human keratinocytes by suppressing interferon signalling [48]. Nonetheless, several *in vivo* studies showed that mosquito saliva can enhance DENV immunogenicity and pathogenesis in interferon-signalling-deficient mice [39,40]. Besides the IFN signalling, an *A. aegypti* salivary factor, LTRIN, facilitates ZIKV transmission by binding to lymphotoxin-β receptor (LTβR), interfering with NF-κB signalling, and subsequent production of downstream inflammatory cytokines [49].

Mosquitoes might also develop strategies to regulate host adaptive immune responses. A reduction in the proliferation of murine splenocytes and production of both Th1 and Th2 cytokines was observed in the presence of *Aedes* salivary gland extracts (SGEs) [50]. In addition, mosquito saliva may modulate antiviral signalling of antigen-presenting cells to impair T lymphocyte proliferation and antiviral activities at the inoculation site [51]. Furthermore, CHIKV infection in mice through mosquito bites induced higher IL-4 (Th2-related cytokine) expression but lower IFN-γ and IL-2 (Th1-related cytokines) expression than did needle inoculation [52]. This suggests that mosquito saliva may alter host T cell immune responses, resulting in a shift from a Th1 to a Th2 response and benefitting CHIKV survival and replication [52].

Mosquito Saliva-Mediated Nonimmune Responses to Arboviral Transmission

In addition to regulating immune responses, mosquito saliva may also contribute to arboviral infection in the host by enhancing viral attachment, inducing cell migration, and regulating host vascular permeability (Figure 1A). A recent study identified that a serine protease in *A. aegypti* saliva, called CLIPA3, augmented DENV infection by proteolysing extracellular matrix proteins, thereby increasing

Figure 1. (A) Arboviruses are transmitted to naive hosts by probing of infected mosquitoes, during which the infectious particles are injected along with mosquito salivary components. The predominant cells that are highly permissive for initial arboviral infection include intradermal fibroblasts, keratinocytes, and immune cells, such as dendritic cell subsets, monocytes, and macrophages. Mosquito salivary factors, including a 34-kDa protein, LTRIN, NeSt1, and *Ag*BR1, facilitate successful acquisition of host blood and also contribute to optimal arboviral transmission by modulating the host immune response, such as the expression of Th1 and Th2 cytokines, as well as the activity of T lymphocytes. Mosquito salivary proteins, such as a serine protease, CLIPA3, may also contribute to arboviral infection in the host by activating autophagy, enhancing viral attachment, inducing cell migration, and regulating host vascular permeability. (B) Mosquitoes acquire arboviruses through haematophagous feeding, and the infectious viral particles enter the mosquito gut together with the host blood. Host blood components or metabolites that modulate the immune and physiological status of the mosquito gut may hinder the infection capacity (IgM, IgG, LDL, or serum iron), or enhance arboviral infection by suppressing mosquito gut antiviral immunity (GABA, NS1), thereby potentially determining the vector competence of mosquitoes to arboviral transmission in nature. The commensal microbiome, including bacteria (*Chromobacterium* sp., *Proteus* sp., *Serratia marcescens*, or *Serratia odorifera*), and fungi (*Talaromyces*, *Beauveria bassiana*, or *Metarhizium anisopliae*), residing in the mosquito gut lumen also plays an important role in modulating vector susceptibility towards arboviruses. The ability to acquire arboviruses by mosquitoes is also determined by gut antiviral immunity, which is modulated by several evolutionarily conserved innate immune pathways, such as the Toll, Imd, JAK-STAT, and RNAi pathways.

viral attachment to heparan sulfate proteoglycans and inducing cell migration [53]. In addition to these mechanisms of direct regulation of viral replication, the mosquito saliva may influence viral dissemination within the host by regulating vascular permeability. Coinoculation of viruses with salivary gland extracts facilitates systemic viral dissemination, which may be related to the effects of salivary components on vascular permeability [54]. Indeed, vascular permeability may help DENV infection of dermal dendritic cells (DCs) and macrophages or influx of monocytes into the bite site [54].

Overall, arboviruses comprehensively utilize the properties of mosquito salivary proteins, such as immune modulation, induction of migration of permissive host cells to the bite site, enhancement of viral attachment, and regulation of vascular permeability, to achieve efficient transmission from infected mosquitoes to hosts.

Arboviral Acquisition from Infected Hosts by Mosquito Vectors

Arboviral acquisition, a process of viral transmission from infected hosts to mosquitoes, is the other essential step in the arboviral life cycle [15]. After viral infection, the host may develop a prolonged viraemia in which numerous infectious viral particles circulate in the host blood. Mosquitoes may incidentally feed on viraemic hosts and acquire a blood meal containing viral particles. The viruses subsequently establish successful infection in the mosquito gut epithelium and then spread to other tissues. Prior to systemic dissemination, the viruses must overcome the gut epithelial barrier to establish their infection in the epithelial cells [16]. Many factors in the midgut, such as the local immunity, resident gut microbial flora, host blood components, and the physical mucosal barrier, may determine the outcomes of arboviral acquisition by a mosquito blood meal, thereby regulating the **vector competence** of the infected mosquitoes and their ability to transmit the viruses to other hosts [15,16] (Figure 1B). Here, we summarize recent progress on the underlying mechanisms of arboviral acquisition from infected hosts by mosquitoes.

Blood-Derived Factors Regulate Arboviral Acquisition by Mosquitoes

Through haematophagous feeding, mosquitoes take up blood with infectious viral particles from infected hosts into the mosquito gut [14]. The host blood is digested and metabolized within days after the blood meal, while the viruses simultaneously establish an infection in the gut epithelium. Therefore, host blood components and metabolites that modulate the immune and physiological status of the mosquito gut may regulate the permissiveness of mosquitoes to viral infection [15,55] (Figure 1B). Several factors derived from host blood have been found to influence viral replication in the mosquito gut epithelium. Increasing titres of IgM and IgG against DENV envelope (E) proteins may modulate the capacity for DENV acquisition by *A. aegypti* mosquitoes from viraemic patients, thereby reducing DENV infection in mosquitoes [55]. Low-density lipopolyprotein (LDL), a highly abundant blood component, acquired from host blood, in the mosquito gut, is endocytosed by the gut epithelial cells and accumulates at the luminal epithelium during blood digestion to impair flavivirus acquisition by *A. aegypti* [56]. In addition, glutamic acid, an amino acid derived from blood protein digestion, is converted to γ-aminobutyric acid (GABA) via decarboxylation. GABA can activate the GABAergic system to modulate the gut antiviral immunity in mosquitoes, thereby generally facilitating arboviral replication in the mosquito vectors [14]. Besides, a recent study showed that the serum iron concentration in human subjects was negatively correlated with DENV acquisition by mosquitoes. Feeding on iron-deficient mice led to a higher DENV prevalence in mosquitoes [57]. Mechanistic studies suggested that serum iron can be utilized by the mosquito iron metabolism pathway to boost the activity of **reactive oxygen species** (**ROS**) in the gut epithelium, subsequently inhibiting DENV infection [58–60]. In addition to host blood components, arboviruses also use their own nonstructural proteins secreted in host blood to efficiently escape the hostile gut immunity in mosquitoes, therefore completing the life cycle from infected hosts to mosquitoes. Flavivirus nonstructural protein-1 (NS1) is made during viral replication inside host cells, but can be also secreted into the sera of infected hosts [58]. The circulating NS1s of DENV [58], JEV [58], and ZIKV [61] in the sera of viraemic hosts can be taken into the mosquito gut lumen together with virions. NS1 downregulates the expression of many antiviral genes in the mosquito gut,

particularly the genes involved in ROS production and the **insect Janus kinase (JAK) - signal transduction and activators of transcription (STAT) pathway**, to enable efficient viral replication in mosquito gut epithelia [58]. Indeed, the mutations in the DENV or ZIKV NS1 protein that result in higher sNS1 production promote viral infectivity and prevalence in mosquitoes [61,62]. Overall, various components of host blood, including proteins, lipids, inorganic constituents, and virus-encoded proteins, play important roles in the regulation of arboviral acquisition by mosquitoes, thereby potentially determining the vector competence of mosquitoes to arboviral transmission in nature.

Gut-Associated Factors Regulate Arboviral Acquisition by Mosquitoes

After a blood meal, the mosquito gut is the pivotal entry site for arboviruses. The intricate ecological gut environment, including local immunity and resident gut commensal microbes, closely interacts with the whole process of arboviral infection in the gut epithelial cells and thus may play a key role in modulating arboviral acquisition by mosquitoes [63,64] (Figure 1B). Indeed, previous studies indicated that the inhabitant commensal microbial flora in the mosquito gut can modulate viral replication in the mosquito gut epithelia. Colonization by a commensal bacterium belonging to the genus *Chromobacterium* in *A. aegypti* compromised the vector competence of mosquitoes to DENV infection [64,65]. Oral introduction of another mosquito-gut commensal bacterium *Proteus* sp. strain upregulated the expression of **antimicrobial peptide** (**AMP**) genes in the gut epithelial cells, resulting in suppression of DENV infection in mosquitoes [66]. Intriguingly, gut residency of certain commensal bacteria facilitates arboviral infection. For example, *Serratia marcescens* is a commensal bacterium common to the *Aedes* mosquitoes in Brazil, Panama, and Guangzhou, China, where dengue is endemic. *S. marcescens* facilitates arboviral infection in the *Aedes* mosquitoes through a secreted protein named *Sm*Enhancin, which digests membrane-bound mucins on the mosquito gut epithelia, thereby enabling arboviruses such as DENV, ZIKV, and SINV to overcome the physical gut barrier and enhance viral dissemination [63]. In addition, oral introduction of another *Serratia* species, *Serratia odorifera*, also rendered mosquitoes highly susceptible to DENV infection. The underlying mechanism might involve specific interactions between a secreted *S. odorifera* polypeptide, the mosquito protein prohibitin, and DENV-2 virions [67]. However, it is still unknown whether the *S. odorifera* polypeptide may play the same role in other arboviral infections.

In addition to commensal bacteria, fungi may also regulate the mosquito permissiveness to arboviruses (Figure 1B). A recent study found that the residency of a *Talaromyces* fungus in the *A. aegypti* gut enhanced DENV infection in mosquitoes, which may be attributed to the modulation of digestive enzymes and trypsin activity in the mosquito gut [68]. An entomopathogenic fungus, *Beauveria bassiana* activates the **Toll pathway**- and JAK-STAT pathway-controlled effector genes that inhibit DENV infection of *A. aegypti* [69]. Another study found that the vectorial capacity of *A. aegypti* to DENV-2 is reduced when coinfected with a mildly virulent fungus, *Metarhizium anisopliae* [70]. Overall, arboviral acquisition by mosquitoes can be markedly affected by multiple commensal microbes residing in the mosquito gut.

The ability to acquire arboviruses by mosquitoes is also determined by gut antiviral immunity (Figure 1B). Several evolutionarily conserved innate immune pathways, such as the Toll, **immune deficiency** (**Imd**) and JAK-STAT pathways, modulate arboviral replication in the gut epithelium. Infection by o'nyong'nyong virus (ONNV, *Alphavirus*, Togaviridae family) in *Anopheles gambiae* was enhanced by silencing the Imd components in the mosquito midgut [71]. Suppression of the Imd pathway by activating GABAergic signalling facilitated infections by multiple arboviruses after a blood meal [14]. Silencing key components in the Toll (*Myd88*) and JAK-STAT (*Dome* and *Hop*) pathways enhanced DENV replication [72], while silencing PIAS (a negative regulator of the JAK-STAT pathway) reduced DENV infection in mosquitoes by a blood meal [73]. In addition, RNA interference (RNAi) serves to resist many arboviral infections in mosquitoes [74]. Silencing RNAi components resulted in more robust viral replication in the mosquito gut [75,76]. However, a recent study suggested that the small interfering RNA (siRNA) pathway cannot efficiently silence DENV in the *A. aegypti* midgut because of defective expression of the double-stranded RNA (dsRNA)-binding proteins Loquacious and r2d2 (Loqs2). Ectopic expression of Loqs2 in the *A. aegypti* midgut gave this organ the

ability to restrict viral replication and dissemination [77]. Moreover, this pathway is intact in other tissues in the haemocoel, thereby enabling effective restriction of systemic viral dissemination in mosquitoes [77]. These pieces of evidence indicate the crucial roles of the aforementioned mosquito immune pathways in regulating arboviral acquisition.

Overall, arboviral acquisition by mosquitoes is affected by multiple intrinsic and extrinsic vectorial factors. Some mosquito-intrinsic factors, such as mosquito immunity and gut commensal microbes, are associated with the vector competence of mosquitoes to arboviruses. Some extrinsic factors, such as environmental status and the quality of host blood, may also play important roles in the permissiveness of mosquitoes to virus infection. The complex interplays between hosts and mosquitoes determine the outcomes of infection in the mosquitoes and therefore regulate arboviral transmission by mosquitoes in nature.

Outstanding Questions

What are the underlying mechanisms of the role of salivary proteins in facilitating arboviral transmission?

Is the difference in salivary components between mosquito species a potential explanation of the preference of arboviruses in certain species?

What are specific mechanisms exploited by different mosquito species in the interplays of arboviruses, host immunity, and salivary factors?

How do other factors regulate the permissiveness of mosquitoes to infectious blood meals, such as vectorial genetic status, other host blood components, additional gut microbiota, and environmental variations?

Concluding Remarks

Mosquito-borne arboviruses maintain a life cycle between permissive hosts and mosquito vectors, in which both viral transmission and acquisition are two essential processes that determine the survival of arboviruses in nature. Therefore, mosquito-borne arboviruses have evolved sophisticated strategies to efficiently accomplish their life journey. During arboviral transmission, the host is simultaneously inoculated with mosquito saliva and infectious viral particles. Accumulating knowledge indicates that mosquito saliva promotes flavivirus transmission and pathogenesis in bitten hosts. Notably, recent studies have identified multiple salivary proteins from mosquitoes that facilitate arboviral transmission by modulating host immunity, inducing the migration of permissive host cells, and regulating the host physiological status. Nonetheless, only a limited number of salivary proteins have been investigated. The underlying mechanisms of salivary proteins in flaviviral transmission remain to be comprehensively understood. In addition, different mosquito-borne arboviruses are specifically carried and transmitted by different mosquito species. For example, *Aedes* mosquitoes present high vector competence for DENV, ZIKV, and YFV transmission, while other viruses, such as JEV, WNV, and SINV, are predominantly transmitted by *Culex* mosquitoes in nature [1]. Previous studies indicated that the salivary components of the two mosquitoes differ substantially, suggesting that arboviruses carried by different mosquito species might exploit distinct mechanisms for their transmission. Therefore, the triple interplay among arboviruses, host, and salivary proteins in certain mosquito species is also an interesting area for future investigation. In addition, arboviral acquisition by mosquitoes, the other essential process in the arboviral life cycle, is affected by multiple intrinsic and extrinsic factors, such as mosquito immunity, gut commensal microbes, environmental status, and the quality of the host blood. Although our understanding of arboviral acquisition has rapidly expanded, there are numerous puzzling questions that must be studied further (see Outstanding Questions). The major remaining challenge is to comprehensively understand the mechanisms that regulate the permissiveness of mosquitoes to viral infection by a blood meal. The mechanisms in regulation of viral acquisition by mosquitoes, such as vectorial genetic status, variations in the environment, host blood components, and additional gut microbiota, still remain to be understood. Overall, an understanding of the sophisticated process of viral transmission in the arboviral life cycle may provide insights into the survival process of arboviruses and may offer an avenue for disease prevention in the future.

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Disclaimer Statement

The authors declare that they have no competing financial interests.

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