REVIEW

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The Role of Host Cytoskeleton in Flavivirus Infection

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Abstract

The family of flaviviruses is one of the most medically important groups of emerging arthropod-borne viruses. Host cell cytoskeletons have been reported to have close contact with flaviviruses during virus entry, intracellular transport, replication, and egress process, although many detailed mechanisms are still unclear. This article provides a brief overview of the function of the most prominent flaviviruses-induced or -hijacked cytoskeletal structures including actin, microtubules and intermediate filaments, mainly focus on infection by dengue virus, Zika virus and West Nile virus. We suggest that virus interaction with host cytoskeleton to be an interesting area of future research.

Keywords Flavivirus · Host cytoskeleton · Actin filaments · Intermediate filaments · Microtubules

Introduction

Flaviviruses, comprised of more than 70 closely related RNA viruses, including human pathogens dengue virus (DENV), Zika virus (ZIKV), West Nile virus (WNV), tick-borne encephalitis virus (TBEV), Japanese encephalitis virus (JEV), yellow fever virus (YFV) that infect several hundred million people annually and cause severe morbidity and mortality in about 1%–5% of them (Bhatt *et al.* 2013; Guzman and Kouri 2003; Rasmussen *et al.* 2016). Attempts to develop antiviral drugs that target viral proteins have been hampered in part by the incomplete understanding of host interaction with these viruses. Viruses take advantage of the cellular machinery at every stage of their life cycle (Bekerman and Einav 2015), and alter the normal functions of a cell to support their replication and production. In addition to standard drug development strategy that targets viral components, an alternative therapeutic strategy is to target host factors which are essential for infection.

The cytoskeleton is the framework of the cell, composed of actin filaments, microtubules and intermediate filaments, as defined based on filaments diameter and assembly pattern. These cytoskeleton elements enable the living cell to have many specialized functions, they are concentrated in different areas, but extensively interconnected by paths of communication. Moreover, cytoskeleton helps organelle localization and vesicle trafficking. Of interest, the cytoskeleton network takes on an active role during the virus infection. For example, (1) it could transport certain substances the viruses need; (2) it could function as scaffolds in cell cortex and perinuclear replication complex; (3) it could function as trafficking paths interconnecting early-late endosome transportation and late endosome-lysosome degradation; (4) it could also function as a shield of protection against attacks from the body's own immune system which destroys foreign genetic material.

Over the years, it has been learned that cytoskeleton dynamics and assembly are prime targets of viral pathogens, and that cytoskeleton disorganization occurs in many cases of virus infection (Taylor *et al.* 2011). For example, various cellular proteins have been identified, including central cytoskeletal-associated proteins and molecular motors, to be utilized by flaviviruses to facilitate their infection (Gerold *et al.* 2017). Other studies of flaviviruses also suggest that virus entry, in-cell transport, intracellular assembly, and release are prominently dependent on interacting/crosstalk with host cytoskeleton systems (Foo

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and Chee 2015; Ploubidou and Way 2001). However, our understanding of infection-triggered cellular cytoskeletal system responses is far from complete. In this review, the cytoskeleton dynamics and regulation during several flaviviruses infection are discussed (Fig. 1).

The Role of Cytoskeleton in DENV–Host Interaction

The Role of Actin Filaments in DENV–Host Interaction

DENV is the most studied flavivirus with respect to its interaction with host cytoskeleton network, among which

Fig. 1 Schematic host cytoskeletal regulating network in flavivirus infection. Detailed regulating steps, including entry, trafficking, replication, assembly and egress, and corresponding references are depicted. Dashed frames indicate the distinct process of infection, and the colors of frames indicate the involved dominating cytoskeleton network (red, actin; blue, intermediate filaments; green, microtubules). Square brackets indicate the relative references as following:

[1] (Zhang *et al.* 2016), [2] (Wang *et al.* 2010), [3] (Xu *et al.* 2009), [4] (Cureton *et al.* 2009), [5] (Mooren *et al.* 2012), [6] (Skruzny *et al.* 2012), [7] (Greber and Way 2006), [8] (Merino-Gracia *et al.* 2011), [9] (Chen *et al.* 2008), [10] (Kanlaya *et al.* 2010b), [11] (Makino *et al.* 2014), [12] (Chu and Ng 2004), [13] (Fraisier *et al.* 2013), [14] (Chu *et al.* 2006), [15] (Chu *et al.* 2003), [16] (Chu and Ng 2002), [17] (Ng *et al.* 1994).

actin filaments have been found to be the most prominent component. Rho GTPases Rac1, Cdc42 and RhoA are the main regulators of actin cytoskeleton on its assembly, dynamics and central structures such as lamellipodia, filopodia and stress fibers. In EAhy926 cells, Rac1 has been reported to play an important role in the DENV serotype 2 (DENV2) life cycle by regulating actin reorganization. DENV2 infection induced dynamic changes in actin organization, and treatment with cytochalasin D or jasplakinolide disrupted actin dynamics, reduced DENV2 entry, and inhibited DENV2 assembly and maturation (Zhang *et al.* 2016). Works in ECV304 cells showed that actin and Rac1 signaling are essential for DENV2 entry into and release from the host cell (Wang *et al.* 2010). In addition, activation of Rac1 and Cdc42 signaling, which occurs upon virus binding, induced reorganization of actin to form filopodia in the cellular periphery. The formation of filopodia is an essential requirement for virus entry and further cell infection (Zamudio-Meza *et al.* 2009).

Consistently, DENV infections induce activation of PI3K/Akt/Rho GTPases signaling pathway with concomitant reorganization of the actin cytoskeleton in hepatocyte-derived carcinoma Huh7 cells. Additionally, inhibition of PI3K/Akt/Rho GTPases and actin filaments significantly reduced new viral progeny (Cuartas-Lopez *et al.* 2018). Moreover, inhibition of the ATPase activity of myosin II greatly decreased the infection efficiency (Zamudio-Meza *et al.* 2009). Myosin is a molecular motor associated with actin filaments that was previously reported to be involved in the process of DENV2 infection, and redistribution of myosin causes reduced release of DENV particles (Xu *et al.* 2009). Cell motility, which was mainly regulated by actin stress fibers as well as intermediate filaments, was considerably increased in DENV infected murine microglial BV2 cell line, indicating the involvement of DENV infection to an integrated cellular function (Jhan *et al.* 2017).

Interestingly, antibody-dependent enhancement of DENV infection in macrophage-like P388D1 cells was dependent on actin and endosomal related Rab5. By live-cell imaging, actin-mediated membrane protrusions were observed to facilitate virus uptake. Actin protrusions were actively searching and capturing antibody-bound virus particles nearby the cell body, which was not observed in the absence of antibodies (Ayala-Nunez *et al.* 2016). Previous study found that DENV structural components are clustered at the interface between plasmacytoid dendritic cells (pDCs) and infected cells, and the actin cytoskeleton is pivotal for both clustering at the contact areas and pDCs activation (Decembre *et al.* 2014).

Another work identified an interaction between the 43-kDa actin and DENV2 enveloped E protein domain III (EDIII) and found that actin network rearranged within

1 h-post-infection (h.p.i.) of DENV2-infected ECV304 cells, indicating a direct contact between DENV2 E protein and the 43-kDa actin may have a crucial function in viral infection (Lei *et al.* 2013; Wang *et al.* 2010; Yang *et al.* 2013). The alteration of actin filament and tight junction arrangement in DENV-infected cells may be one of the causes of endothelial permeability leading to plasma leakage (Cudmore *et al.* 1997).

The Role of Microtubules in DENV–Host Interaction

Polymerization of actin was seen to be involved in the clathrin-mediated endocytosis pathway by coupling with the plasma membrane, making an invaginated path specially for virus internalization (Cureton *et al.* 2009; Mooren *et al.* 2012; Skruzny *et al.* 2012). The function of clathrin in facilitating virus entry into host cells has been well documented, and disruption of clathrin interrupted viral entry (Acosta *et al.* 2008; Chu *et al.* 2006; Chuang *et al.* 2015).

Microtubules and their associating proteins play important roles in trafficking viral particles into host cells, including being responsible for transporting virions in infected cells (Greber and Way 2006). One study focused on the role of microtubules and its motor protein dynein in the early events of DENV2 replication in BHK-21 cells (Shrivastava *et al.* 2011). Association of E protein with α -tubulin was observed from 8-h post-infection, indicating that it was the newly translated protein that trafficked on the microtubules. The tubulin was also suggested to assist viral scaffold assembly through interactions with the DENV 2 E protein (Chee and AbuBakar 2004).

Dynein was found to be associated with the DENV2 E protein from 4-h post-infection in the cytoplasm to 48-h post infection in the perinuclear region and dissociate at 72-h post-infection. Over expression of dynamitin disrupts the dynein complex, resulting in a loss of trafficking of DENV E and core proteins (Shrivastava *et al.* 2011). It has been suggested that viral capsids utilize dynein transport system to move around and exit the cell (Merino-Gracia *et al.* 2011). In addition, the microtubule-associated protein dynamin II was found to aid DENV2 internalization through an association with DENV E protein.

In another work, researchers identified that miR-223 inhibits DENV replication by negatively regulating the microtubule-destabilizing protein STMN1 (Wu *et al.* 2014). Disorganization of actin precluded DENV2 infection, while microtubule depolymerization had no effect in endothelial HMEC-1 cells (Zamudio-Meza *et al.* 2009). Moreover, colocalization of DENV2 antigens with microtubules or vimentin were observed in ECV304 cells. By using drug inhibition assay, it was demonstrated that

vimentin is required for DENV2 infection, whereas microtubule may be not, and microtubules disruption may promote DENV2 release. However, the involvement of microtubules is suggested to have little to no effect on DENV2 entry into ECV304 cells by using demecolcine, nocodazole, and paclitaxel drugs that are capable of disrupting microtubule network (Chen *et al.* 2008).

The Role of Intermediate Filaments in DENV–Host Interaction

The involvement of cytoskeletal intermediate filaments in DENV replication has been less explored. Vimentin intermediate filaments are thought to be involved in the infectivity and replication of DENV. One work revealed a direct interaction between host cellular vimentin and DENV nonstructural protein 4A (NS4A), a known component of the viral replication complex (RC), during DENV infection by tandem affinity purification, coimmunoprecipitation and scanning electron microscopy methods (Teo and Chu 2014). Further studies specified the region of NS4A that interacts with vimentin lies within the first 50 amino acid residues at the cytosolic N-terminal of NS4A. Furthermore, vimentin reorganization and phosphorylation occur during DENV infection, signifying that vimentin reorganization is important in maintaining and supporting the DENV RCs. Gene silencing of vimentin induced a significant disruption of RCs in DENV-infected cells (Teo and Chu 2014). It has been shown that vimentin is involved in the assembly of DENV2 particles (Chen *et al.* 2008). Acrylamide, which disrupts the organization of vimentin network, caused a reduction of colocalization of DENV2 antigen in host cell cytoplasm (Chen *et al.* 2008). In addition, DENV NS1 protein was found to have a strong association with vimentin, which plays a crucial role in replication as well as the egress of DENV (Kanlaya *et al.* 2010b).

Other than utilizing cytoskeletons directly, DENV also makes use of cell membrane microdomains, such as lipid raft, to enter host cells (Reyes-Del Valle *et al.* 2005). Microtubules, intermediate filaments, and cytoskeletal proteins are the few lipid rafts associated proteins, and this perchance indicates secondary involvement of cytoskeletons during the internalization of viruses exploiting lipid rafts (Foster *et al.* 2003).

Cytoskeletal Proteins were Identified in the Screens of DENV–Host Interaction

Several screen studies were performed and revealed multiple host interactors during DENV infection. Actin cytoskeletal protein Tropomyosin-1 (TPM-1) was identified from a Sengenics Immunome protein array screen

recently to have elevated more than twofold in severe dengue infection, indicating its potential as a biomarker for the progression from mild dengue to severe dengue (Soe *et al.* 2018). In another screen, several differentially expressed lncRNAs were identified in DENV infection (Wang *et al.* 2017). Since host cell cytoskeleton reorganization was reported to be tightly associated with these lncRNAs, the screens provide interesting hints for exploring the cytoskeletal related molecular machinery and mechanisms. A proteomics screen of *Aedes albopictus* salivary gland, midgut, and C6/36 cells infected by dengue virus has found varied mRNA level of cytoskeletal related genes. The abundant expression of these cytoskeleton proteins in salivary gland may be related to its high susceptibility (Zhang *et al.* 2013).

A high-throughput yeast two-hybrid screen was performed against a human liver activation domain library and identified 139 interactions, of which the vast majority are novel. Among these hits, human proteins with functions related to the cytoskeleton were enriched (Khadka *et al.* 2011). Two-dimensional polyacrylamide gel electrophoresis (PAGE) and quantitative intensity analysis revealed some significantly altered protein spots in DENV-2-infected cells, and cytoskeleton assembly related components were successfully identified by mass spectrometry (Kanlaya *et al.* 2010a). Decreased expression and disorganization of the actin-cytoskeleton were observed in the infected cells, whereas the increase in actin stress fibers was found in adjacent noninfected cells (Kanlaya *et al.* 2009). By using yeast two-hybrid assay, NS3 or NS5 proteins of flavivirus were found to interact with many major cellular proteins such as vimentin and myosin, but no further studies were performed to investigate the mechanism of these interactions (Le Breton *et al.* 2011).

The Role of Cytoskeleton in ZIKV–Host Interaction

The Role of Actin Filaments in ZIKV–Host Interaction

In the central nervous system, disruption of the actin cytoskeleton results in defects of the blood brain barrier, facilitating infection by neurotropic viruses including ZIKV and WNV (Al-Obaidi *et al.* 2018). In addition, actin cytoskeleton signaling related proteins were found in the interactome of IFITM1 and IFITM3, both of which have been shown to repress the replication of flaviviruses such as ZIKV (Ganapathiraju *et al.* 2016). Moreover, there is a possible connection between cytoskeleton changes and microcephaly as well as other neurodegenerative damages occur in congenital ZIKV infection.

The Role of Microtubules and Intermediate Filaments in ZIKV–Host Interaction

An exciting work reported that ZIKV infection causes a drastic reorganization of microtubules and intermediate filaments cyokeratin 8 forming cage-like structures surrounding the viral replication factory, and ZIKV replication is suppressed by microtubule-stabilizing drug paclitaxel. This work, for the first time, tightly linked ZIKV replication factories to rearrangements of the host cell cytoskeleton, leading to the hypothesis that the cytoskeleton may serve as an involuntary aid to the Zika virus (Cortese *et al.* 2017). However, by assessing the effect of ZIKV infection on microtubule morphology using immunostaining, another study showed that there is no significant disruption of the microtubule network by ZIKV infection in human lung epithelia A549 cells (Hou *et al.* 2017).

In macrophages, ZIKV was enclosed in cytoplasmic vacuolar-like structures, while in the other primary cells the viral envelope was either diffused in the cytoplasm or surround the nucleus. Thus, it is hypothesized that the observed viral localization may be related to different organization of host cell cytoskeleton dynamics that are cell type specific (El Costa *et al.* 2016). By using transmission electron microscopy, people found that ZIKV infection could cause widespread morphological disruption and endoplasmic reticulum membrane remodeling in host cells (Rossignol *et al.* 2017). In addition, the viroplasm-like structures of Brazilian strain ZIKV infected C6/36 and Vero cells, were mainly localized to the perinuclear area and contained abundant microtubules in near proximity (Barreto-Vieira *et al.* 2017). The increasing number of microcephaly reinforces the relevant cellular researches of ZIKV infection (Alcantara and O'Driscoll 2014). One study pointed out that flavivirus infection can cause mispositioned spindles and augmented centrosome numbers, which indirectly contributed to microcephaly of ZIKV infection (Wolf *et al.* 2017).

A very recent study revealed that lymphocyte antigen 6 loci E (LY6E) promotes the internalization of large cargoes, mainly flaviviruses, including ZIKV, DENV and WNV. Of importance, LY6E adopts a microtubule-like organization upon flavivirus infection, and such microtubules are essential for flavivirus uptake and large-cargo internalization (Hackett and Cherry 2018). Interestingly, people found that intermediate filaments surround ZIKV replication factory in the perinuclear area, arguing for differences between ZIKV and DENV mediated cytoskeleton remodeling (Cortese *et al.* 2017; Teo and Chu 2014).

Cytoskeletal Proteins were Identified in the Screens of ZIKV–Host Interaction

A recent study applied viscRNA-Seq method, which is capable of sequencing and quantifying the whole transcriptome at single cell level together with the viral RNA from the same cell, and thus screening virus–host interactions in an unbiased, high-throughput manner. Correlation between intracellular viral load and gene expression within single cells revealed that numerous genes are components of actin and microtubules, and downregulation of cytoskeleton organization is observed at 48 h.p.i. (Zanini *et al.* 2018). Other study presented BioID/IP-MS ZIKV–host interactome and revealed that there is a correlation between specific ZIKV polypeptides and host-cell lipid metabolism reprogram, during which actin network was heavily involved (Coyaud *et al.* 2018).

The Role of Cytoskeleton in WNV–Host Interaction

The Role of Actin Filaments in WNV–Host Interaction

WNV is a vector-borne pathogen that causes systemic infections and serious neurological disease in human and animals. WNV enters cell by clathrin-mediated endocytic pathway and egresses from cell by budding from the plasma membrane of infected cells. Ng and colleagues found that actin filaments are essential during the initial penetration of the WNV across the plasma membrane, whereas microtubules are involved in the trafficking of internalized WNV from early endosomes to lysosomes for uncoating. WNV internalization into uninfected cells was shown to be prevented by actin microfilament agitation using cytochalasin D which disrupts actin filaments. Using atomic force microscopy (AFM), it was observed during the budding process of WNV infected Vero cells that there was an increased actin filament bundles and enhancement of the filopodia around the cell periphery (Lee and Ng 2004). Other team also verified that actin polymerization could assist clathrin-mediated endocytosis of WNV into host cells, and the organization and dynamics of cytoskeleton were changed by WNV infection via Rho GTPase signaling (Fraisier *et al.* 2013).

In mosquito cells, disruption of the microtubule using nocodazole drastically affects the entry process of WNV, but not the disruption of actin filaments by cytochalasin D (Chu *et al.* 2006). Actin filaments were reported to co-localize and co-immunoprecipitated with viral envelope and capsid proteins. Perturbation of actin network by

cytochalasin B significantly inhibited the release of WNV from host cells, confirmed the key role of actin filaments in the late stage of WNV egress (Chu *et al.* 2003). Moreover, WNV was previously showed to interact with filopodia, which is composed of a bundle of actin filaments, during the budding process at the plasma membrane of host cells (Ng *et al.* 1994).

The Role of Microtubules in WNV–Host Interaction

Makino and colleagues developed a tracking system of WNV particle in live cells by using fluorescent subviral particles (SVPs). They revealed that there are two phases of SVP cellular entry: early, slow phase and late, fast phase. Interestingly, fast movements at the late phase were found to correlate with microtubule network (Makino *et al.* 2014). WNV infection also negatively impacts the barrier function of tight junctions of epithelial and endothelial cells, and the process was dependent on the GTPase dynamin and microtubule-based transport (Xu *et al.* 2012). In addition, microtubule was reported to transport internalized WNV from endosomes to lysosomes (Chu and Ng 2004). Furthermore, microtubules were involved in virus trafficking in the late stage. Both the E and C proteins of WNV were transported from the perinuclear region towards the plasma membrane along the microtubules. Further studies revealed that the transportation of WNV E protein was also associated with the microtubules-based motor protein, kinesin. Microtubules were perceived to assist WNV envelope and capsid proteins transported from the perinuclear region to plasma membranes, probably for assembly before releasing virus particles; consistently, disruption of microtubules could markedly reduce virus titers (Chu and Ng 2002). Additionally, WNV exploits lipid rafts by interacting with either receptors or accessory proteins in the lipid rafts to promote virus internalization into host cells (Medigeshi *et al.* 2008).

The role of intermediate filaments in WNV–host interaction has been really obscure and further studies are needed.

Cytoskeleton Dynamics in Other Flavivirus–Host Interaction

Cytoskeleton Dynamics in JEV–Host Interaction

Japanese encephalitis virus (JEV) is a major cause of viral encephalitis in Asia. Works using human neuroblastoma cell line IMR32 revealed the different role of actin and microtubules during JEV infection. JEV internalization of host neuronal cells uses clathrin-independent mechanism

where actin rearrangement takes place when JEV binds to neuronal cells for viral entry (Kalia *et al.* 2013). However, clathrin-dependent pathway was observed when JEV infects fibroblast and Vero cells (Nawa *et al.* 2003). Depolymerization of the actin cytoskeleton at the early stage of infection inhibits JEV infection, but has no effects at the later stage.

On the other hand, microtubule acts as the transport system of the virus to the replication site and facilitates mature virus release. Recently, microtubule has been shown to be involved in the release of mature JEV (Henry Sum 2015). Since microtubules serve as a bidirectional pathway between cell organelles and the viral polyprotein of JEV, it is deduced that the interaction between viral polyprotein and the microtubules leads to in-cell transport of proteins from endoplasmic reticulum to Golgi apparatus during JEV replication. NS3 is associated with microtubules and TSG101, which also participates in JEV replication (Chiou *et al.* 2003). JEV, DENV, and WNV were tested to interact with the light chain of dynein (Tctex-1) by binding to its membrane protein. Knocking down of Tctex-1 indicated that Tctex-1 is involved in the trafficking of these flaviviruses inside the host cell. However, when the same method was tested for yellow fever virus (YFV), there was no similar interactions found (Brault *et al.* 2011). Moreover, one study pointed out that the pathway of JEV invading cells is different between fibroblasts and neuronal cells. JEV infects fibroblasts by clathrin-dependent pathway, whereas infects neuronal cells by clathrin-independent pathway. They also put forward an idea that small GTPase RhoA and actin rearrangement play an important role in JEV entry (Kalia *et al.* 2013). After that, Xu and colleagues discovered that RhoA and Rac1 activation can trigger actin cytoskeleton remodeling, and subsequently stimulation of caveolin-1-mediated endocytosis which allows JEV's entry into human neuronal cells (Xu *et al.* 2016). In addition, other research team also found that JEV entry into BHK-21 cells is related to the Rab proteins that control vesicle trafficking dynamics, and that dynamin-, actin- and cholesterol-dependent clathrin mediated endocytosis is triggered by Rab5 and Rab11 (Liu *et al.* 2017).

Cytoskeleton Dynamics in USUV–Host Interaction

Usutu virus (USUV) is a flavivirus belonging to the Japanese encephalitis complex, having similar ecology as WNV (Nikolay *et al.* 2011). Recently, researchers have explored the interaction between USUV and host cells from the perspective of autophagy which is one of the first line of defense against intracellular pathogens and it can contribute to viral clearance. For example, an accumulation of microtubule-associated protein 1 light chain 3 (LC3)

increased in the cytoplasm of Vero cells infected with USUV, and LC3 stimulated the autophagic process (Blazquez *et al.* 2013).

Cytoskeleton Dynamics in Kunjin Virus–Host Interaction

The Kunjin virus protein was first known to be associated with microtubules cytoskeleton (Ng and Hong 1989). Cytoskeleton rearrangement induced by the Kunjin virus was suggested to aggregate fibers in the infected cells as demonstrated by an increase in vacuole numbers in the cytoplasm, which contains whorls of fibers; when infection progresses, clusters of matured Kunjin virus particle inside the vacuoles in the cytoplasm are possibly released (Ng 1987). Kunjin virus yield was slightly reduced when cytochalasin B drug disrupted the network of actin filaments (Ng *et al.* 1983).

Cytoskeleton Dynamics in TBEV–Host Interaction

Electron tomography-based 3D reconstructions using tick-borne encephalitis virus (TBEV) -infected human neurons revealed a connection between microtubules and virion-containing vesicles (Bily *et al.* 2015). Disrupting microtubules severely impeded virus production, highlighting the importance of this cytoskeleton network for the TBEV life cycle (Bily *et al.* 2015). Host cell cytoskeleton is proposed to play an important role in TBEV maturation process as microtubulin is observed to have increased density in the assembly area of TBEV proteins (Ruzek *et al.* 2009). Another researcher proposed that TBEV causes reorganization of actin in rat astrocyte instead of microtubules (Potokar *et al.* 2014). However, the proper function of cytoskeleton for TBEV is still not fully understood.

Perspective

Broadly inhibiting cytoskeleton assembly/disassembly is the most commonly used experimental method, but it is not a reasonable consideration for therapeutic use. We believe that thorough investigations are needed to explore the precise mechanisms of action that cytoskeleton plays during flavivirus infection. There are numbers of further direction worth pursuing:

- (1) Since three distinct cytoskeletal networks, actin filaments, microtubules and intermediate filaments, exist simultaneously within one cell and their interplay are involved in many key cellular functions, it would be interesting to study during flavivirus infection how these three cytoskeleton

systems are regulated individually or in combination;

- (2) Since different structures of actin filaments play relatively distinct roles, it might be worthy of narrowing down the role of specific actin structures (such as filopodia, lamellipodium, dorsal stress fibers, ventral stress fibers, transvers arcs, Arp2/3 nucleated actin and formin nucleated actin) in the regulation of flavivirus entry, cellular transport and egress;
- (3) Since motor proteins are involved in various dynamic process, it is also worthy of studying how flaviviruses adjust the activities of actin motor protein myosin family and microtubules motor protein kinesin and dynein family to fulfill their own transport needs;
- (4) Since intermediate filaments are expressed in a cell specific manner and comprised over 70 genes, it would also be interesting to examine the functions of different intermediate filaments during flavivirus infection;
- (5) Since flaviviruses target different host cell types resulting in divergent consequences, it would be important to study the cytoskeleton function in the context of specific host cell types;
- (6) Since cytoskeleton is spatial- and temple- dynamic system, it would also be essential to consider the complication of 3D structures and time-course dependent processes during flavivirus infection.

The last few years have witnessed a substantial increase in our understanding of how flaviviruses exploit host cytoskeleton network, as well as its indirect regulation through clathrin and caveolae endocytotic pathways, to facilitate key steps of infection, as summarized in Fig. 1 and Table 1. These results may indicate that almost all flaviviruses could have followed a similar rule of invading into, assembly within, and egress from the host cells, despite having some minor differences in these processes. On the other hand, there are flaviviruses, such as powassan encephalitis virus, that have hardly been studied from the host cell perspective. Therefore, it will be of great benefit to explore deeply and thoroughly of the regulation of host cytoskeleton during viral infection from the cell biology point of view. Collectively, cytoskeleton network sheds light on the spatial dynamics of virus–host interactions at the single cell level and represents an attractive direction for discovery of novel host-targeted antiviral strategies.

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Table 1 Summary of cytoskeletal proteins in flaviviruses infection.

Virus species	Cytoskeleton	Proteins	Resources	Results	References
DENV	Actin filaments	Akt	Huh7 and Vero cells	DENV2 induces Akt phosphorylation (on Ser-473) and disassembly of the actin filaments early after infection	Cuartas-Lopez <i>et al.</i> (2018)
		Rac1	EAhv926 cells	Rac1 activity decreases in the early phase and gradually increases by the late phase of infection	Zhang <i>et al.</i> (2016)
		Rac1	HMEC-1 cells	Induce reorganization of actin to form filopodia	Zamudio-Meza <i>et al.</i> (2009)
		Cdc42	HMEC-1 cells		
	Microtubules	Myosin II	HMEC-1 cells		
		TPM-1	Blood sample from patients		
		Dynein	BHK-21 cell	Important role in the pathogenesis of severe dengue infection	Soe <i>et al.</i> (2018)
	Intermediate filaments	STMN1	EAhv926 cells	Associates with E protein of DENV	Shrivastava <i>et al.</i> (2011)
		Vimentin	EAhv926 cells	miR-223 inhibits DENV2 replication by negatively regulating the STMN1	Wu <i>et al.</i> (2014)
	Screens	Myosin-9	Huh7 cells	Associates with NS1 protein of DENV	Kanlaya <i>et al.</i> (2010b)
ZIKV	Actin filaments	ACTA2	Huh7 cells	Interacts with NS4A protein of DENV2	Teo and Chu. (2014)
		ACTB	ECV304 cells	Involves in the assembly of DENV2 particles	Chen <i>et al.</i> (2008)
		ACTG1	Human cDNA libraries from liver, brain, spleen and bronchial epithelia	Interact with NS3 or NS5 protein or both	Le Breton <i>et al.</i> (2011)
		KIF3B			
	Microtubules	LMNA			
		KRT19			
		Actin	Central nervous system	Vital for neurotropic viral infection	Al-Obaidi <i>et al.</i> (2018)
	Intermediate filaments	α -tubulin	Huh7 cells	Microtubule displacement and cage formation	Cortese <i>et al.</i> (2017)
		Keratin	Huh7 cells	Thicker filaments surround the virus-induced structure	Cortese <i>et al.</i> (2017)
	Screens	Nestin	Huh7 cells	Accumulates in the periphery of virus-induced structure, whereas the diffuse nestin signal presents within the NS3-positive region	Cortese <i>et al.</i> (2017)
		ACTB	Huh7 cells	Actin and microtubule networks negatively correlate with virus	Zanini <i>et al.</i> (2018)
		ACTG1			
		TUBB1			

Table 1 (continued)

Virus species	Cytoskeleton	Proteins	Resources	Results	References
WNV	Actin filaments	Actin	Vero cells	Directly involves in virus budding	Chu and Ng (2004)
	Microtubules	Rho GTPase	Vero E6 cells	Modified during the course of WNV infection	Fraiser <i>et al.</i> (2013)
		Dynamin	CACO-2 cells	Dynamin and microtubules are required for WNV induced degradation of claudin-1 and JAM-1	Xu <i>et al.</i> (2012)
		Kinesin	Vero cells	Associates with E protein during its trafficking along the microtubules	Chu and Ng (2002)
	Screens	ACTB	Twenty-nine days old female C57/BL6 mice brain	Regulates during late time point of infection	Fraiser <i>et al.</i> (2013)
		ARPC5			
		GNAQ			
		GIT1			
		GFAP			
		MYO18A			
		Moesin (MSN)			
		SPTBN1			
		SPNA2			
JEV	Actin filaments	VIM			
		STXBPI			
		DYN1			
		TBB5			
		RhoA	SK-N-SH cells	Helps JEV enters into human neuronal cells	Xu <i>et al.</i> (2016)
		Rac1			
		ROCK2			
		Tctex-1	Human spleen cDNA library	Plays a role in late stages of viral replication through its interaction with the membrane protein	Brault <i>et al.</i> (2011)
		LC3	Vero cells	Stimulates the autophagy process	Blazquez <i>et al.</i> (2013)
		Tubulin	Vero cells	Associates with NS3 protein	Ng and Hong (1989)
USUV	Microtubules				
Kunjin virus	Microtubules				
TBEV	Actin filaments	Actin	Three days old Wistar rat astrocytes	Reorganization of actin filaments upon infection	Potokar <i>et al.</i> (2014)
	Microtubules	α -tubulin	Cultured human neural cells	Disrupts and partially degrades in infected cells	Ruzek <i>et al.</i> (2009)

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Compliance with Ethical Standards

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