Bunyaviruses: from transmission by arthropods to virus entry into the mammalian-host first-target cells

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Abstract

The *Bunyaviridae* constitute a large family of animal RNA viruses distributed worldwide, most members of which are transmitted to vertebrate hosts by arthropods and can cause severe pathologies in humans and livestock. With an increasing number of outbreaks, arthropod-borne bunyaviruses (arbo-bunyaviruses) represent a global threat to public health and agricultural productivity. Yet transmission, tropism, receptors, and cell entry remain poorly characterized. The focus of this review is on the initial infection of mammalian hosts by arbo-bunyaviruses from cellular and molecular perspectives, with particular attention to the human host. We address current knowledge and advances regarding the identity of the first-target cells and the subsequent processes of entry and penetration into the cytosol. Aspects of the vector-to-host switch that influence the early steps of cell infection in mammalian skin, where incoming particles are introduced by infected arthropods, are also highlighted and discussed.

Keywords

Arbovirus; arthropod; bunyavirus; cell entry; emerging disease; transmission; vector.

Introduction

The *Bunyaviridae* is one of the largest families of RNA viruses, with over 350 identified isolates assigned to five genera (*Hantavirus*, *Nairovirus*, *Orthobunyavirus*, *Phlebovirus*, and *Tospovirus*) (**Figure 1**) [1,2]. The *Bunyaviridae* is a unique group of viruses whose members have a global distribution and infect a wide range of hosts, including plants, invertebrates, and vertebrates. With the exception of hantaviruses, which are mainly transmitted through inhalation of aerosols from urine, feces, and saliva of infected rodents, the *Bunyaviridae* members are all arthropod-borne viruses (arboviruses) (**Figure 1**) [1,3-7]. While tospoviruses are plant-specific and spread via non-hematophagous vectors, namely thrips, orthobunyaviruses, nairoviruses, and phleboviruses are transmitted to vertebrates by blood-feeding arthropods [1,2]. Several arthropod-borne bunyaviruses (arbo-bunyaviruses) represent a threat to livestock, agricultural productivity, and human public health, causing a broad spectrum of illness, ranging from mild syndrome to serious life-threatening disease and death. Because of their mode of transmission, these viruses are considered potential emerging agents of disease. Some are classified as potential biological weapons and listed as category A, high-priority pathogens, by the National Institute of Allergy and Infectious Diseases (NIAID) of the United States.

In this review, we first illustrate the significance and diversity of the arbo-bunyaviruses using the best documented species infecting humans and domestic animals as examples (thus excluding *de facto* hanta- and tospoviruses from the review). We then highlight and discuss different aspects of the vector-to-host switch that most likely influence the identity and infection of the first-target cells in the skin dermis, where incoming particles are introduced through arthropod bites. We finish with an extensive review of current knowledge and advances addressing the subsequent cellular and molecular processes that drive virus penetration into cells, the ultimate steps of the initial infectious entry.

A global threat to human and veterinary public health

A limited number of arbo-bunyaviruses has been investigated, most of the available information coming from studies of a sprinkling of isolates, predominantly those introduced in the following paragraphs. However, it is apparent that there is a wide variety of isolates, vectors, hosts, diseases, and geographical distributions. This diversity is also

manifested at the cellular and molecular levels in the genomic organization, virion structure and architecture, transmission, tropism, cellular receptors, and cell entry, which are all discussed in the following chapters. It is obvious that there will be exceptions to some of the generalizations below.

The *Orthobunyavirus* genus contains over 170 viruses divided into 18 serogroups. Most are transmitted by mosquitoes and midges, and a few by ticks and bed bugs [5]. More than 30 isolates in the genus are responsible for several diseases in humans, from acute but self-limiting febrile illnesses, e.g. Oropouche virus (OROV) in South America, to neurologic diseases, e.g. La Crosse virus (LACV) in North America [5]. In domestic animals, abortion, offspring with congenital malformations, or stillbirth are observed following infection by some orthobunyaviruses such as Schmallenberg virus (SBV), recently identified in the north of Europe and now present all over the continent [8].

The *Nairovirus* genus comprises seven serogroups, which together represent more than 30 viruses, all transmitted by ticks [7,9]. Ticks are classified in *Arachnida*, a class distinct from that of insects (*Insecta*). These arthropods are of huge economic significance worldwide, both as harmful parasites and as vectors of several emerging agents of diseases [10-13]. This includes not only viruses, but also parasites and bacteria, causing for instance Lyme disease. Nairobi sheep disease and Crimean Congo hemorrhagic fever viruses (CCHFV) are arguably the most important nairoviruses from a public and veterinary public threat perspective. Nairobi sheep disease virus induces acute hemorrhagic gastroenteritis in sheep and goats in Central and East Africa. CCHFV is the most geographically widespread tick-borne virus that causes outbreaks of severe hemorrhagic disease in humans, with mortality approaching 30% [7]. Cases in humans have been reported in Africa, the Middle East, Asia, and Eastern Europe. Recently the virus has been detected in ticks collected in Spain [10,14,15].

The *Phlebovirus* genus comprises 70 viruses [16]. Although many phleboviruses are transmitted by sandflies, some are vectored by other arthropods. This is the case of Rift valley fever (RVFV) and Uukuniemi (UUKV) viruses, respectively transmitted by mosquitoes and ticks [16,17]. RVFV is an emerging pathogen affecting livestock and humans [18-20]. Infected domestic animals develop hepatitis, hemorrhage, and abortion with often fatal outcomes. In humans, the virus is responsible for an extensive variety of

symptoms ranging from febrile illness to severe disease, including among others hepatitis, retinitis, encephalitis, and hemorrhagic fever [21]. In recent outbreaks, the mortality rate in humans went up to 45%, though this may reflect exclusion of less severe cases from calculations rather than a change in virulence [20,22]. Whilst RVFV has spread from Africa to the Arabian Peninsula in the past decade, it now presents a risk of introduction into Southern Europe [18-20].

UUKV and related viruses are all transmitted by ticks. Uukuniemi-like viruses were initially grouped in a separate genus (*Uukuvirus*). After 1991, they were incorporated into the *Phlebovirus* genus because of molecular similarities with the other phleboviruses. Indeed UUKV has served for many decades as a major model for phleboviruses. Its investigation has led to major insights into many aspects of the phlebovirus life cycle, e.g. virion structure and morphogenesis, cell entry, and viral replication [23-32]. Recently, a number of novel tick-borne phleboviruses, all closely related to UUKV, have emerged in distinct parts of the world, some of which are highly pathogenic in humans [33-41]. Recent illustrations are Heartland virus (HRTV) in North America and severe fever with thrombocytopenia virus (SFTSV) in Asia, latter causing a fatality rate of up to 60% in some outbreaks [33-37,40]. In this context, there is a renewed interest in using UUKV to study these emerging viruses [42]. UUKV can be efficiently amplified in tick *Ixodes ricinus* cell lines (personal communication), it is not associated with any disease in humans and other animals, and therefore, allows for state-of-the-art elegant approaches, such as live cell and animal imaging, which are nearly impossible with the emerging, pathogenic tick-borne phleboviruses.

Virus transmission

Arbo-bunyaviruses infecting humans and other animals are generally maintained in arthropod vectors and amplified in non-human vertebrates, although a few reports suggest that humans may serve as amplifying reservoirs during urban epidemics [1,9,43-46]. Horizontal human-to-human transmission has rarely been reported and only occurs under specific circumstances, e.g. exposure of healthcare personnel to CCHFV-infected patients [46]. Confirmed cases of vertical transmission in humans are also rare. In general, humans are ‘dead-end’ hosts [43]. Livestock-to-human transmission can happen during outbreaks such as those of RVFV. RVFV can be transmitted to humans through contact with the

blood or organs of infected animals, e.g. slaughtering, butchering, or veterinary procedures, etc. The transmission of RVFV via aerosol has also led to infection in laboratory workers. However, natural transmission of arbo-bunyaviruses to vertebrates mostly occurs through the bite of infected arthropods [1,9,47].

With few exceptions, viruses are spread within arthropod populations both venereally and transovarially. In contrast to vertebrate hosts, there is no clear evidence of pathology or lethal outcomes following infection in arthropod hosts. Infection of arthropods is usually asymptomatic, predominantly persistent, and necessary for efficient propagation to other hosts. A few reports suggest a possible alteration in the general behavior of infected arthropods. Some reports show that insects can present significant modifications in their way to fly and feed following infection by dengue virus (DV), an important human pathogenic arbovirus of the *Flaviviridae* family [48,49]. Regarding bunyaviruses, infection of *Aedes* mosquitoes by LACV seems to cause a higher biting activity and also a longer probing time [50,51]. Authors propose that these changes in the behavior of mosquitoes may result in an enhancement of horizontal transmission of LACV. The molecular and cellular determinants of such behaviors in infected arthropod populations as well as the impact on the virus transmission and spread remain largely uncharacterized. The phosphorylation of the flavivirus NS5 protein by the mosquito protein kinase G has been recently correlated with the alteration of the flight behavior in *Aedes aegypti* and *Anopheles gambiae* [52].

Because of human activity and the changes in global climate, several of the bunyavirus arthropod vectors are spreading to new geographical locations, notably in more northerly regions of Europe and North America [19,43,53]. It is known that once the arthropod vectors are established in a new area, the viruses that they carry will shortly follow. Moreover intensive deforestation, overpopulation, and introduction of susceptible military personnel or settlers into new environments and wild territories are also factors favoring new contact with arthropods, and therefore the emergence, reemergence, and rapid spread of arboviral diseases. Consequently, the exposure of non-immune populations to pathogenic arbo-bunyaviruses as well as new outbreak episodes seem inevitable. Though our knowledge of the complex interactions between arthropods and these viruses has substantially increased during the last decade, our current view of the transmission to

mammals remains essentially based on studies performed *in vitro* or involving virus stocks produced in mammalian cells. Clearly much further work involving arthropod-derived bunyaviruses is needed to improve our global understanding of the bunyavirus life cycle and transmission to humans and other vertebrates.

Genome organization and viral proteins

Like all the other *Bunyaviridae* members, arbo-bunyaviruses are enveloped with a tri-segmented single-stranded RNA genome that encodes a minimum of four structural proteins in a negative-sense orientation [1]. The largest genomic RNA segment (L) codes for a RNA-dependent RNA polymerase (protein L) that is essential for initiating the viral replication, the smallest segment (S) for a nucleoprotein (protein N), and the medium segment (M) for a precursor polypeptide that is further processed into two envelope glycoproteins (G_N and G_C) (**Figure 2A**). A third structural glycoprotein is sometimes encoded by the M segment of nairoviruses, such as Hazara and Clo Mor viruses [7,54,55]. Arbo-bunyaviruses also encode some non-structural proteins. Those for which the most data is available are briefly described in the following paragraphs (reviewed in [56]).

The number of non-structural proteins varies from a genus to another, and sometimes among isolates within a genus. The NSm and NSs proteins are by far the most documented. When expressed, NSm results from the maturation cleavage of the M precursor polypeptide, usually in the order G_N-NSm-G_C or NSm-G_N-G_C (**Figure 2A**) [5-7]. Most orthobunyaviruses encode an NSm protein [5]. Phleboviruses transmitted by dipterans (sandflies and mosquitoes) also encode an NSm, but not the tick-borne phleboviruses (Uukuniemi-like viruses) [6]. With the vector of transmission, the presence of an NSm protein appears as one of the main distinctions between tick- and dipteran-borne phleboviruses [6]. In the *Nairovirus* genus, an NSm seems to be expressed in cells infected by CCHFV [57,58]. Not much is known about other nairoviruses and possible NSm proteins [7]. An additional non-structural protein, named NSs, is found in cells infected by phleboviruses and most orthobunyaviruses [5,6]. However some orthobunyaviruses present a truncated form of NSs or do not even encode an NSs protein [5]. Although the S segment of both phlebo- and orthobunyaviruses contains the NSs open reading frame (ORF), the expression of NSs involves divergent coding strategies. The NSs ORF of phleboviruses is non-overlapping and in a positive-sense orientation in the genomic viral

RNA, while that of orthobunyaviruses is internal to the N coding sequence and in a negative-sense orientation in the genomic S segment (**Figure 2A**) [5,6]. In the case of nairoviruses, the NSs ORF is simply absent [7]. Further non-structural highly O-glycosylated proteins have been shown to be released from cells infected by CCHFV following maturation cleavages of the N terminal part of the M precursor polypeptide, also designated as the mucin-like domain (**Figure 2A**) [59]. These viral factors, the role and significance of which remain unclear, seem to be a specificity of CCHFV, and most likely, of other nairoviruses.

The NSs protein of RVFV has been extensively studied, but a substantial amount of work has also been done regarding that of orthobunyaviruses and other phleboviruses [56,60]. While they seem dispensable for the production of viral progenies, the NSs proteins are considered as an important factor of virulence. An accumulation of evidence indicates that NSs contributes to the disease outcome by modulating host cell functions and antiviral responses [56,60]. These proteins have the ability to counteract the host innate immune defense. They interfere with the type I interferon induction, block the function of the protein kinase R (PKR), and inhibit the general transcription of host cells [56]. Mice inoculated with RVFV strains lacking the NSs sequence survive infection, in stark contrast to those exposed to the wild type virus, which typically die within a couple of days [61]. The NSm proteins have been studied to a lesser extent. Little is known about the function of these proteins. The few existing studies suggest a role in the assembly of orthobunyavirus particles [62,63]. The NSm of RVFV seems to play a key role in anti-apoptotic processes, and therefore, is proposed to be the second virulence factor of the virus [64,65]. While the NSm of RVFV is important for virus replication and dissemination from the midgut of *Aedes aegypti* mosquitoes, NSs is rapidly silenced in RNA interference-competent mosquito cell culture [66,67]. The NSs protein of Bunyamwera virus (BUNV), the prototype member of the *Orthobunyavirus* genus, is not able to shut off the transcription of infected insect cells as is the case in mammalian cells by blocking the activity of the RNA polymerase II [68]. The function of NSs and NSm in mammalian cells thus appears fundamentally distinct from that in arthropod cells.

Virion structure

All bunyaviruses exclusively replicate in the cytosol and bud from the Golgi apparatus where virions acquire their lipid bilayer membrane and where maturation occurs. In the viral particles, the protein N is associated with the virus RNA genome, and together with the viral polymerase L, constitutes the pseudo-helical ribonucleoproteins (RNPs) [2]. A major distinction of bunyaviruses from other enveloped viruses is that the virions are devoid of any classical matrix or capsid. The protein N thus has an important role in the protection of the viral genetic information. In the last five years, the crystal structure of N has been solved for many bunyavirus members, providing new insights into the mechanism of RNP assembly [69-80] (reviewed in [81]). Briefly, the protein N of orthobunyaviruses binds to RNA in a positively charged cleft, formed by two-helical lobes [69-73,75]. In these cases, both the N- and C-terminal arms of N mediate the oligomerization of the viral nucleoprotein. Recently the structures of the N proteins of RVFV, SFTSV, and Toscana virus (TOSV) have also provided a new understanding of the assembly of phlebovirus RNP [74,76,77,82,83]. The general features of oligomerization and RNA binding in the N proteins are conserved among phleboviruses. The protein N comprises a C-terminal core domain, which binds to RNA, and an N-terminal single arm responsible for the oligomerization of the protein. Interestingly, the CCHFV N is closest to the nucleoprotein of Lassa virus, a member of the *Arenaviridae* family, and not that of a bunyavirus [78,79]. In the absence of additional information about the N proteins of other nairoviruses, it is difficult to say whether it is a specificity of CCHFV or of the nucleoproteins of all members in the genus.

Electron microscopy (EM) pictures of bunyaviruses show particles roughly spherical and heterogeneous in size, with an average diameter of 80-140 nm and spike-like projections between 5 and 10 nm (**Figure 2B and 2C**) [1,2,23]. These protrusions are composed of the two glycoproteins, G_N and G_C, responsible for virus attachment to target cells and for penetration by membrane fusion. Recent ultrastructural studies confirmed the high degree of pleomorphism previously observed for bunyaviruses [23,84-87]. Cryoelectron tomography analyses of the phleboviruses RVFV and UUKV have disclosed that the most regular particles harbor surface glycoprotein protrusions arranged on an icosahedral lattice, with an atypical T = 12 triangulation [23,84-86]. Contrasting with these previous

observations, tomography data obtained for the orthobunyavirus BUNV revealed non-icosahedral particles with glycoprotein spikes exhibiting a unique tripod-like arrangement [87].

Besides the several cryoelectron tomography studies recently reported for bunyavirus particles, the molecular structure of the glycoproteins remains largely unknown for these viruses. With the exception of a partial crystal structure obtained for the cytoplasmic tail of the CCHFV glycoprotein G_N, the only X-ray structure available is for the complete ectodomain of the RVFV glycoprotein G_C, solved at a resolution of 1.9 Å [88,89]. The overall fold of the protein shows a strong resemblance to the class II membrane fusion E proteins of flaviviruses such as DV and West Nile virus (WNV) [90,91]. This novel finding is in agreement with previous bioinformatics predictions and of major importance [92,93]. It provides for the first time direct evidence that the glycoprotein G_C of a bunyavirus member belongs to the group of class II membrane fusion proteins. Although the degree of amino acid homology is rarely superior to 30% among the glycoproteins of bunyaviruses, one can reasonably postulate that the peptide responsible for bunyavirus membrane fusion is present in the glycoprotein G_C. Mutation-based functional investigations into the glycoproteins of orthobunyaviruses, involving mainly cell-cell fusion assays and virus-like particles, support this view [94-98]. However, there are still outstanding questions concerning the global, highly-ordered arrangement and interactions of the glycoproteins G_N and G_C at the surface of virus particles [99]. To this extent, the X-ray structure of G_N is unfortunately lacking.

In the last decade, reverse genetics systems were developed to rescue BUNV and LACV from plasmids, enabling the modification of viral genomes and the studies of bunyavirus gene function [100-102]. These systems were recently adapted to other bunyavirus members, including RVFV, UUKV, SFTSV, and SBV [42,103-105]. Reverse genetics has revolutionized negative-sense RNA virology. The discovery potential has not yet been entirely tapped into in the bunyavirus field, especially in the domain of transmission and early virus-host cell interactions. This system indeed offers many opportunities to label the structural proteins of the virions so as to track single particles by microscopy, and to generate mutants of G_N and G_C so as to study the virus fusion mechanisms in living cells.

Arthropod vector-to-mammalian host switch

The typical picture, which is largely employed to illustrate the life cycle of a virus, is often restricted to the productive infection of a single cell. This limited representation is particularly misleading in the case of arbo-bunyaviruses. This image tends to minimize the importance of the arthropod vector-vertebrate host alternation in the complete virus life cycle. The intrinsic cell biology of the arthropods differs from that of vertebrates in many aspects, such as growing temperature, lipid membrane, and glycan motifs. The host-switch, therefore, results in important changes in the molecular composition of the viral particles that are transmitted between the different host species. Indeed, the complete life cycle of an arbovirus consists of two essential components that are intricately interdependent, 1- the transmission cycle between, and within, non-vertebrate and vertebrate host populations, and 2- the productive cycle in the host cells (**Figure 3**). The host alternation appears to be critical for the genetic stability of RVFV and the infectivity of the virus [106]. Similar observations have been made for other arboviruses [107,108]. In the following paragraphs, some examples are given and discussed to illustrate the importance of taking into account and recapitulating the arthropod-to-mammal switch in investigations into the initial infection of mammalian host cells by arbo-bunyaviruses.

The viral particles transmitted by arthropods to humans and other mammals are clothed with a glycan coat gained in tick or insect vectors. The *N*-glycans of viral glycoproteins derived from ticks remains nearly uncharacterized, whereas those produced in insect cells are notoriously known to be essentially composed of mannose residues [109]. When produced in mammalian cells, arboviruses are believed to lose their high-mannose coat to gain a complex glycosylation, meaning that the *N*-glycans on viral particles consist of diverse carbohydrate residues and not only mannoses [110-112]. Indeed, the mannose residues that are added onto the nascent glycoproteins of mammalian cells in the endoplasmic reticulum (ER) undergo several modifications by glycosidases and glycosylases through the Golgi apparatus [113]. *N*-glycosylations have several functions in the virus life cycle. Among others they are involved in the correct folding of viral glycoproteins through the quality control machinery, in hiding virus epitopes to escape the host humoral immune system response, and also in mediating virus interactions with cell surface lectin receptors. Many bunyaviruses use the human C type lectin DC-SIGN to enter

and infect dermal-like dendritic cells (DCs) (see the chapters 'First-target cells' and 'Early virus-host cell interactions: receptors for bunyaviruses') [25,114]. Not much more is known about the *N*-glycosylations of bunyaviral particles and the relation with the first-target cells following transmission.

The evidence gives *N*-glycosylations a critical role in the assembly, infectivity, and propagation of the unrelated arboviruses DV and WNV within both arthropod vectors and mammalian hosts, which is likely also true for the arbo-bunyaviruses [115-117]. A recent study has established the importance of host alternation in lectin switching during DV infection [111]. Only incoming insect-derived DV particles, which have a high-mannose coat, can infect human DCs via specific lectin-mannose interactions, while progeny viruses with a complex *N*-glycosylation are no longer able to infect DCs [111,112,118]. In line with these observations, RVFV presents a higher potency to infect goat DCs when derived from insect cells rather than mammalian cells [119]. Interestingly, some bunyaviruses have been shown to keep a highly mannosylated coat, even during production in mammalian cells [24,25,59]. This raises several questions: how can bunyaviral particles remain with this type of glycosylation when they egress through specialized compartments such as the ER and Golgi apparatus? How does the nature of *N*-glycans impact the virus spread throughout mammalian hosts in the further rounds of amplification after initial infection?

Another important constituent of the bunyaviral particles is the lipid bilayer envelope. Similarly to the carbohydrates, lipids are acquired from the host cells during virus assembly, with substantial differences between insect and vertebrate cells in terms of fatty acid transport, metabolism, and lipid composition [120,121]. For instance, the plasma membrane of Sf9 insect cells contains 20 times less cholesterol than those isolated from mammalian cells [122,123]. Again, little information is available regarding the lipid composition of the arbo-bunyavirus membranes, the identity of lipids that are important for the virus fusion, if any, and the importance of host alternation in these processes. The virion structure and infectivity might effectively diverge according to the cell origin of viruses. Mammal-derived bunyaviral particles are mainly pleomorphic [23,84,85,87]. That bunyaviruses do not have any classical capsid or matrix proteins forming rigid structures underneath the viral envelope likely explains the relative fragility of virions observed in EM pictures. It is tempting to postulate that the arthropod cell-derived lipids contribute a

stronger protection of the RNPs to the particle envelope, and indirectly, a higher infectivity to the virions compared to those originating from mammalian cells. Infectivity also strongly depends on the capacity of the particles to fuse with cell membranes. In this sense, the lipid composition of target membranes has a major impact. UUKV has been shown to critically rely on late endosomal lipids for fusion and penetration (Bitto et al., personal communication, European Society for Virology 2013, Lyon, France).

Additional differences exist between arthropod- and mammal-derived arbo-bunyaviral particles. In addition to sugars and lipids, the body temperature of the vertebrate and invertebrate hosts infected by arbo-bunyaviruses is also a major distinction. Arthropods are poikilothermic, meaning that their body temperature depends on that of the environment. Thereby one can imagine that virus variants have proteins correctly folded and functional in the typical range of temperature associated with these hosts (28-32 °C), but not at a higher temperature, such as that in mammalian bodies (37 °C). Temperature-sensitive virus mutants obtained in laboratory have been reported for UUKV and RVFV [124,125]. While remaining important for the infection of the first target-cells, these mutants might be unable to propagate throughout mammalian hosts. The selection of specific viruses and the importance of host alternation in the stability of arbovirus genomes may, in part, find an explanation in such a bottleneck [106-108]. In the case of RVFV, a third structural glycoprotein of 78 kDa is found on virions matured in mosquito C6/36 cells, but not on those derived from mammalian cells [126-128]. The expression of this protein, also referred to as P78/NSm-G_N, actually results from the translation of an alternative ORF overlapping both sequences of NSm and G_N in the M segment [126,127]. While its function remains unclear, P78/NSm-G_N appears critical for virus production in insect cells and dispensable in mammalian cells [129]. This makes P78/NSm-G_N a distinct determinant of virus propagation in insect vectors and mammalian hosts. This discussion points out the work that remains to be achieved to improve our understanding of the initial infection in the human dermis, where arbo-bunyaviruses are introduced by infected arthropods, the subsequent spread throughout the host, and at the end, the progression of the disease.

Release of viruses into the host dermis by infected arthropods

Arbo-bunyaviruses are introduced into the mammalian skin through the saliva of infected arthropods during a blood meal. The bite of arthropods usually triggers local host defenses

in the skin such as hemostasis and immune responses. Insects and ticks have all developed similar strategies to counteract these defenses. The saliva of arthropods contains a number of molecules with angiogenic, immunomodulatory, anti-inflammatory, and anti-hemostatic abilities [130-133]. In this context, resident immune skin cells, such as macrophages (MPs) and DCs, are most likely rendered silent [134,135]. As discussed in the following paragraphs, these cells are considered key players in the progression of infection by many arbo-bunyaviruses. Their inefficiency to trigger the immune response arguably confers a definitive advantage to arboviruses for the initial infection and spread throughout the host. Together, the effects of the arthropod saliva create a propitious, ideal environment in the site of virus transmission for the establishment of infection [136]. This is particularly well documented for insect-borne viruses such as DV, WNV, and the vesicular stomatitis virus (VSV), an arbovirus from the *Rhabdoviridae* family. Infection by these arboviruses through mosquito saliva results in an increase in virus transmission, host susceptibility, viraemia, disease progression, and mortality [136-144]. In the case of arbo-bunyaviruses, the infection of mice is potentiated by the co-injection of mosquito saliva with RVFV or Cache Valley virus, an orthobunyavirus [145,146]. Similarly, co-injection with LACV potentiates infection of white-tailed deer and chipmunks [147].

The immune defenses of arthropods differ from those of mammals in the sense that they do not involve antigen-presenting cells and also lack an interferon-based innate immune response [148-150]. Remarkably, insects make use of RNA interference as the main immune pathway to control arbovirus infections [151]. Microsymbionts, mostly bacteria living in symbiosis with their host, have also been shown to play an important role in arthropod immune defenses, mainly in the saliva, and to a higher extent in the midgut [152-154]. For instance, the bacteria *Wolbachia* increases host resistance to RNA viruses and other insect-transmitted pathogens [155-159]. In some cases, viral replication can, however, be enhanced by the presence of microbial symbionts [160]. Despite the importance of arthropod-associated microbiota in the general behavior of arthropods, how it affects the transmission of arbo-bunyaviruses and other arboviruses to mammalian hosts remains to be explored.

Other physiological aspects of the introduction of arboviral particles into the skin, like the diameter of syringes used for injection, are often neglected in animal studies. The smallest

diameter is still much larger than the proboscis of mosquitoes or the hypostome/chelicera of ticks. Syringes most likely trigger inflammatory responses in animals, and with this regard, are inappropriate to mimic an arthropod bite. Significant differences in terms of viraemia have been observed when LACV is injected into deer and chipmunks either by syringes or through infected-mosquito bites [147]. Many experimental challenges thus remain for studying arbovirus transmission under conditions identical to those of natural infection in the host skin.

First-target cells

The mammalian skin is a highly complex organ that consists of the epidermis underlain by the dermis and subcutaneous fatty tissues (**Figure 4**). The epidermis and the dermis are sprinkled with a large variety of organ-like structures such as hair follicles and sweat glands as well as nerves and blood vessels. Many diverse cell types reside in each layer of the skin and together these cells coordinate the human defense against invading microbes. For a comprehensive view of the different innate and adaptive immune cells associated with the skin, we recommend the excellent review by Heath et al. [161].

Arbo-bunyaviruses are thought to be released from the arthropod saliva directly into the dermis layer where the blood meal occurs and blood vessels, capillary beds, and lymphatics spread. The dermis is essentially composed of elastin and collagen fibers as well as of an extracellular matrix produced by the subcutaneous fibroblasts. This layer of the skin is interspersed with many cell types, including MPs, various subpopulations of DCs and T cells, mast cells, and innate lymphoid cells [161]. These cells are most likely the first to encounter incoming viruses. However, while many reports support a prominent role for immune cells in the pathogenesis of arbo-bunyavirus-induced diseases, the identity of the first-target cells during the initial infection remains largely unknown.

Whilst B-, T-, and natural killer cells appear resistant to CCHFV, human monocyte-derived MPs have been shown to support productive infection by CCHFV and Dugbe virus (DUGV), a nairovirus closely related to CCHFV [162,163]. RVFV and rhabdoviral particles pseudotyped with the glycoproteins of SFTSV have also been seen to infect MPs [114,164]. Furthermore the role of MPs in RVFV dissemination is supported by a study involving the use of a virus strain lacking the virulence factor NSs in combination with

mice deficient in the interferon receptor [61]. Monocyte-derived DCs, which are commonly used as a model for dermal DCs, are productively infected by nairoviruses, including DUGV and CCHFV, as well as by phleboviruses like RVFV and UUKV [25,119,162,163]. In addition, monocyte-derived DCs also support infection by rhabdoviral particles pseudotyped with the glycoproteins of LACV and SFTSV [114]. Not much is known about the potential interactions between arbo-bunyaviruses and different skin cell types.

Dermal DCs are believed to be of principal importance for the initial infection and dissemination of unrelated arboviruses [112]. As sentinels patrolling in the peripheral tissues, DCs constantly look for foreign bodies, acting at the frontier between innate and adaptive immune systems. Once an antigen is captured, DCs undergo a complex maturation process that results in the migration of DCs from the skin to the lymphoid organs where the presentation of the processed antigens to T cells triggers the adaptive immune response. A substantial amount of work on DV and WNV supports the model that arboviruses use dermal DCs as carriers to spread throughout the host [112]. These cells may play a similar role in the early stages of arbo-bunyavirus infection. Infected DCs release high amounts of arbo-bunyavirus progenies rapidly after exposure to the viruses, as soon as 6 hours in the case of RVFV and between 6 and 24 hours in that of CCHFV [25,119,162]. The virus would continue to spread during the 24 hours that it takes for DCs to reach lymph nodes [165].

While an accumulation of evidence supports a role for MPs and DCs in arbo-bunyavirus infection, it is still not completely clear whether these cells are the first to be targeted and infected following the delivery of incoming viral particles by arthropods into the host skin. In other words, if these cells represent an entry door for arbo-bunyaviruses into the mammalian hosts, or if they are involved in later stages and subsequent rounds of infection. Our current knowledge is essentially based on *in vitro* investigations, and the available data obtained *in vivo* for RVFV often involves either NSs-defective viruses or vaccine strains, the propagation of which in mammals is without doubt substantially different from that of the wild type strain [61,166]. Additionally, data on other skin cell types is insufficient to rule out their participation, direct or indirect, in the initial infection. Therefore it is not possible to exclude that arbo-bunyaviruses employ alternative strategies to spread throughout the host. Arbo-bunyaviruses and other arboviruses may replicate in some skin

cells before spreading or simply jump into the blood vessels or lymphatics to reach other organs.

Early virus-host cell interactions: receptors for arbo-bunyaviruses

Independently of the cell type in which the initial infection occurs, viruses need to attach and enter cells to amplify and propagate within the host. Animal viruses are usually simple in structure and composition. Their variety is, however, wide in size, structure, tropism, and mode of replication. This diversity is also manifested at the level of host entry into target cells. The penetration of viruses into the cytosol relies on complex interactions with the host cells and involves hundreds of cellular factors and processes. The virus entry program begins with the attachment of the viral particles to cell surface proteins, carbohydrates, or lipids. Although some viruses have the ability to penetrate into the cytosol directly from the plasma membrane, an immense majority of viruses, including bunyaviruses, are sorted into one of several endocytic pathways (**Figure 5**). After delivery into the endosomal lumen, low pH triggers changes in arbo-bunyavirus particles that ultimately result in the delivery of the virus genome into the cytosol. The receptors, cellular factors, and pathways used by arbo-bunyaviruses to enter their host cells remain largely unidentified and poorly characterized. Only a few surface receptors on target cells have been proposed to initiate the endocytic processes used by arbo-bunyavirus (**Table 1**). Heparan sulfate has been involved in RVFV and TOSV attachment to cells [167,168]. SFTSV has also been shown to use non-muscle myosin heavy chain IIA during early infection, while CCHFV seems to require cell surface nucleolin to target cells [169,170]. It remains unknown whether these proteins serve as entry receptors or merely attachment factors.

Recent work has shown that many arbo-bunyaviruses, including RVFV, make use of the C type lectin DC-SIGN to target and infect DCs [25,114]. Using the couple UUKV-DC-SIGN, it was possible for the first time to visualize virus-receptor interactions in live cells and analyze their dynamics [25]. This represents a powerful tool to study general virus-receptor interactions. In addition, DC-SIGN provides an interesting bridge between arbovirus amplification in insect vectors and initial infection in humans. This C type lectin is expressed on immature dermal DCs, which are present in the anatomical site of virus transmission, and is specialized in pathogen capture and antigen presentation [171]. DC-

SIGN binds high mannose or fucose *N*-glycans in foreign glycoproteins through its C-terminal carbohydrate recognition domain, as those of insect-derived glycoproteins. For these reasons, interactions between DC-SIGN and insect-borne pathogens are thought to be the most relevant although several studies have suggested a role for the lectin in infection by various microbes that are not transmitted by arthropods [112,171].

That some arbo-bunyaviruses are even rich in high-mannose glycans when produced in mammalian cells suggests that they do not require production in insects to be recognized by DC-SIGN. It is unlikely that viral progeny coming from dermal DCs can superinfect mother-infected DCs after spreading into the host. Whilst the immature DCs express high levels of DC-SIGN, maturation of DCs leads to downregulation of the lectin. In further rounds of infection *in vivo*, it is apparent that arbo-bunyaviruses use other receptors than DC-SIGN. They can infect a wide spectrum of tissues that do not express this immune receptor, and progeny virions coming from DCs could use other C-type lectins with high affinity for mannose residues to target and infect new tissues in hosts. A recent study has established that rhabdoviral particles pseudotyped with the glycoproteins of SFTSV, but not with those of RVFV and LACV, can subvert L-SIGN [114]. This C-type lectin is closely related to DC-SIGN, but expressed on liver sinusoidal endothelial cells. In part, this interaction may explain the tropism of many arbo-bunyaviruses in the liver.

Viral particle uptake

Virus-receptor interactions are often specific and multivalent. Binding to multiple receptor molecules clustered within microdomains can enhance avidity of low affinity interactions [172]. Additional receptor molecules can be recruited to the virus binding site, as observed for UUKV and DC-SIGN [25]. Cholesterol and other lipids also play an important role in these mechanisms by promoting the formation of the docking site for specific proteins. Infection by CCHFV and two orthobunyaviruses, OROV and Akabane virus, is abolished in cells depleted of cholesterol [173-176].

In general, virus attachment to the cell surface induces receptor-mediated signaling. For example, DC-SIGN is able to trigger selective signal transduction pathways, which seem to depend on the nature and glycosylation pattern of the captured antigens [177,178]. The host and cell identity from which viruses originate is of particular importance, i.e.

arthropods vs. mammals, and may selectively influence subsequent events, including those related to endocytosis. In fact, the receptor-mediated cascade of signaling results in the generation of membrane curvature important for triggering the internalization of the viral particles into the endocytic machinery [25,172].

Sequence motifs in the cytoplasmic tail of receptors are critical to drive the internalization of cargo and viruses. DC-SIGN and UUKV can serve again as a perfect illustration. The short cytoplasmic tail of the lectin carries several motifs involved in signaling, endocytic internalization, and intracellular trafficking [171,177-179]. DC-SIGN critically relies on two leucines (LL) for the endocytosis of cargo [25,179,180]. In contrast to the lack of evidence for the role of DC-SIGN in internalization of other viruses, the lectin has clearly been demonstrated to act as a true entry receptor for UUKV [25,179-181]. UUKV is no longer able to enter cells expressing the endocytic-defective LL mutant of DC-SIGN [25,181]. This is the only evidence of a direct role for DC-SIGN, beyond attachment, in productive virus internalization. The implication of DC-SIGN in UUKV infection is thus fundamentally different from any other viruses.

By interacting with specific adaptor proteins, endocytic motifs determine in general the internalization pathways of cargo. The motif LL is a typical docking site for adaptor proteins required in the formation of clathrin-coated endocytic vesicles [182]. The LL motif in the DC-SIGN cytoplasmic tail is of particular interest. It is present within the sequence QXXXLL that is different from any others known to involve a LL motif ([DE]XXXL[LI] or DXXLL) [182]. However, the internalization processes of DC-SIGN remain debatable; phagocytosis, lipid raft-mediated endocytosis, and clathrin-mediated endocytosis have each been proposed to be involved [171,180,183,184]. In cells stably expressing DC-SIGN, EM pictures do not exclusively show UUKV particles in endocytic clathrin-coated vesicles (CCVs) [25,26]. In cell types lacking endogenous expression of lectin, the virus is rarely seen in CCVs and silencing of clathrin does not significantly impact infection [26]. The marginal effect of clathrin knockdown on UUKV infection could reflect the diversity of endocytic pathways used by UUKV or differences in the role of clathrin in other processes, such as receptor recycling.

In contrast to UUKV, a body of data suggests that arbo-bunyaviruses mainly use clathrin-mediated endocytosis to enter cells (**Figure 5** and **Table 1**). Some studies based on the use

of chemical inhibitors, small interfering RNAs (siRNAs), and dominant-negative (DN) mutants indicate that clathrin-mediated endocytosis is used by CCHFV and many orthobunyaviruses, including OROV and LACV [173-176,185,186]. The situation is unclear regarding RVFV uptake. Clathrin-mediated endocytosis has been implied in the internalization of genetically modified, non-spreading RVFV, whereas two independent studies suggest that the vaccine strain MP12 enters cells through macropinocytosis and caveolin-dependent mechanisms [187-189]. Beyond the different RVFV strains used, the apparent discrepancy between these reports probably points out a general ability of viruses to use alternative endocytic pathways in a single cell or distinct tissues. The divergent endocytic processes by which virus receptors are internalized into the cells as well as the expression pattern of virus receptors on the cell surface certainly influence the capacity of arbo-bunyaviruses to use one or more entry pathways to infect cells and tissues.

Virus intracellular trafficking and penetration

After internalization and arrival in the lumen of endosomal vesicles, viruses must find their way through the endocytic machinery in order to reach the appropriate location for penetration into the cytosol. The endosomes provide a milieu in which the decreasing pH provides a cue for virus activation (**Figure 5**) [190,191]. Inhibitor studies have clearly shown that arbo-bunyaviruses rely on vacuolar acidification for infection (**Table 1**) [26,114,173-176,187,188]. Many are sensitive to very low concentrations of ammonium chloride, bafilomycin A1, or concanamycin B, which are weak bases and inhibitors of vacuolar-type H+ ATPases that all neutralize the endosomal pH. In addition to the endosomal acidification, some arbo-bunyaviruses may require proteolytic cleavage in their envelope glycoproteins for penetration [114].

Several lines of evidence support the idea that early endosomes (EEs) represents a necessary step in the journey of arbo-bunyaviruses into the endocytic machinery (**Table 1**). Expression of DN and constitutively active mutants against endogenous Rab5, a small GTPase critical for the trafficking and maturation of EEs, blocks infection by many arbo-bunyaviruses, including UUKV, CCHFV, and LACV [26,175,185,186]. Furthermore, confocal microscopy shows that UUKV enters Rab5-positive EEs while OROV and CCHFV transit through EEA1-positive EEs [26,174,175].

An accumulation of data indicates that many arbo-bunyaviruses are late-penetrating viruses (L-PVs), a large group of viruses that rely on late endosomal maturation for productive infection [192]. Acid-activated viral membrane fusion for RVFV, UUKV, and CCHFV occurs typically at pH levels below 6.0, which are characteristic of late endosomal vacuoles [26,186,187,192,193]. Acid-activated penetration after internalization of RVFV and UUKV takes place within 20-40 min, a timing compatible with that of late endosome (LE) maturation [26,187]. Microtubules are required for productive infection by CCHFV and UUKV, suggesting the involvement of LE mobility in virus entry [26,194]. Furthermore, OROV was seen in endosomal vacuoles positive for Rab7, the most critical small GTPase for the function of LEs [174,195].

LE formation and cargo transport to lysosomes is inhibited when Rab7 is perturbed. However, while some arbo-bunyaviruses can be confidently considered L-PVs, Rab7 does not seem to be required for their penetration. The expression of a Rab7 DN mutant does not impair infection by CCHFV [175,186]. Although live-cell imaging and confocal microscopy clearly demonstrates the presence of UUKV particles in Rab7- or LAMP1-positive endosomes and lysosomes, the Rab7 DN mutant T22N has no significant effect on UUKV infection [26]. In contrast, the expression of the constitutively active form of Rab7 results in an increase in UUKV infection [26]. Rab7 appears to be also unessential for infection by LACV, though little information is available on intracellular trafficking for this virus [185]. There could be many reasons for Rab7 being dispensable, such as the presence of multiple isoforms of Rab7, the DN mutants' lack of effect, mislocalization of the mutant Rabs, etc. An alternative explanation would be that viruses simply escape the degradative branch of the endocytic machinery earlier, e.g. during sorting from the EE platform to the nascent multivesicular bodies (MVBs), the first intermediates in LE maturation (**Figure 5**). That Rab7 is less present on LEs during the early stages of maturation may explain why Rab7 mutants do not affect infection [190,191]. This seems to be the case for CCHFV, which has been recently shown to penetrate cells from MVBs [175]. Interestingly unrelated L-PVs, such as Lymphocytic choriomeningitis and Lassa viruses, two arenaviruses, do not rely on Rab5 and Rab7 to reach late endosomal compartments [196,197]. Therefore, the interpretation of the effects of Rab7 perturbations is not always entirely straightforward and should be regarded with caution.

As the final step of the entry process, bunyaviruses make use of membrane fusion to transfer their genome and accessory proteins into the cytosol [26]. The viral glycoproteins G_N and G_C undergo important conformational changes upon acidification [23,187]. The structural similarities between the RVFV G_C protein and class II fusion glycoproteins probably indicate that fusion mechanisms resemble those determined for other arboviruses involving class II fusion proteins such as the flavivirus E and alphavirus E1 proteins [3,198]. However, the details of the fusion mechanisms allowing bunyavirus penetration remain not well characterized [3,198]. Once the virus is uncoated and the viral core gains access to the cytosol, the replication begins. The cell and then the host are infected.

Conclusion

Arbo-bunyaviruses and other arboviruses constitute a large group of viruses that share dependence on arthropod vectors for transmission. In this review, we have summarized current knowledge of the initial infection by arbo-bunyaviruses in humans and other mammals, from virus introduction into the skin to entry into the first cells. While arbo-bunyavirus transmission and spreading are complex processes not yet thoroughly understood, it is already clear that hundreds of cellular factors with a wide range of functions are involved in the transmission and cell entry program of these viruses. Progress in this area can be driven by results from siRNA-based high throughput screens that help to identify host cell proteins and functions critical for infection, such as those recently published for RVFV and UUKV [27,199].

In part, progress will also require detailed cell biological analysis of the infection process, and of the whole pathway in different types of cells and hosts. However, single inhibitors cannot accurately define a cellular pathway, and perturbants have many side effects or simply impair different processes in cells. It will be possible to determine specific endocytic mechanisms only by a combination of well-defined inhibitor profiles, preferentially through various independent approaches. This will not happen without the development of quantitative and qualitative assays allowing for the monitoring and analysis of the very first minutes of infection, i.e. from virus attachment to internalization and fusion. All the cell factors and mechanisms identified can potentially be used as targets to block the initial steps of transmission and the subsequent early virus-host cell interactions. The information gained from studies on arbo-bunyaviruses and other arboviruses may, on

638 the other hand, provide valuable information about hard-to-investigate, basic cellular and
639 molecular mechanisms that control the immune response in hosts as well as various
640 endocytic processes.

641 Future perspective

642 The increasing number of outbreaks underscores the importance of understanding the
643 cellular mechanisms involved in arbo-bunyavirus transmission and infection. Much further
644 work is needed in the characterization of particles derived from arthropods in order to
645 understand the very first stages of transmission to human and other mammalian hosts, and
646 in order to find a novel means to control bunyaviral diseases. In this respect, tick-derived
647 viruses remain insufficiently characterized given the central role played by tick vectors in
648 emerging diseases. One can easily anticipate that the tick cell biology of viruses is going
649 to be an ever-growing field of inquiry. While it is clear that arbo-bunyaviruses use many
650 receptors to target and infect a large panel of tissues, only a few receptors have been
651 documented in humans, and not a single one in the arthropod vectors. The quest to identify
652 arbo-bunyavirus receptors is the key to broadening our knowledge of viral dissemination
653 and tissue tropism.

654 At the molecular level, many aspects of the cell biology of bunyavirus entry await further
655 investigation. The uptake mechanisms must be clarified, or simply uncovered, for most
656 bunyaviruses. Beyond entry into EEs, progression of particles into the endosomal network
657 remains to be further defined. Virus fusion and uncoating are evident areas of possible
658 investigation for future research and the X-ray structure recently published for the
659 glycoprotein G_C of RVFV is a crucial first step. Ultimately, all of these approaches need to
660 be ascertained under a relevant physiological context. The complete picture of arbo-
661 bunyavirus transmission, entry, and spread will not be achieved without *in vivo* approaches.
662 In this regard, live animal imaging represents the future of studies. Multiphoton
663 microscopy and other state-of-the-art microscopy techniques will shed new light on very
664 early interactions between arbo-bunyaviruses and hosts.

Executive summary

A majority of bunyaviruses belongs to the super group of arboviruses (arthropod-borne viruses)

- Arbo-bunyaviruses infect a large spectrum of hosts, including plants, invertebrates, humans, and other vertebrates.
- Arbo-bunyaviruses are mainly transmitted by mosquitoes, flies, and ticks.
- Due to their mode of transmission and the increasing number of outbreaks, arbo-bunyaviruses are considered as emerging agents of diseases.

Arthropod-to-mammal host switch

- Host-switch is a prominent part of the arbo-bunyavirus life cycle.
- The cell biology in mammalian hosts is different from that in arthropod vectors, the consequence being that arbovirus particles can change some components and the composition of their lipid and glycan coats during host switch.
- Arbo-bunyaviruses are introduced into the human skin through the saliva of infected arthropods during a blood meal.
- Arthropod saliva has major effects on arbo-bunyavirus infection.

First-target cells in mammalian hosts

- Arboviruses are believed to target resident dendritic cells in the skin dermis, the anatomical site of virus transmission, for the initial infection and the later spread throughout the host. However one cannot exclude that arbo-bunyaviruses use alternative strategies for dissemination within hosts.
- It is paramount to recapitulate arthropod-to-mammal host switch in experimental approaches. The initial infection of mammalian cells by arbo-bunyaviruses in the host skin should not be addressed outside the context of the physiological conditions of arthropod-to-mammal conditions.

Cell biology of bunyavirus entry

- Only a few receptors have been documented for arbo-bunyaviruses (non-muscle myosin heavy chain IIA, surface nucleolin, DC-SIGN, and L-SIGN).

- Arbo-bunyaviruses penetrate host cells by endocytosis and acid-activated membrane fusion.
- Clathrin-mediated endocytosis appears to be used by a majority of arbo-bunyaviruses to enter cells. However, the uptake mechanisms remain to be uncovered for most bunyaviruses and clarified for many others.
- While it is clear that several arbo-bunyaviruses present the features of late-penetrating viruses, a group of viruses that share dependence on late endosomal maturation for productive infection, the details of the intracellular trafficking and uncoating are still missing for many of them.

Future perspective

- Most of the findings regarding arbo-bunyavirus transmission and infectious entry need to be ascertained *in vivo*, under more relevant physiological conditions. Live animal imaging represents the future in this area of research and should shed new light on the early interactions between arbo-bunyaviruses and their hosts.

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● of interest

●● of considerable interest

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This study and Ref. [27] report the first genome-wide siRNA screens in drosophilia and human cells and identify hundreds of new cellular factors involved in infection by RVFV and UUKV.

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Figure and table legends

Figure 1 – The *Bunyaviridae* family.

The *Bunyaviridae* family comprises five genera with more than 350 identified isolates worldwide. These viruses are mainly transmitted to vertebrate hosts, including humans, by arthropod vectors.

Abbreviations: ANDV, Andès virus; BUNV, Bunyamwera virus; CCHFV, Crimean Congo hemorrhagic fever virus; DUGV, Dugbe virus; HAZV, Hazara virus; HTNV, Hantaan virus; INSV, Impatiens necrotic spot virus; LACV, La Crosse virus; NSDV, Nairobi sheep disease virus; OROV, Oropouche virus; PTV, Punta Toro virus; PUUV, Puumula virus; RVFV, Rift Valley fever virus; SBV, Schmallenberg virus; SFTSV, Severe fever with thrombocytopenia virus; TOSV, Toscana virus ; TSWV, Tomato spotted wilt virus; UUKV Uukuniemi virus

Figure 2 – Bunyavirus genome and particles.

A. Schematic representation of the genome of arthropod-borne bunyaviruses. The arrows indicate the open reading frames. **B.** Schematic representation of a generic bunyavirus particle. **C.** The electron microscopy picture shows the phlebovirus Uukuniemi (kind gift of Dr. Radosav Pantelic, Laboratory of Henning Stahlberg, University of Basel, Switzerland, 2010).

Abbreviations: CCHFV, Crimean Congo hemorrhagic fever virus; RVFV, Rift Valley fever virus; UUKV Uukuniemi virus

Figure 3 – Arbo-bunyavirus life cycle and host-switch.

The life cycle is depicted here for a generic arthropod-borne bunyavirus, which includes the transmission cycle between, and within, non-vertebrate and vertebrate host populations, and the productive cell cycle in arthropod vectors and mammalian hosts. Arthropod cell-derived viruses appear in shades of blue and those originating from mammalian cells in shades of red.

Figure 4 – Delivery of arboviral particles into human skin.

During natural transmission, arboviruses are introduced into the human skin dermis through the saliva of infected arthropods. DC and NK stand for dendritic cells and natural killer, respectively.

Figure 5 – Arbo-bunyaviruses in the endocytic machinery.

The figure shows an overview of the degradative branch of the endocytic machinery. The gray dash lines indicate the location for the penetration of some bunyavirus members (LACV, OROV, RVFV, and UUKV). On the left, the scale indicates the time required for a cargo to traffic from the plasma membrane to an organelle and the pH inside the vacuoles (pH).

Abbreviations: CCHFV, Crimean Congo hemorrhagic fever virus; EEA1, early endosome antigen 1; EL, endolysosome; ER, endoplasmic reticulum; ESCRT, endosomal sorting complex required for transport; ILV, intraluminal vesicle; LACV, La Crosse virus; MVB, multivesicular body; Lamp1, lysosomal-associated membrane protein 1; LY, lysosome; OROV, Oropouche virus; Rab, Ras-related in brain; RE, recycling endosome; RVFV, Rift Valley fever virus; TAHV, Tahnya virus; UUKV Uukuniemi virus; * Jamestown, Inkoo, California encephalitis, Serra do Navio, Melao, Keystone, Trivittatus, and Snowshoe Hare viruses.

Table 1 – Cell factors and processes important for arbo-bunyavirus infectious entry.

Table 1 – Cellular factors and processes important for bunyavirus infectious entry

	Virus	Receptor (s)	Acid-activated penetration	Penetration (t1/2 min)	Cellular factors and processes		Ref
					Involved	Not involved	
Orthobunyavirus	AKAV		Yes^{WT}		Clathrin$^{WT, c}$, Dynamin 2^{WT}, Cholesterol^{WT}, vATPases^{WT}	Caveolin-1^{WT}	[176]
	BUNV		Yes^{ccf}				[96]
	GERV	DC-SIGN^{WT}					[25]
	LACV	DC-SIGN**	Yes^{ccf} (pH ~6.0-6.2 for fusion)^{ccf}		Clathrin^{WT}, Dynamin 2^{WT}, Eps15^{WT}, Rab5^{WT}, Serine protease**	Actin^{WT}, Caveolin-1^{WT}, Cholesterol^{WT}, L-SIGN**, Na^+/H^+ antiporters^{WT}, Rab7^{WT}	[94,95,97, 98,114,185]
	OROV		Yes^{WT}		Clathrin$^{WT, \$}$, Cholesterol^{WT}, Rab7$^{WT, c}$, vATPases^{WT}	Caveolin-1^{WT}, EEA1$^{WT, c}$	[174]
	TAHV		Yes^{ccf}		Clathrin^{WT}, Dynamin 2^{WT}		[94,185]
	Others*				Clathrin^{WT}, Dynamin 2^{WT}		[185]
Nairovirus	CCHFV	Nucleolin^{WT}	Yes$^{WT, \#}$ (pH ~5.5-6.0 for penetration)^{WT}		Alix/Aip$^{\#, c}$, AP2^{WT}, CD63$^{WT, c}$, Cholesterol$^{WT, \#}$, Dynamin 2$^{\#}$, Clathrin$^{WT, \#}$, EEA1 $^{WT, c}$, ESCRT$^{\S, \#}$, Microtubules^{WT}, PI3K$^{\#}$, Rab5$^{WT, \#}$, vATPases$^{WT, \#}$	Caveolin-1^{WT}, LAMP1^{WT}, Na^+/H^+ antiporters$^{\#}$, Rab7^{WT}	[170, 173, 175,186, 194]
Phlebovirus	PTV	DC-SIGN^{WT}					[25]
	RVFV	Heparan sulfate***, DC-SIGN$^{\P, **}$	Yes$^{***, \&}$ (pH ~5.7 for penetration)***	16-24***	Actin$^{***, \&}$, Ca^{2+} and K^{2+} channels$^{\&}$, Caveolin-1$^{\&}$, Cholesterol$^{\&}$, Clathrin***, Dynamin 2$^{***, \&}$, Microtubules$^{\&}$, Na^+/H^+ antiporters$^{\&}$, PI3K$^{\&}$, PKC$^{\&}$, vATPases$^{\#, ***, \&}$	Actin$^{\&}$, Cholesterol***, Clathrin$^{\&}$, L-SIGN**, Eps15$^{\&}$, Na^+/H^+ antiporters$^{\&}$, PAK-1$^{\&}$, PI3K$^{\&}$, Rac-1$^{\&}$	[25,114, 167,187, 188,189]
	SFTSV	NMMHCIIA^{WT}, DC-SIGN**, L-SIGN**	Yes**		Dynamin 2**, Serine protease**, vATPases**	Cathepsin B and L**, PI3K**	[114,169]
	TOSV	Heparan sulfate^{WT}, DC-SIGN^{WT}					[25,168]
	UUKV	DC-SIGN^{WT}	Yes^{WT} (pH ~5.4 for penetration)^{WT}	10-15	Clathrin$^{WT, @}$, LAMP1$^{WT, c}$, Microtubules^{WT}, PI3K^{WT}, Proteasome^{WT}, Rab5^{WT}, Temperature^{WT}, VAMP3^{WT}, vATPases^{WT}	Rab7^{WT}	[25,27]

The red lettering indicates the cellular factors that have been shown to be both important and dispensable for RVFV entry.

***Abbreviations*:** AKAV, Akabane virus; BUNV, Bunyamwera virus; CCHFV, Crimean Congo hemorrhagic fever virus; GERV, Germiston virus; LACV, La Crosse virus; NMMHCIIA, Non-muscle myosin heavy chain IIA; OROV, Oropouche virus; PTV, Punta Toro virus; RVFV, Rift Valley fever virus; SFTSV, Severe fever with thrombocytopenia syndrome virus; TAHV, Tahnya virus; TOSV, Toscana virus; UUKV, Uukuniemi virus.

Virus model: * Jamestown, Inkoo, California encephalitis, Serra do Navio, Melao, Keystone, Trivittatus, and Snowshoe Hare viruses; ** Rhabdoviral particles pseudotyped with the glycoproteins G_N and G_C; *** Genetically modified, non-spreading RVFV; ¶ RVFV ZH548 strain; $^{\&}$ RVFV MP12; $^{\#}$ Genetically modified CCHFV to express the red fluorescent protein mKate2; ^{WT} Wild type or parental virus strain.

^{ccf} cell-cell fusion assay; § Tsg101, Vps24, and Vps4B; ^{c} Confocal microscopy-based analysis of colocalization events; $^{@}$ Depletion of clathrin has a weak effect on infection (30% <); $^{\$}$ Electron and confocal microscopy pictures also show OROV in a clathrin-coated pit and co-localizing with the clathrin heavy chain, respectively.

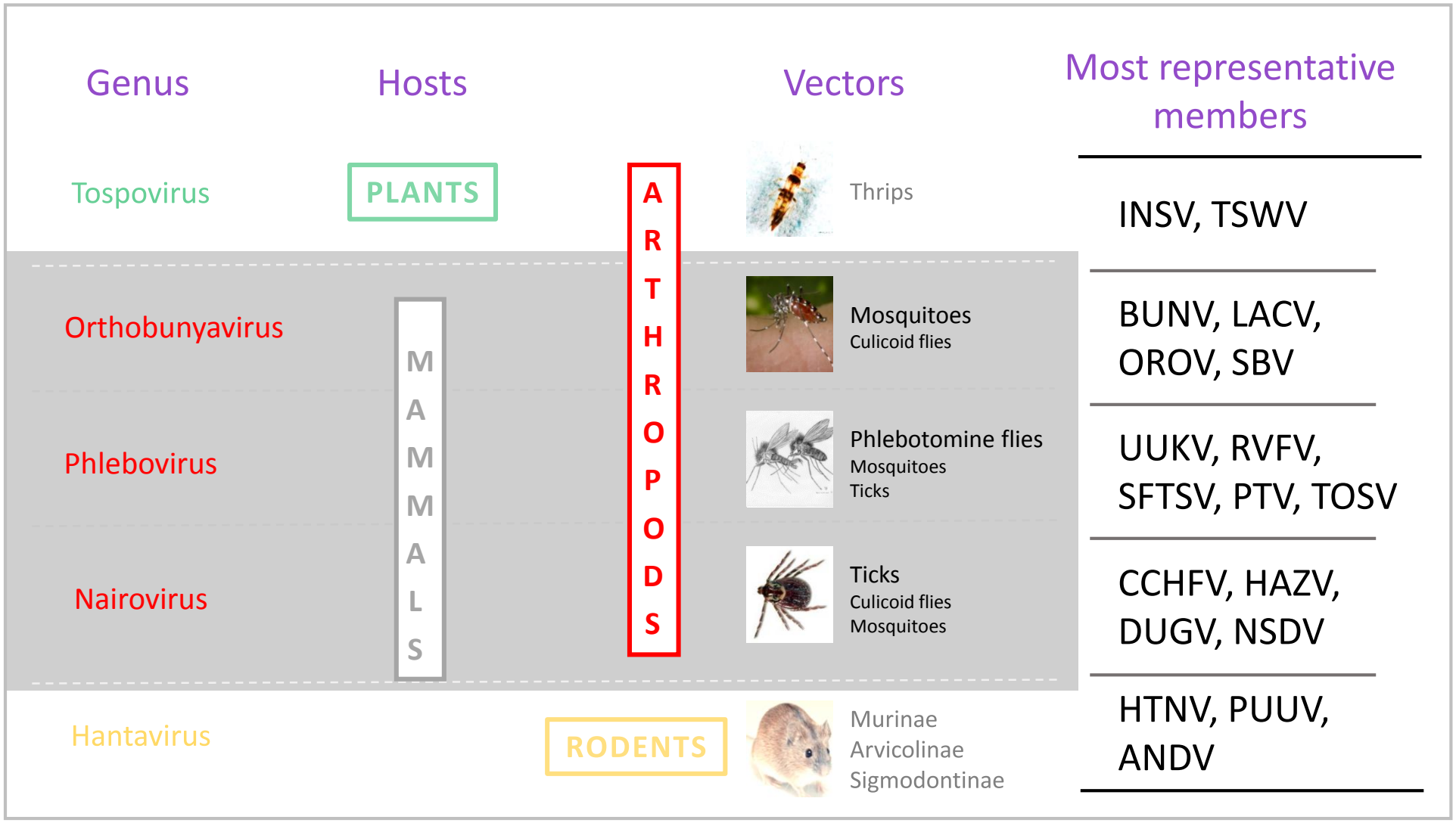
Genus
Hosts
Vectors
Most representative members
Tospovirus
PLANTS
Thrips
INSV, TSWV
Orthobunyavirus
Phlebovirus
Nairovirus
MAMMALS
ARTHROPODS
Mosquitoes
Culicoid flies
BUNV, LACV, OROV, SBV
Phlebotomine flies
Mosquitoes
Ticks
UUKV, RVFV, SFTSV, PTV, TOSV
Ticks
Culicoid flies
Mosquitoes
CCHFV, HAZV, DUGV, NSDV
Hantavirus
RODENTS
Murinae
Arvicolinae
Sigmodontinae
HTNV, PUUV, ANDV

Figure 1

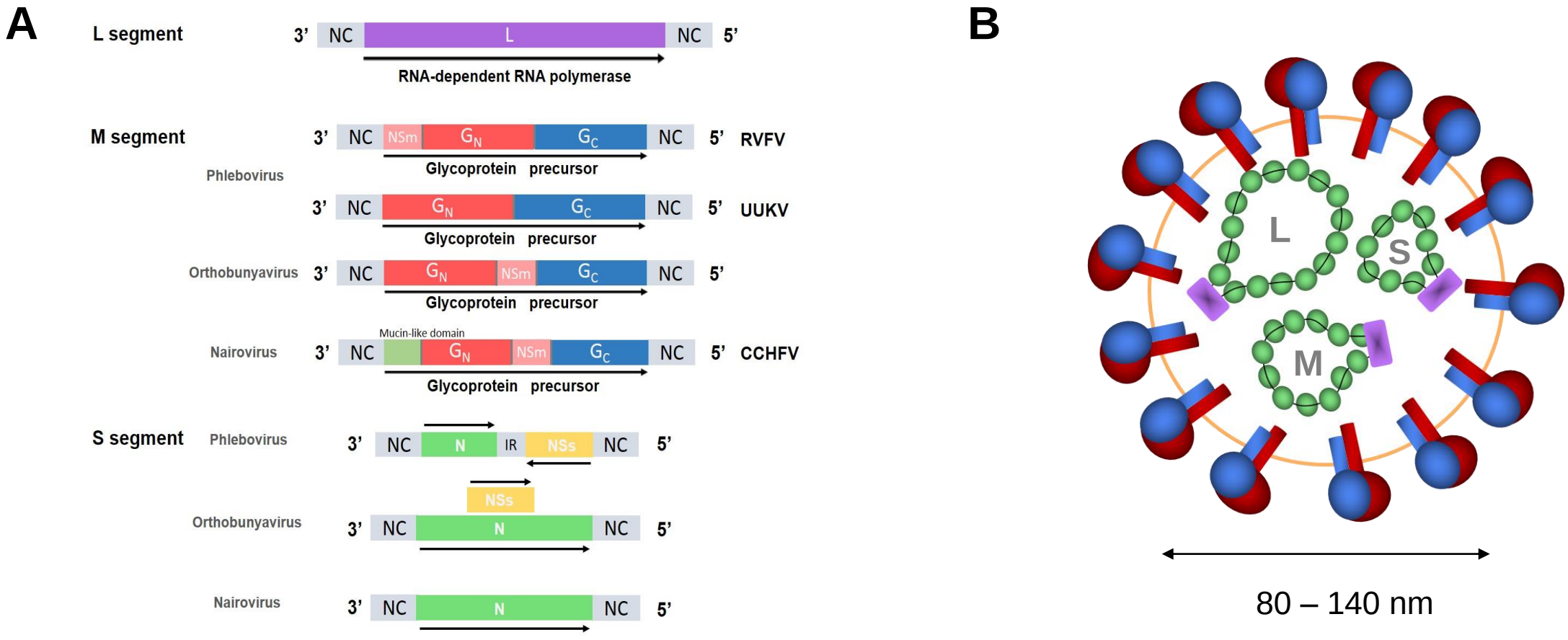
A
L segment
3'
NC
L
NC
5'
RNA-dependent RNA polymerase
M segment
Phlebovirus
NSm
G_N
G_C
RVFV
Glycoprotein precursor
UUKV
Orthobunyavirus
Mucin-like domain
Nairovirus
CCHFV
S segment
N
IR
NSs
B
L
S
M
80 – 140 nm
C
100 nm
UUKV

Figure 2

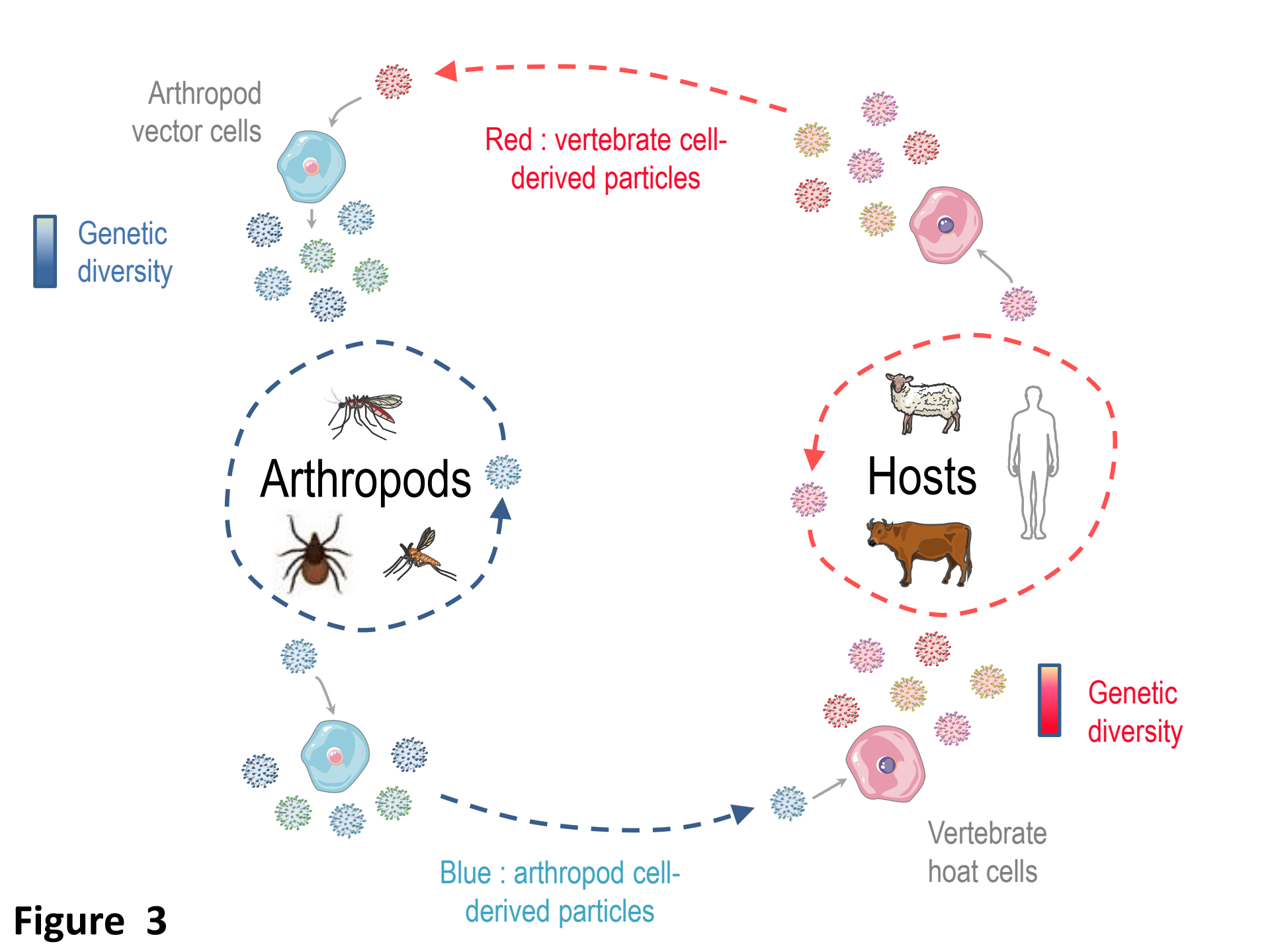
Arthropod vector cells
Red : vertebrate cell-derived particles
Genetic diversity
Arthropods
Hosts
Genetic diversity
Vertebrate hoat cells
Blue : arthropod cell-derived particles

Figure 3

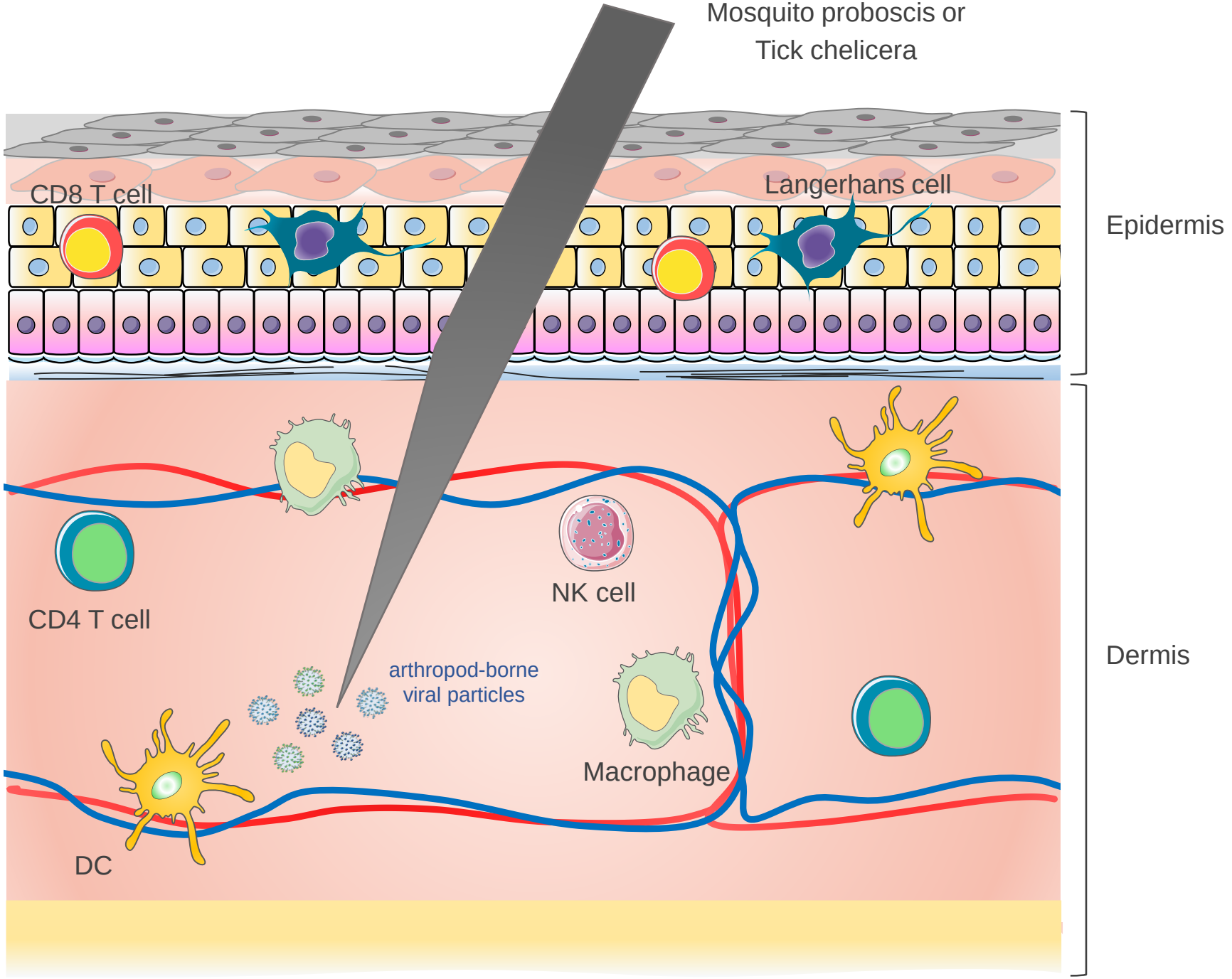
Mosquito proboscis or
Tick chelicera
CD8 T cell
Langerhans cell
Epidermis
CD4 T cell
NK cell
arthropod-borne
viral particles
Macrophage
DC
Dermis

Figure 4

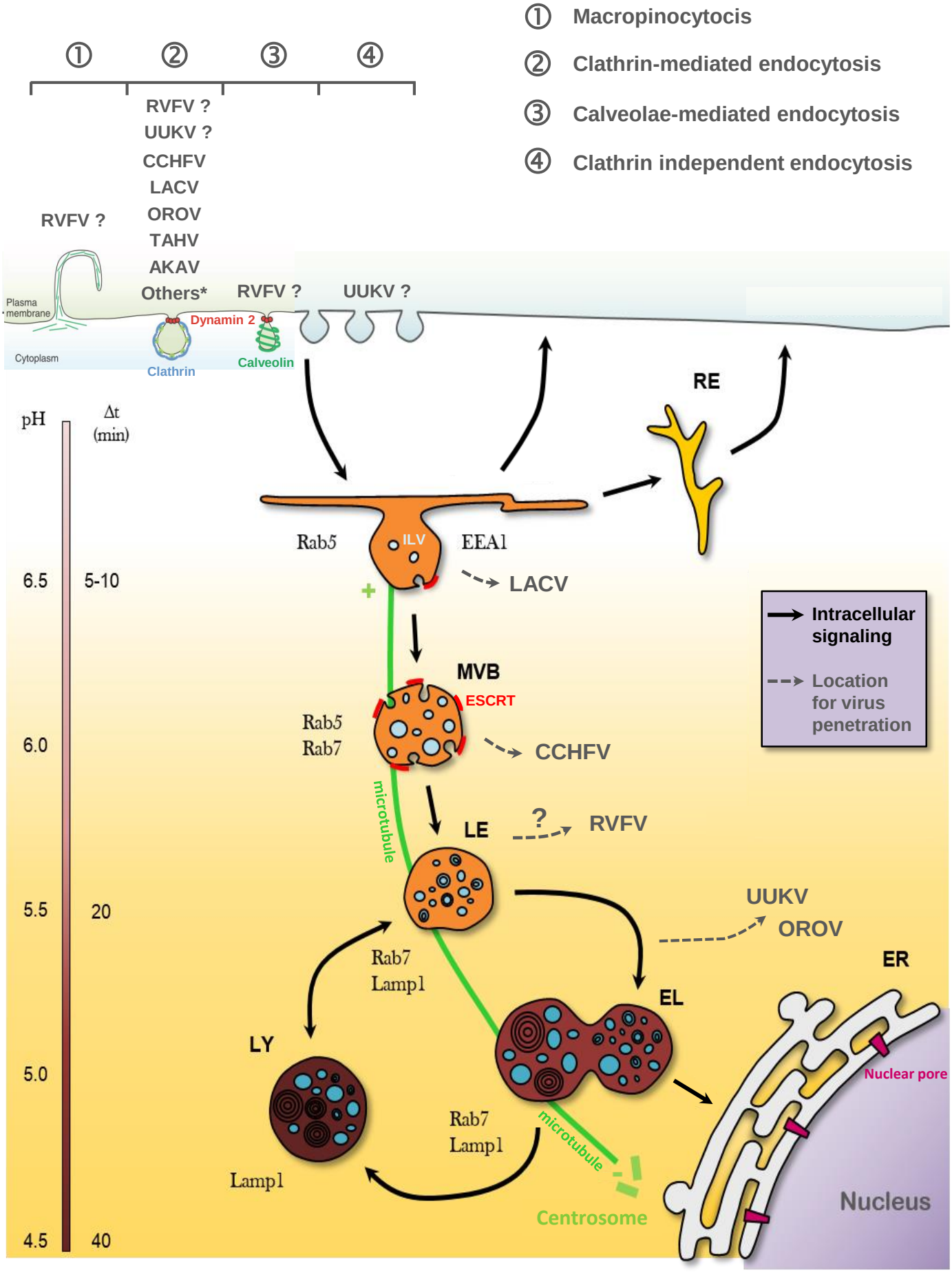
① Macropinocytocis
② Clathrin-mediated endocytosis
③ Calveolae-mediated endocytosis
④ Clathrin independent endocytosis
① ② ③ ④
RVFV ?
UUKV ?
CCHFV
LACV
OROV
TAHV
AKAV
Others*
RVFV ?
Plasma membrane
Cytoplasm
Dynamin 2
Clathrin
Calveolin
RVFV ?
UUKV ?
RE
pH
Δt (min)
6.5
5-10
6.0
5.5
20
5.0
4.5
40
Rab5
ILV
EEA1
LACV
MVB
ESCRT
Rab5
Rab7
CCHFV
microtubule
LE
?
RVFV
UUKV
OROV
Rab7
Lamp1
EL
ER
LY
Nuclear pore
Rab7
Lamp1
microtubule
Lamp1
Centrosome
Nucleus
Intracellular signaling
Location for virus penetration

Figure 5