An introduction to Rift Valley fever virus

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i. Abstract

Rift Valley fever virus (RVFV) is a pathogen transmitted to humans and livestock via mosquito bites. This virus, which was discovered in Kenya in 1930, is considered by the World Health Organization (WHO) and the World Organization for Animal Health (WOAH) to be associated with a high risk of causing large-scale epidemics. However, means dedicated to fighting RVFV have been limited, and despite recent research efforts, the virus remains poorly understood at both the molecular and cellular levels as well as at a broader scale of research in the field and in animal and human populations. In this introductory chapter of a methods book, we aim to provide readers with a concise overview of RVFV, from its ecology and transmission to the structural and genomic organization of virions and its life cycle in host cells.

ii. Key Words

Bunyavirus, *Phenuiviridae*, *Phlebovirus*, Rift Valley fever virus.

1. Biological and health significance

Discovered by Daubney and Hudson in 1930 within the Great Rift Valley in Kenya, Rift Valley fever virus (RVFV) is a pathogen that affects both humans and a wide range of animals, including both farmed and wild species [1-3]. In animals, particularly young ruminants, the infection manifests as fetal malformations, leading to a high rate of abortion and mortality [2]. In humans, the infection can progress to severe forms, resulting in long-lasting conditions such as retinitis, hepatitis, and even hemorrhagic fevers and encephalitis [4]. Some past epidemics have had mortality rates among hospitalized patients reaching 40% [2].

RVFV is primarily transmitted to animals by mosquitoes of the *Aedes* and *Culex* genera during blood meals (**Fig. 1**) [5,6]. Over forty mosquito species have been identified as competent vectors for RVFV and able to transmit the virus [7]. Humans most often contract RVFV through contact with infected body fluids or tissues, rather than through mosquito bites. Since its discovery, RVFV has spread beyond the African continent, reaching Madagascar in the 1970s and, more recently, Saudi Arabia and Turkey [2]. RVFV epidemics have disastrous socioeconomic consequences beyond the human morbidity and mortality [8]. For instance, the 2006 epidemic in Somalia resulted in a loss of agricultural productivity of $471 million, equivalent to 5% of the country's GDP [8].

Due to human activity and climate change, several mosquito species involved in RVFV transmission have been identified in Europe, and their geographical expansion has continued [9]. In 2017, the World Health Organization (WHO) classified RVFV as one of the most dangerous pathogens with the potential to cause future epidemics. Nevertheless, research and prevention efforts remain limited, and the virus has not been fully characterized. For more information on the epidemiology, clinical manifestations, and socioeconomic impact of this virus, we recommend the review by Wright and colleagues [2].

2. Structural and genomic organization of viral particles

RVFV belongs to the genus *Phlebovirus*, of the family *Phenuiviridae* and order *Bunyavirales* (**Fig. 2**) [10]. The viral particles are enveloped, generally spherical, and heterogeneous in size, with an average diameter of 100 nm (**Fig. 3**) [11]. The particles are composed of four structural proteins [12], including two transmembrane glycoproteins, Gn and Gc, that form protuberances on the surface. The viral genome consists of three single-stranded RNA segments, S, M, and L, corresponding to size; L is the largest; M is intermediate-sized; and S is the smallest. Each segment contains conserved complementary sequences at the 5' and 3' ends, which have the potential to form short double-stranded structures. The terminal sequences serve as binding sites for the viral L polymerase, resulting in a pseudocircularized loop for each segment [13,14]. Covered by nucleoprotein (N), these segments form ribonucleoproteins, often resembling hairpins. RVFV lacks a rigid internal structure, and in this context, N plays a crucial role in protecting the virus's genetic information.

3. Virus entry into host cells

Viruses must enter host cells to initiate infection (**Fig. 4**). Through utilization of OMICS and large screening methodologies for gene silencing and inactivation, a multitude of cellular factors have been identified for their ability to facilitate RVFV entry into host cells and subsequent stages of the viral infection cycle [15-18]. Heparan sulfates likely serve as cell-surface attachment factors promoting RVFV infection [19,20]. The human C-type lectins DC-SIGN and L-SIGN also function as entry receptors for RVFV [21,22]. DC-SIGN is expressed on the surface of dendritic cells located in the skin dermis, the anatomical site of virus transmission. L-SIGN, which is present on the surface of the liver endothelium, may partially explain the hepatic tropism of the virus.

Recently, low-density lipoprotein receptor-related protein 1 (LRP1) was identified as a viral entry factor [23,24]. Given its broad tissue expression profile, LRP1 may contribute to

the extensive tropism of RVFV and to several associated pathologies, such as lethal acute hepatitis [25,26]. Nevertheless, tissues lacking these receptors are still permissive to RVFV infection, suggesting that the virus may utilize other receptors. The apparent multiplicity of receptors likely contributes to the broad tissue tropism of RVFV.

RVFV particles undergo endocytosis following attachment to the cell (**Fig. 4**) [27], and interferon (IFN)-induced transmembrane proteins (IFITMs) may contribute to host restriction [28]. The surrounding acidity of late endosomes triggers multiple conformational changes in Gn and Gc on the surface of viral particles [29,30], which leads to fusion of the envelope of viral particles with the endosomal membrane, and recent resolution of the crystal structures of Gn and Gc has significantly improved our understanding of viral fusion [31,32]. A lipid-binding pocket in Gc has been proposed to play an important role in this process by recognizing cholesterol in target cell membranes [33]. This final stage of viral entry occurs at approximately 20 minutes after the onset of particle internalization [29]. A pore opens in the endosomal membrane, releasing viral ribonucleoproteins into the cytosol to establish cellular infection.

4. Replication and assembly of viral particles

Several cellular proteins contribute to the infection and replication of RVFV [17,15], and viral RNA replication occurs exclusively in the cytosol. The trisegmented genome of RVFV encodes at least six proteins (**Fig. 5**). The M and L segments, which are of negative polarity, encode a polypeptide precursor and viral RNA-dependent RNA polymerase L (RdRp L), respectively. RdRp L is a large protein exceeding 230 kDa. Its complex structure was recently elucidated by cryo-electron microscopy, revealing three distinct domains: an endonuclease domain, an RdRp domain, and a cap-binding domain [34].

Within the endoplasmic reticulum, the polypeptide precursor undergoes proteolytic cleavage, resulting in the glycoproteins Gn and Gc, the nonstructural protein NSm, and a 78 kDa glycoprotein (GP78). NSm facilitates viral propagation by inhibiting the apoptotic

response triggered by infection [35]; GP78 is believed to play a structural role exclusively in mosquito cells [36]. The S segment encodes the N protein and the nonstructural NSs protein using an ambisense strategy (**Fig. 5**). Replication of RVFV, as well as assembly and release of new particles, involve complex mechanisms that are poorly characterized. Comprehensive reviews provide the most recent knowledge on these subjects [2,13,14].

In brief, the negative polarity of the virus does not permit direct translation of viral genomic RNA segments by the infected cell. Therefore, intermediate messenger RNAs (mRNAs) are required for viral protein synthesis (**Fig. 5**). Viral mRNAs, which are shorter than genomic RNAs, contain a 5' cap followed by a short heterogeneous sequence derived from the host cell but lack a poly(A) tail at their 3' end. The N protein plays a pivotal role in virus replication through its interactions with viral RNAs during viral protein synthesis and in their subsequent embedding into new viral particles [37,38]. The virus buds from the membrane of the Golgi apparatus, whereby the viral particles acquire their lipid envelope and enter the secretion pathway to exit the cell (**Fig. 4**) [37,38]. A resident protein of the Golgi, GBF1, has been implicated in virus assembly and exit [39].

5. Cellular stress responses and immunity

Mosquito vectors, including those for RVFV, predominantly rely on small RNA-mediated interference for antiviral defense [40-42]. In mammalian hosts, acquired immunity to RVFV has not been well documented. However, production of neutralizing antibodies appears to be the primary defense mechanism in all mammalian hosts [43-45]. The surface glycoproteins Gn and Gc are the main targets. Neutralizing antibodies have been reported to block fusogenic Gn-Gc rearrangement by specifically targeting Gn [46]. Dendritic cells and macrophages, which are among the first cells to encounter the virus when it is introduced into the dermis, are susceptible to infection [47,22]. Moreover, rapid proliferation of neutrophils, CD4+ and CD8+

T cells, and B cells is associated with less severe forms of infection in humans and primates [2].

Innate immunity plays a crucial role in preventing Rift Valley fever disease [13]. Early induction of the type I IFN response correlates with viral control in animal models [13]. The three RNA segments of RVFV can be detected in their hairpin conformation by RIG-I, an RNA helicase responsible for activating the IFN response [48]. However, RVFV has developed strategies to delay and evade the IFN response in infected cells, primarily through its nonstructural NSs protein as reviewed in [13,49].

NSs, a small 30 kDa protein, is expressed in infected cells but not incorporated into viral particles. NSs acts as a potent antagonist of the innate immune response. The Clone 13 isolate, a natural strain of RVFV lacking the NSs gene, induces strong type I IFN responses and become highly attenuated in cattle and humans [13,50]. BALB/c mice survive infection with the Clone 13 strain because their IFN system suppresses production and spread of infectious virus [50]. In contrast, mice inoculated with the wild-type virus develop acute hepatitis, which is not fatal but is typically followed by fatal encephalitis [50]. Therefore, NSs is considered the major virulence factor of RVFV in mammals.

The NSs protein is known to form large filamentous structures in the cell nucleus, a hallmark of RVFV infection (**Fig. 6A**) [51]. Although their involvement in chromosome cohesion and segregation defects has been reported [52], the ultrastructure and function of these filaments remained poorly characterized until recently. An X-ray study proposed a structure for the core domain of the protein [53], approximately half of the protein, and more recent findings have revealed that the filamentous structures formed by NSs are assemblies of multiple fine, roughly parallel amyloid-like fibrils (**Fig. 6B** and **6C**) [54,55]. Formation of these fibrils appears to be critical to the ability of NSs to silence host cell innate responses [54].

6. Prevention, diagnosis, and research: multiple needs remain

The COVID-19 pandemic is a dramatic reminder of the problems that can be caused by viral zoonoses worldwide. Furthermore, the rapid development of vaccines and antivirals against SARS-CoV-2 was only possible because the structural and functional biology of other coronaviruses was already known. This knowledge is lacking for other emerging viruses, including RVFV. An illustration of this phenomenon is the super group of arthropod-borne viruses (arboviruses), to which RVFV belongs and spread of which has accelerated due to climate change.

Our understanding of some arboviruses, *e.g.*, flaviviruses and alphaviruses, is somewhat similar to that of coronaviruses. However, arboviruses such as RVFV and other phenuiviruses in the *Bunyavirale*s order have been largely neglected [27], yet these viruses have high potential for emergence in humans and livestock. Recent examples include outbreaks of Schmallenberg virus in western Europe, Toscana virus in the Mediterranean Basin, severe fever with thrombocytopenia syndrome virus (SFTSV) throughout Asia, the Heartland virus in the U.S., and RVFV in Africa. Thus, preventive, therapeutic, and research strategies against emerging and re-emerging phenuiviruses need to be established for an integrated response [56].

Substantial technological advancements have been achieved in detection of RVFV [57-59], and these advancements necessitate large-scale application to formulate effective prevention and diagnostic strategies, especially for monitoring the virus in wildlife and livestock populations. Overall, the virus in its arthropod vectors remains poorly characterized. The advent of reverse genetics for RVFV in the 2000s [60-63], based on the pioneering work of Richard Elliott [64], not only enhanced our understanding of viral pathogenesis and the functions of viral proteins but also led to significant progress in development of vaccines against this virus [65,66]. Nevertheless, to date, no RVFV vaccine or treatment has been approved for human use.

Methods and protocols serve as instruments for answering questions, and they are not objectives in themselves. A book of this nature justifies its existence only if there are still unanswered questions that require the attention of an expanding community of researchers. For RVFV, as with most zoonotic infectious agents, the motivation to continue asking questions can originate from various sources. The first and simplest source is personal interest. If you are reading this book, it is likely because you are wrestling with at least one unknown aspect of RVFV biology, a mystery that compels you to develop new tools in your laboratory.

Another compelling reason to engage with this book lies in the broader relevance of resolving biological problems beyond RVFV, with the aim of applying the generated knowledge universally. For instance, viruses are functional cargo carriers elegantly designed and remarkably efficient. In general, study of the transmission and replication of numerous pathogens has improved our understanding of host biology, a feat unachievable by any other means. Furthermore, our understanding of RVFV implies that the virus and its derivatives can be used as tools for investigating other biological mysteries, such as protein aggregation with NSs. A final rationale is to provide methods and protocols for comprehending RVFV and assisting governments and public authorities in their functions and decision-making processes. RVFV can cause illness and death in many humans and animals and poses a serious threat to Europe and Asia. We sincerely hope that the contents of this book will help in answering your questions.

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Figure legends

Figure 1 – Rift Valley fever virus (RVFV) ecology and transmission between hosts. Created with BioRender.com.

Figure 2 – RVFV in the phylogenic tree of the *Bunyavirales* order. The phylogenetic tree was obtained by comparing the amino acid sequences of RNA-dependent RNA polymerase [10].

Figure 3 – RVFV particles. Viral particles are enveloped and coated with heterodimers of the glycoproteins Gn and Gc. Within the viral particles, the trisegmented single-stranded RNA genome is protected by the nucleoprotein N. N, together with RNA-dependent RNA polymerase (RdRp L), assembles into pseudohelical ribonucleoproteins that exhibit a hairpin-like conformation. The three viral genomic RNA segments are named according to their size: S (small), M (medium), and L (large). Created with BioRender.com.

Figure 4 – RVFV life cycle in host cells. Viral particles attach to the cell surface via various receptors, such as heparan sulfates, DC-SIGN, L-SIGN, and LRP1 (1). The particles are then internalized into the cell before entering the cytosol from late endosomes (2). Once the genome and viral material have been released, replication and transcription of genomic RNA begin (3). This is followed by expression of viral proteins (4) and assembly of new particles in the Golgi (5). The new particles reach the cell surface via intracellular vesicles, which, once fused to the plasma membrane, release their contents outside the cell (6). Created in part with BioRender.com.

Figure 5 – RVFV genomic organization. Viral messenger RNAs (mRNAs) are capped 5' and flanked by short heterogeneous host cell-derived sequences (blue dots and lines). The mRNAs transcribed from genomic RNAs are represented by the green lines. Thus, the L segment encodes the RNA-dependent RNA polymerase L gene (RdRp L). The M segment codes for the precursor polypeptide, whose proteolytic cleavage of which results in the nonstructural protein NSm and the two envelope glycoproteins Gn and Gc. Use of an alternative AUG initiation codon in the M segment leads to expression of a second precursor polypeptide that includes a 78 kDa protein and Gc. The S segment encodes the nucleoprotein N and the nonstructural

protein NSs. NSs mRNA (green line) is synthesized from antigenomic RNA (light blue line). In this figure, the black lines symbolize translation into protein.

Figure 6 – RVFV protein NSs. A. Confocal microscopy image of Vero cells infected with RVFV after Hoechst labeling of the nucleus (blue) and immunostaining against the viral proteins N (red) and NSs (green). **B.** Electron micrograph showing large nuclear filaments of NSs. **C.** Image of a nuclear filament, similar to that shown in B, at higher magnification. The large nuclear filaments of NSs are composed of multiple thin fibrils, roughly parallel to each other.

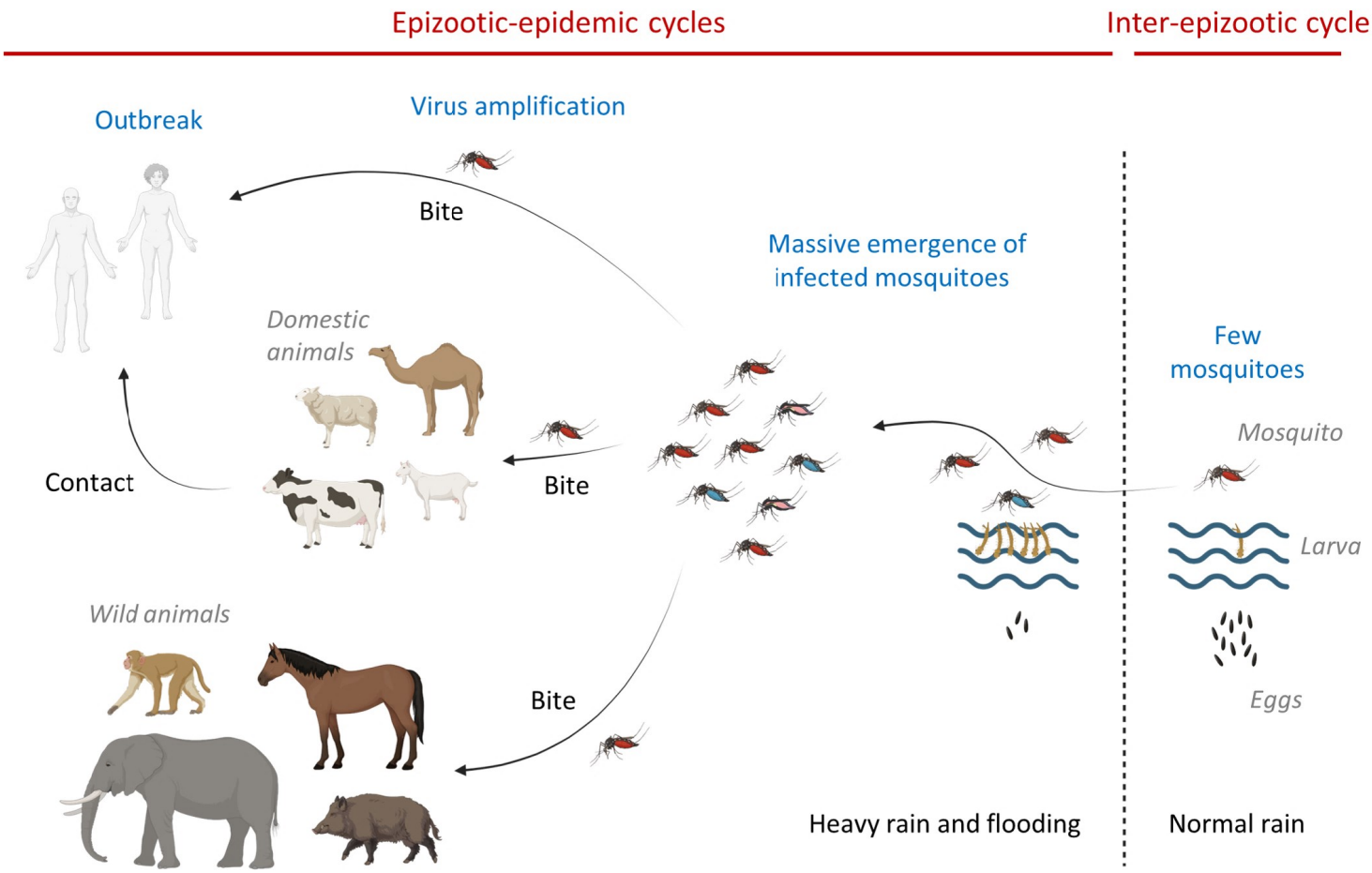
Epizootic-epidemic cycles
Inter-epizootic cycle
Outbreak
Virus amplification
Bite
Massive emergence of infected mosquitoes
Domestic animals
Few mosquitoes
Mosquito
Contact
Bite
Larva
Wild animals
Bite
Eggs
Heavy rain and flooding
Normal rain

Figure 1

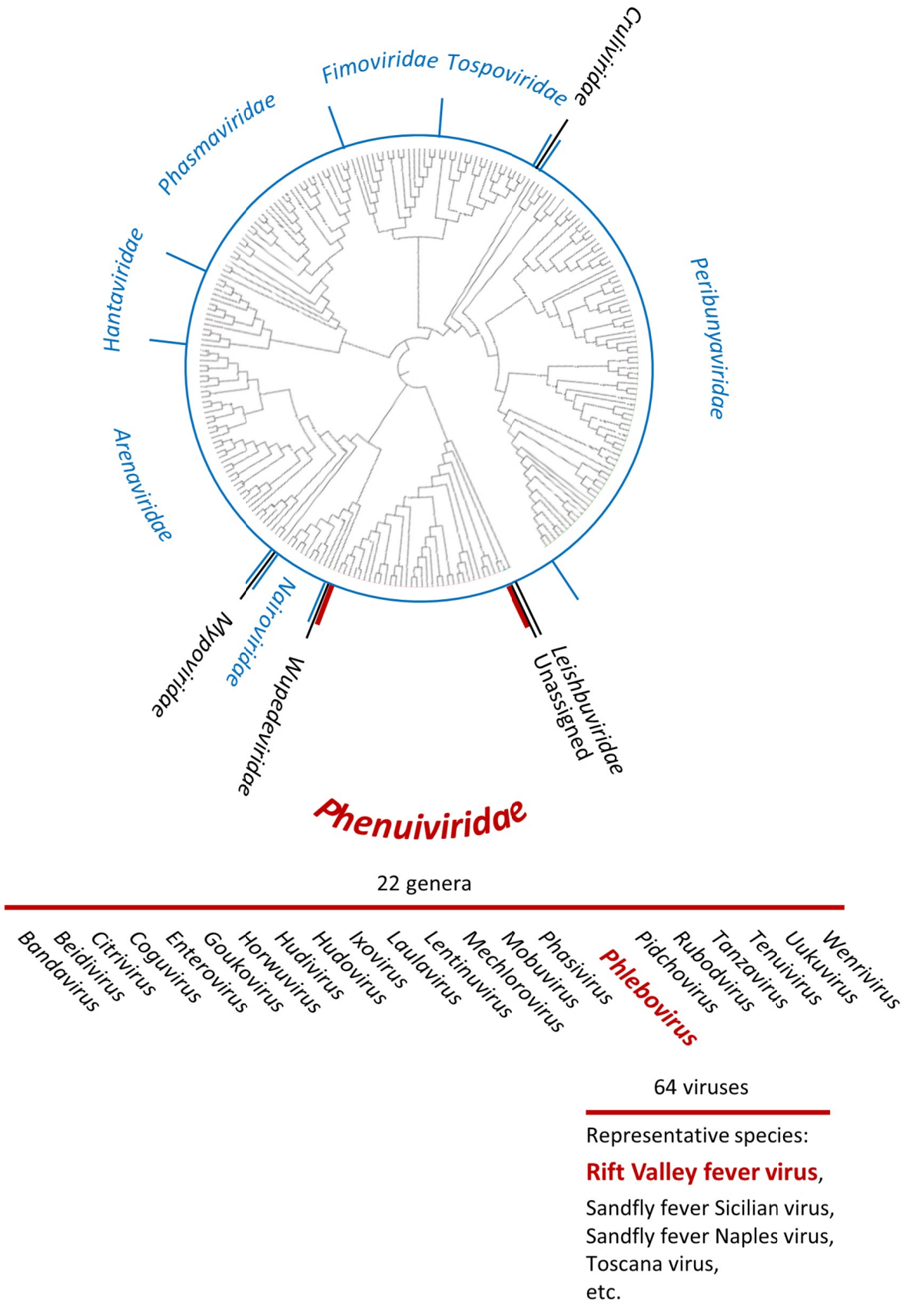
Bunyavirales
Fimoviridae
Tospoviridae
Cruliviridae
Phasmaviridae
Hantaviridae
Peribunyaviridae
Arenaviridae
Nairoviridae
Mypoviridae
Wupedeviridae
Leishbuviridae
Unassigned
Phenuiviridae
22 genera
Bandavirus
Beidivirus
Citrivirus
Coguvirus
Enterovirus
Goukovirus
Horwuvirus
Hudivirus
Hudovirus
Ixovirus
Laulavirus
Lentinuvirus
Mechlorovirus
Mobuvirus
Phasivirus
Phlebovirus
Pidchovirus
Rubodvirus
Tanzavirus
Tenuivirus
Uukuvirus
Wenrivirus
64 viruses
Representative species:
Rift Valley fever virus,
Sandfly fever Sicilian virus,
Sandfly fever Naples virus,
Toscana virus,
etc.

Figure 2

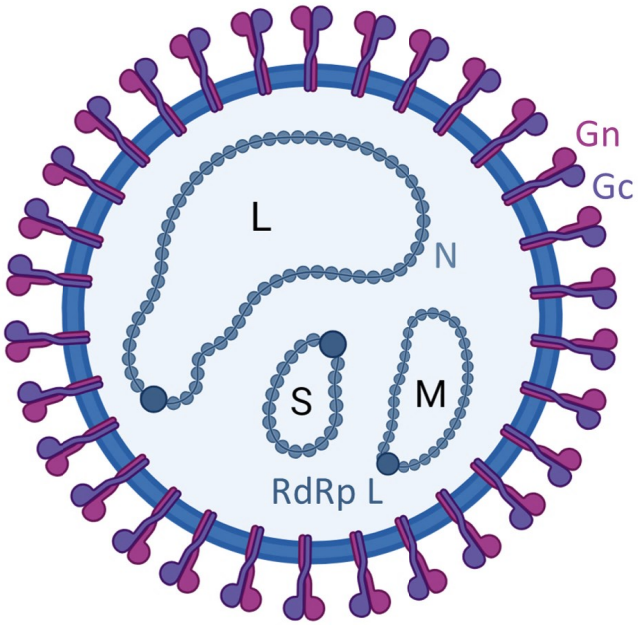

Figure 3

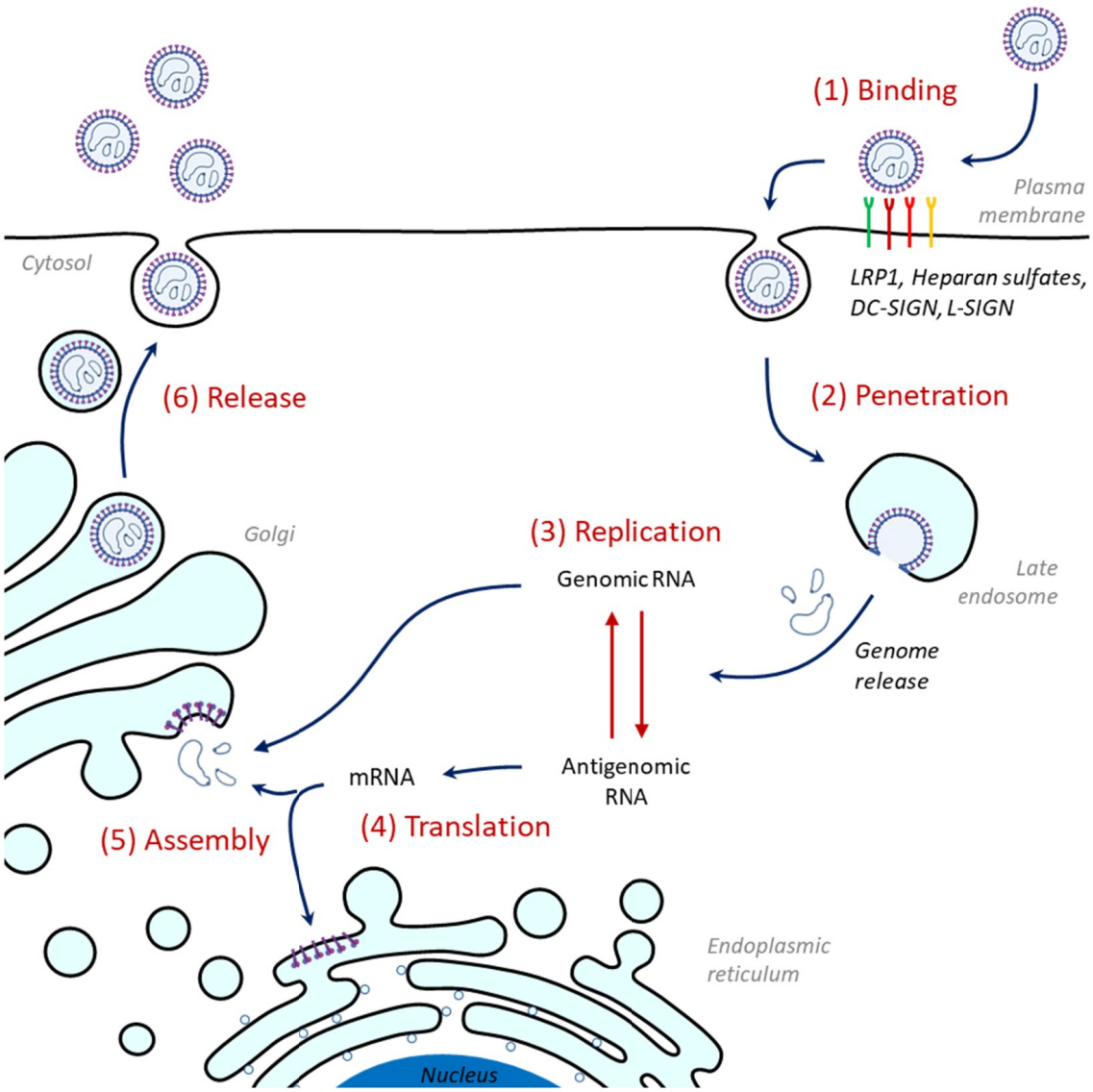
(1) Binding
Plasma membrane
LRP1, Heparan sulfates, DC-SIGN, L-SIGN
Cytosol
(2) Penetration
(6) Release
Golgi
(3) Replication
Genomic RNA
Late endosome
Genome release
mRNA
Antigenomic RNA
(4) Translation
(5) Assembly
Endoplasmic reticulum
Nucleus

Figure 4

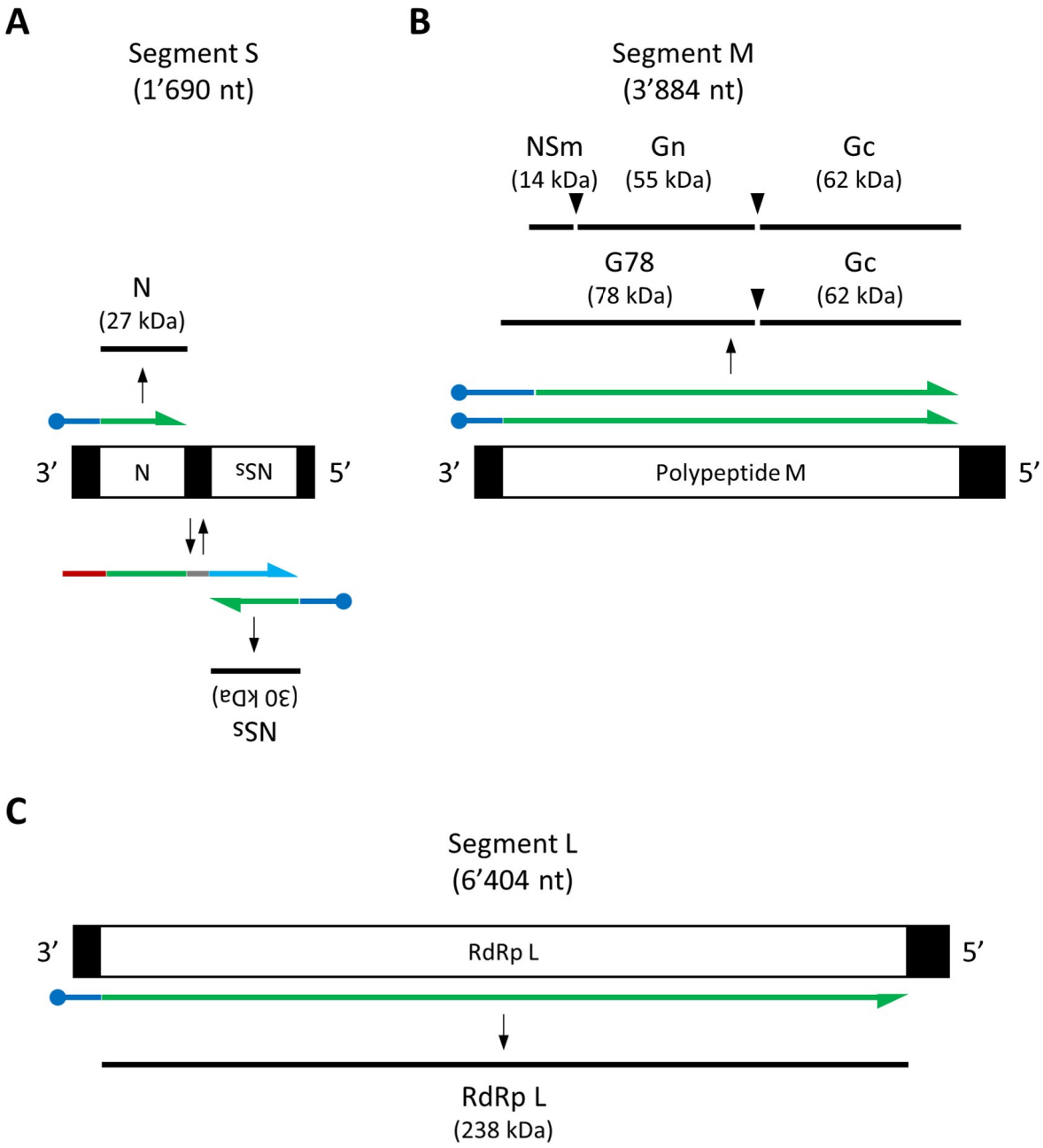
A
Segment S
(1'690 nt)
N
(27 kDa)
3'
N
NSs
5'
(30 kDa)
NSs
B
Segment M
(3'884 nt)
NSm
(14 kDa)
Gn
(55 kDa)
Gc
(62 kDa)
G78
(78 kDa)
Gc
(62 kDa)
3'
Polypeptide M
5'
C
Segment L
(6'404 nt)
3'
RdRp L
5'
RdRp L
(238 kDa)

Figure 5

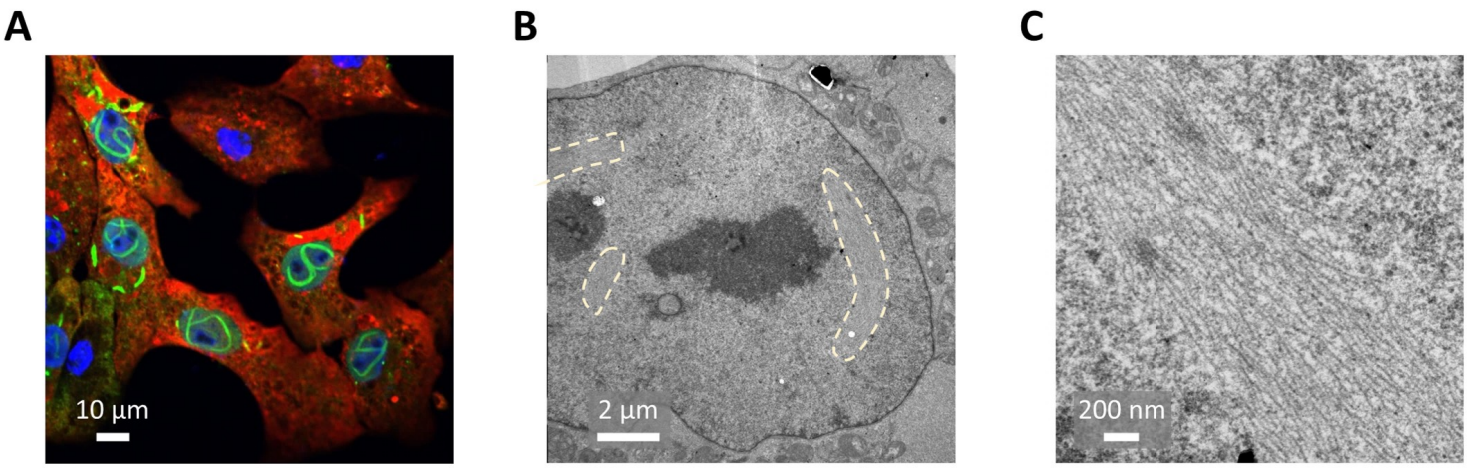

Figure 6