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Future VIROLOGY

Perspective

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Rift Valley fever virus: a new avenue of research on the biological functions of amyloids?

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Rift Valley fever is a mosquito-borne viral zoonosis that was first discovered in the Great Rift Valley, Kenya, in 1930. Rift Valley fever virus (RVFV) primarily infects domestic animals and humans, with clinical outcomes ranging from self-limiting febrile illness to acute hepatitis and encephalitis. The virus left Africa a few decades ago, and there is a risk of introduction into southern Europe and Asia. From this perspective, we introduce RVFV and focus on the capacity of its virulence factor, the nonstructural protein NSs, to form amyloid-like fibrils. Here, we discuss the implications for the NSs biological function, the ability of RVFV to evade innate immunity, and RVFV virulence and neurotoxicity.

Tweetable abstract: The Rift Valley Fever virus protein NSs is an amyloid-like fibril-forming protein. From this perspective, the authors discuss the implications for the NSs biological functions and Rift Valley Fever virus virulence.

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Keywords: amyloid • arbovirus • emerging virus • fibril • NSs • Rift Valley fever • virulence

Rift Valley fever virus

Rift Valley fever virus (RVFV) was discovered by Daubney and Hudson in the Great Rift Valley, Kenya, in 1930 [1]. RVFV primarily infects wild animals and livestock such as goats, cattle, and sheep [2]. In these animals, especially young ruminants, RVFV infection causes fetal malformations, birth defects, and high abortion and mortality rates [2]. Infection is generally asymptomatic or benign in humans. However, 1–2% of infected patients develop severe forms with long-lasting conditions such as retinitis, hepatitis, hemorrhagic fevers, and encephalitis that require hospitalization [2]. In some recent outbreaks, the case fatality rate among hospitalized persons has reached up to 50% [2,3].

RVFV is transmitted to animals by numerous mosquito species, mainly *Aedes* and *Culex*, during their blood meal [4]. Humans most often contract the virus through contact with infected body fluids or tissues rather than through mosquito bites. Since the 1930s, RVFV has spread throughout multiple African countries and it left Africa to reach Madagascar in the 1970s and Saudi Arabia and Turkey more recently [2]. In addition to the direct cost in terms of morbidity and human lives, Rift Valley fever epidemics have significant socioeconomic consequences [5]. For example, the 2006 outbreak cost Somalia $471 million in agricultural productivity and 5% of the country's gross domestic product [5].

As a result of human activity and climate change, several of the mosquito species responsible for Rift Valley fever zoonosis, including *Aedes albopictus*, have been established in Europe and continue to spread and expand to new geographical areas [6]. In 2017, the WHO classified RVFV among the most dangerous pathogens that have a high potential to cause epidemics and pandemics. However, research and prevention efforts are still limited, and the

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virus remains poorly characterized. From this perspective, we will first introduce some general aspects of RVFV at the molecular and cellular levels, including the genomic and structural organization of the virus and the main steps of its cell life cycle. We will then focus on its virulence factor, the nonstructural protein NSs and address the most current knowledge on the astonishing ability of this viral protein to form amyloid-like fibrils and circumvent cellular responses [7]. We will finally discuss the implications for RVFV virulence and the neurotoxicity observed in some infected animals. More information on the epidemiology, clinical manifestations and socioeconomic impact of RVFV can be found in a recent review by Wright and colleagues [2].

RVFV cellular life cycle

RVFV is a member of the genus *Phlebovirus* (family Phenuiviridae, order Bunyavirales) [8]. The viral particles are enveloped, roughly spherical and heterogeneous in size, with an average diameter of 100 nm [8,9]. The viral genome consists of three single-stranded, mainly negative-sense, RNA segments, the names of which refer to their size in nucleic acids, in other words, 'L' for the largest one, 'M' for that with a medium size, and 'S' for the smallest one. Together the three viral genomic segments encode two nonstructural proteins, NSm and NSs, and four structural proteins, namely, the RNA-dependent RNA polymerase L, the nucleoprotein N and the two transmembrane glycoproteins Gn and Gc [10].

Each of the three viral RNA segments has conserved complementary sequences at the 5′ and 3′ ends that anneal into short double-stranded structures to which the viral polymerase L binds [11]. This assembly results in the pseudocircularization of each segment, which typically has a panhandle or hairpin appearance in the viral particles (Figure 1). The nucleoprotein N, together with the viral polymerase associated with pseudohelical viral RNA segments, forms the ribonucleoproteins (RNPs). On the surface of the viral particles, the glycoproteins Gn and Gc constitute protrusions responsible for the attachment of the virus to host cells (Figure 1) [12]. After attachment to the plasma membrane, viral particles are internalized into cells by endocytosis [12–14]. RVFV shares with many other phenuiviruses the dependence on late endosomal maturation and low pH for the infection of target cells [12,14], suggesting that RVFV penetrates the cytosol from late endosomal compartments. After the fusion of the viral and endosomal membranes, viral RNPs are released into the cytosol. The cell is infected, and RVFV replication begins.

RVFV viral replication as well as the assembly and release of new progeny particles relies on complex mechanisms that remain poorly characterized overall. Excellent reviews address the most recent knowledge on these topics [2,11,15]. Briefly, the RVFV trisegmented RNA genome replicates exclusively in the cytosol of infected cells. While the M and L segments have a negative orientation, the S segment codes for the nucleoprotein N and nonstructural protein NSs through an ambisense translation strategy (Figure 2). The negative polarity of the viral genome prevents the translation of viral RNA segments by the host cell machinery. Intermediate messenger RNAs (mRNAs) are therefore necessary for the synthesis of viral proteins (Figure 2). These intermediate mRNAs exhibit a 5′-cap and a short stretch of a heterogeneous sequence, both derived from the host cell, but no poly(A) tail [11]. The nucleoprotein N interacts with viral RNAs while being synthesized. In addition, N favors the incorporation of viral RNA genomic segments into nascent viral particles, which bud in the Golgi network, from which the viral particles acquire their lipidic envelope and enter the secretion pathway to leave the host cell (Figure 1) [16].

RVFV & immunity

Acquired immunity against RVFV remains poorly documented. However, the production of neutralizing antibodies appears as the first defense in all mammalian hosts [2]. The glycoproteins Gn and Gc on the surface of viral particles are the main targets. Neutralizing antibodies seem to block the fusogenic Gn–Gc rearrangement through Gn [17]. Dermal dendritic cells and macrophages are among the first cells to encounter the incoming viral particles, and both type cells are highly sensitive to infection [18,19]. It has also been reported that rapid proliferation of neutrophils, B cells and $CD4^+$ and $CD8^+$ T cells, which together trigger antiviral host defenses, correlates with a less severe disease outcome in both humans and primates [2].

Innate immunity is also a key player in preventing the development of the pathophysiology of Rift Valley fever disease [11,15]. In animal models, the early induction of type I interferon (IFN) response is indeed correlated with control of the virus and its proliferation [11,15]. The hairpin conformation within the three RVFV RNA segments can be detected by RIG-I, an RNA helicase responsible for the activation of the IFN response following infection. RVFV has, however, developed strategies to delay and evade this response of infected cells, mainly with the help of its nonstructural protein NSs [11,15].

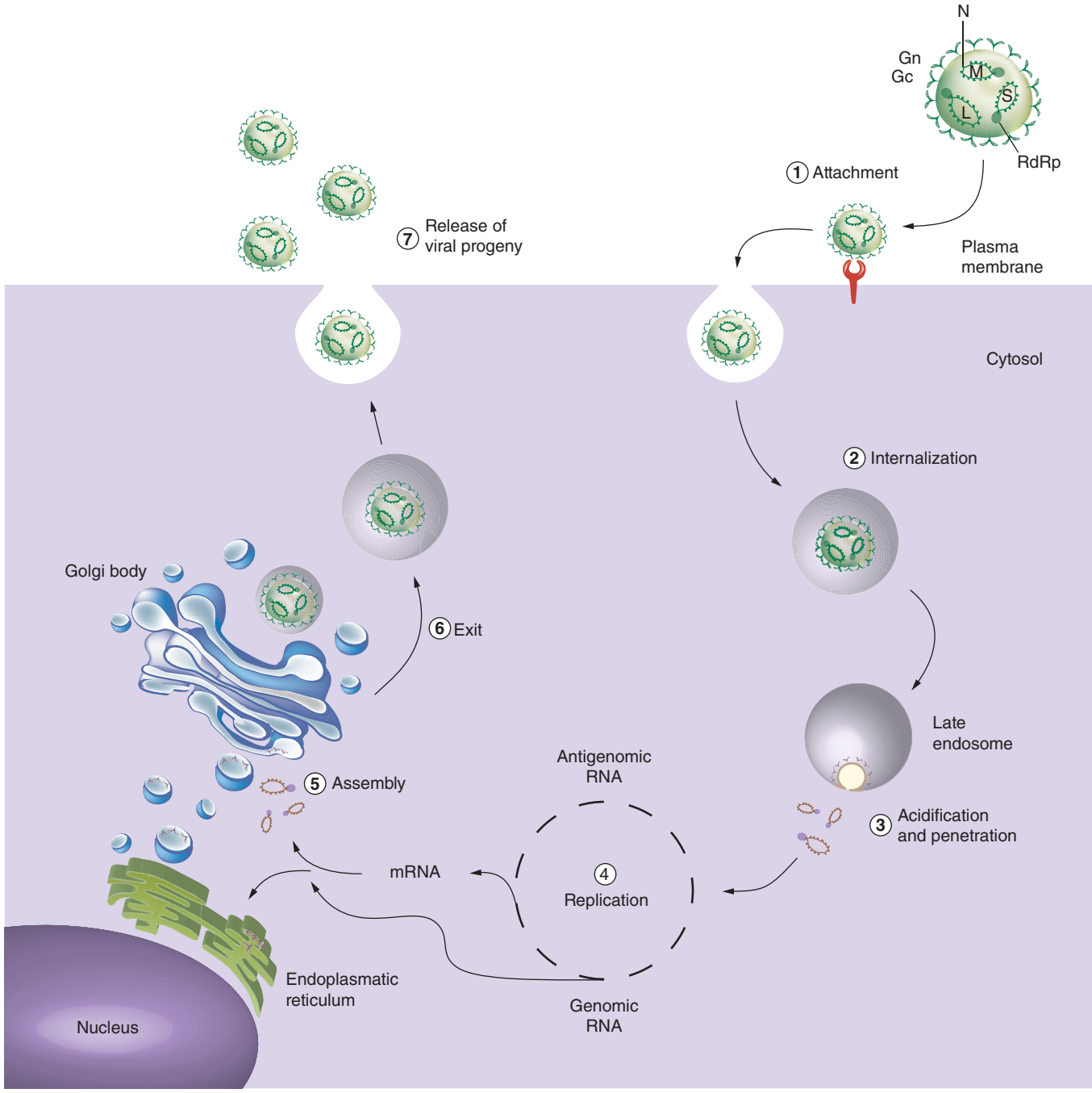


Figure 1. Rift Valley fever virus cell life cycle. (1) Viral particles attach to the cell surface via different receptors, such as heparan sulfates, DC-SIGN and L-SIGN. **(2)** The virus is then sorted into the endocytic machinery. **(3)** The journey of the virus ends in the late endosomal compartments, from which the virus penetrates and releases its genomic material into the cytosol. **(4)** Viral replication and transcription begin and are followed by the synthesis of viral proteins. **(5)** The assembly of viral progenies occurs in the Golgi network. **(6)** The new viral particles then reach the cell surface within intracellular vesicles. **(7)** Once fused with the plasma membrane, the intracellular vesicles release their contents outside the cell.
Gc: Glycoprotein Gc; Gn: Glycoprotein Gn; mRNA: Messenger RNA; N: Nucleoprotein N; RdRp: RNA-dependent RNA polymerase.

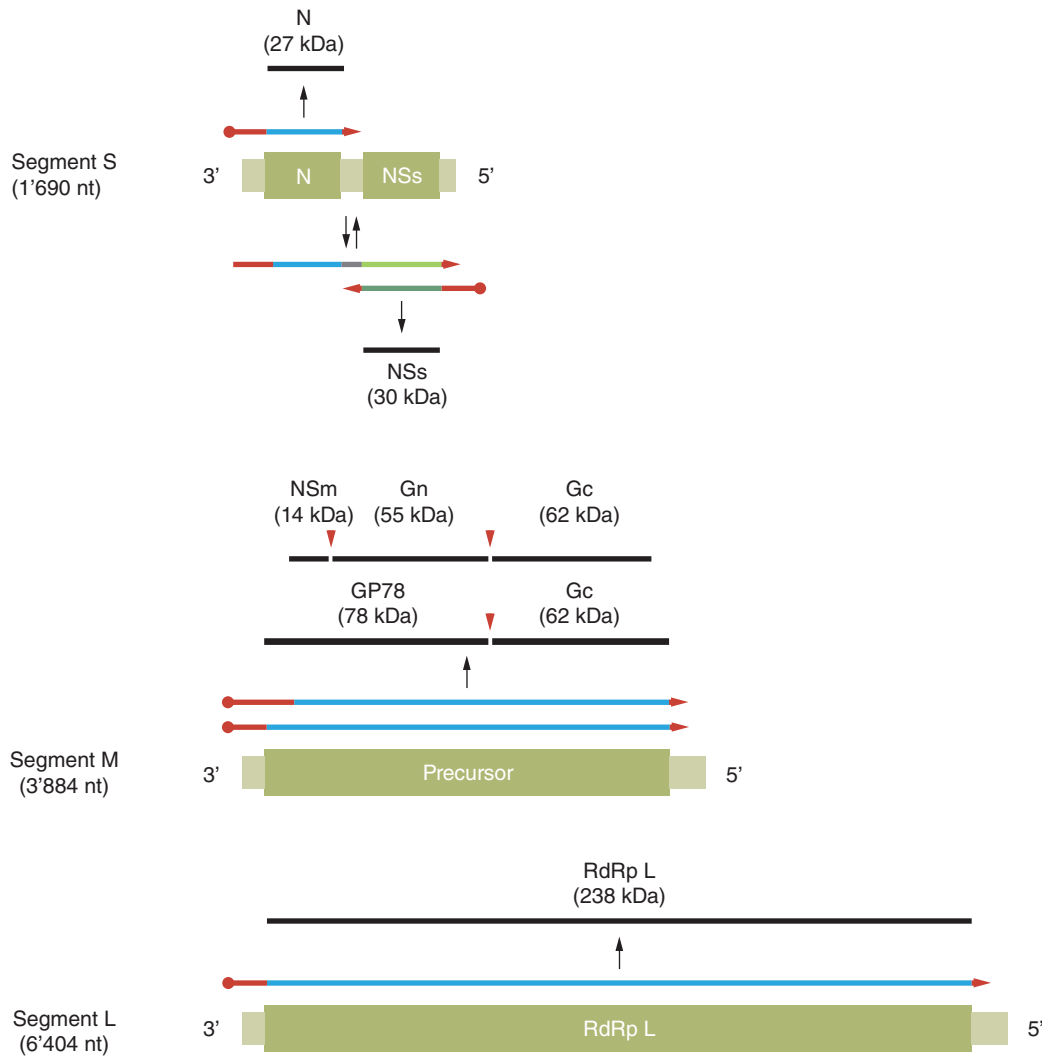


Figure 2. Rift Valley fever virus genomic organization. The viral mRNAs are capped at the 5′ end and flanked by short heterogeneous sequences derived from the host cell (red dots and lines). The virus mRNAs transcribed from genomic RNAs are depicted by the light blue lines. The L segment includes the gene coding for the RNA-dependent RNA polymerase (RdRp) L. The M segment encodes a precursor polypeptide, whose proteolytic cleavage results in the nonstructural protein NSm and the two envelope glycoproteins Gn and Gc. The use of an alternative AUG initiation site in the M segment leads to the expression of a second precursor polypeptide that results in a 78 kDa protein (GP78) and Gc. The S segment codes for the nucleoprotein N and nonstructural protein NSs. The viral mRNA of the nonstructural protein NSs (green line) was synthesized from viral antigenomic RNA (light green line). In this figure, the black lines represent translation into proteins.
Gc: Glycoprotein Gc; Gn: Glycoprotein Gn; N: Nucleoprotein N; NS: Nonstructural protein; NSm: Nonstructural protein m; RdRp L: RNA-dependent RNA polymerase L.

The RVFV nonstructural protein NSs

NSs is a small protein of 30 kDa that is expressed in infected cells but not incorporated into viral particles. In infected cells, NSs has been shown to hamper nuclear transport [20,21]. It has also been reported that the expression of NSs leads to destabilization of the actin cytoskeleton through blockade of the Abl2 protein, an important regulator of the actin network [22]. The result is a dramatic change in the morphology of infected cells and an increase in their mobility. NSs has also been shown to be a remarkably effective antagonist of the innate immune response [11,15,23]. In stark contrast to the wild-type (wt) virus, a natural strain of RVFV lacking most of the NSs sequence, clone 13, was shown to induce a strong type I IFN response [24,25]. In addition, the infectivity of RVFV clone 13 was reported to be strongly attenuated in cattle and humans compared with that of the wt strain [11,15,23]. Similar results have been observed in BALB/c mice inoculated with this natural mutant [24]. Mice survive infection despite

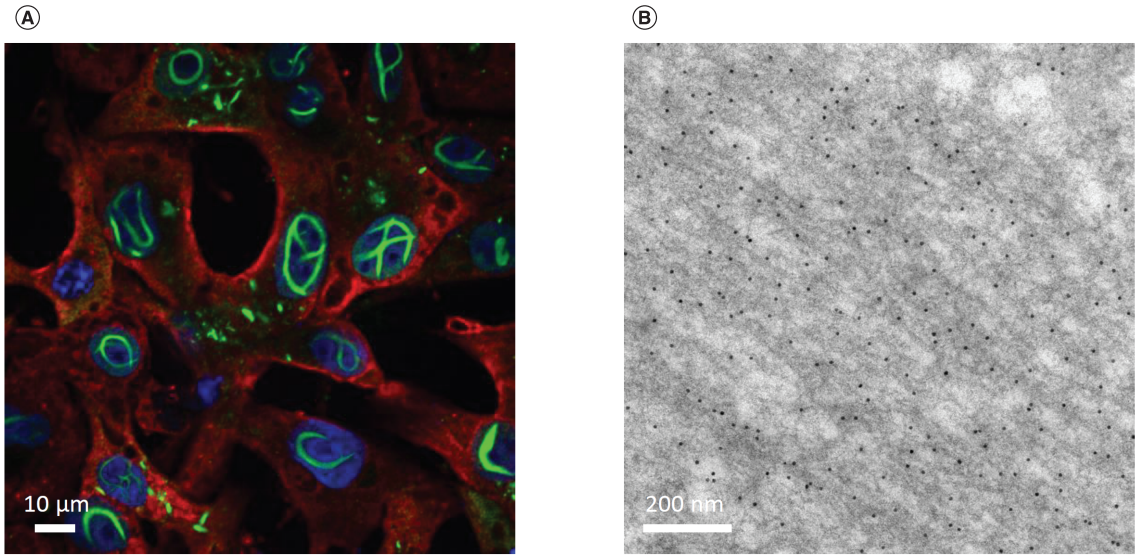


Figure 3. The nonstructural protein NSs assembles into bundles of thin, roughly parallel fibrils. (A) Confocal microscopy image of RVFV-infected Vero cells after immunostaining of the nucleoprotein N (red) and the nonstructural protein NSs (green). Nuclei (blue) were stained with Hoechst. **(B)** Electron micrograph showing a large nuclear filament of NSs after immunogold staining of NSs. Colloidal gold beads appear as black dots. Note that large NSs nuclear filaments are bundles of individual, thin fibrils.

the production and spread of infectious viral progenies. BALB/c mice constitute an interesting animal model to investigate Rift Valley fever disease. When they are infected with the wt strain of RVFV, BALB/c mice recapitulate the acute, nonlethal hepatitis followed by fatal encephalitis observed in severe human cases [24]. Altogether, these studies led to the consideration of NSs as the main factor in RVFV virulence in mammalian hosts.

NSs interferes with the innate immune response in different ways. NSs has been described to recruit the F-Box protein FBXO3 to degrade subunit p62 of the general transcription factor IIH (TFIIH) [26,27]. This results in the blockade of TFIIH assembly, causing a complete arrest of host cell mRNA transcription. The virus thus hijacks the transcription machinery for the exclusive synthesis of its own messenger and genomic RNAs, thereby promoting viral replication and amplification. The general arrest of host cell mRNA transcription also results in the silencing of cellular factors that participate in the innate immune response. For instance, only a few hours after the onset of infection, NSs is highly expressed and suppresses the expression of the IFN-β gene through interactions with the repressor complex SAP30, which together with the transcription factor YY1 regulates IFN-β gene expression [28].

NSs not only contributes to RVFV evasion of innate immune responses by controlling the expression of host cell genes but also degrades protein kinase R (PKR) through the F-box proteins FBXW11 and β-TRCP1 [29–31]. PKR is usually activated by double-stranded RNAs, typical of viral RNA replication complexes, and in turn triggers signaling pathways leading to inhibition of the general synthesis of cellular proteins. This mechanism aims to protect neighboring cells, preventing virus amplification and spread. The degradation of PKR hence allows preservation of the integrity of the cellular machinery, which is essential for RVFV propagation.

NSs assembles into amyloid-like fibrils

The RVFV protein NSs disrupts not only the immune response and the cellular machinery but also induces formation of large filamentous structures in the nuclei of infected cells, a hallmark of RVFV infection (Figure 3A) [32]. The ultrastructure and function of these filaments have so far remained poorly characterized, apart from a report demonstrating their involvement in chromosome cohesion and segregation defects [33]. A study recently revealed that large nuclear filaments are bundles of thin fibrils, reminiscent of amyloid fibrils [7]. Using various advanced imaging techniques, the authors provide with this study a first detailed description of the ultrastructure and cell biology of NSs in infected cells and mice. They were able to link the formation of NSs amyloids in the brain to the neurotoxicity observed in RVFV-infected animals.

Little information is available on the NSs structure at the molecular level. Full-length NSs protein largely form aggregates in solution, making NSs difficult to produce and therefore limiting structural investigations. Only a truncated form of NSs devoid of most N- and C-terminal domains could be analyzed by x-rays [34]. The observations were further confirmed by a nuclear magnetic resonance approach showing that NSs has disordered internal regions and that its C-terminal domain exhibits a strong propensity to form leaflets [34,35]. The C-terminal domain has been reported to be important for NSs oligomerization and filament formation [36]. Altogether, these observations are consistent with recent work establishing NSs as an amyloidogenic protein.

Amyloid proteins

Amyloid fibrils result from the misfolding and aberrant assembly of proteins into highly ordered, linear fibrils [37,38]. To date, approximately 50 proteins have been identified as distinct amyloid fibril-forming proteins and amyloid tissue deposits in humans. They can be either located in certain tissues or systemic, in other words, spread throughout the body [38]. Many of these fibrils and tissue deposits are associated with diseases known as protein-misfolding diseases (PMDs) or amyloidoses, which are often neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and Creutzfeldt–Jakob disease. With the increase in life expectancy and the aging of populations, amyloidoses are becoming increasingly prevalent globally. However, none of these diseases is curable.

Whether amyloid protein aggregation is the cause or the consequence of the deregulation of the cellular machinery and the associated diseases remains unclear. Amyloidoses generally occur spontaneously and, less often, are genetically inherited [39]. While amyloidogenic proteins are host-encoded and present in both prokaryotes and eukaryotes, the only case of infection described for amyloidosis thus far involved acquisition through environmental exposure to prions [39]. Whether viruses encode such proteins remains unclear [40]. Although truncated forms of genetically engineered viral proteins were shown to exhibit some amyloid characteristics *in vitro* [41,42], no virus-encoded amyloid-like fibrils had yet been described *in vivo*, in other words, during authentic viral infections in cell model systems or animals and without any genetic or chemical modifications. In this respect, RVFV and the ability of NSs to assemble into amyloid-like fibrils opens new avenues of research on protein aggregation and toxicity in general.

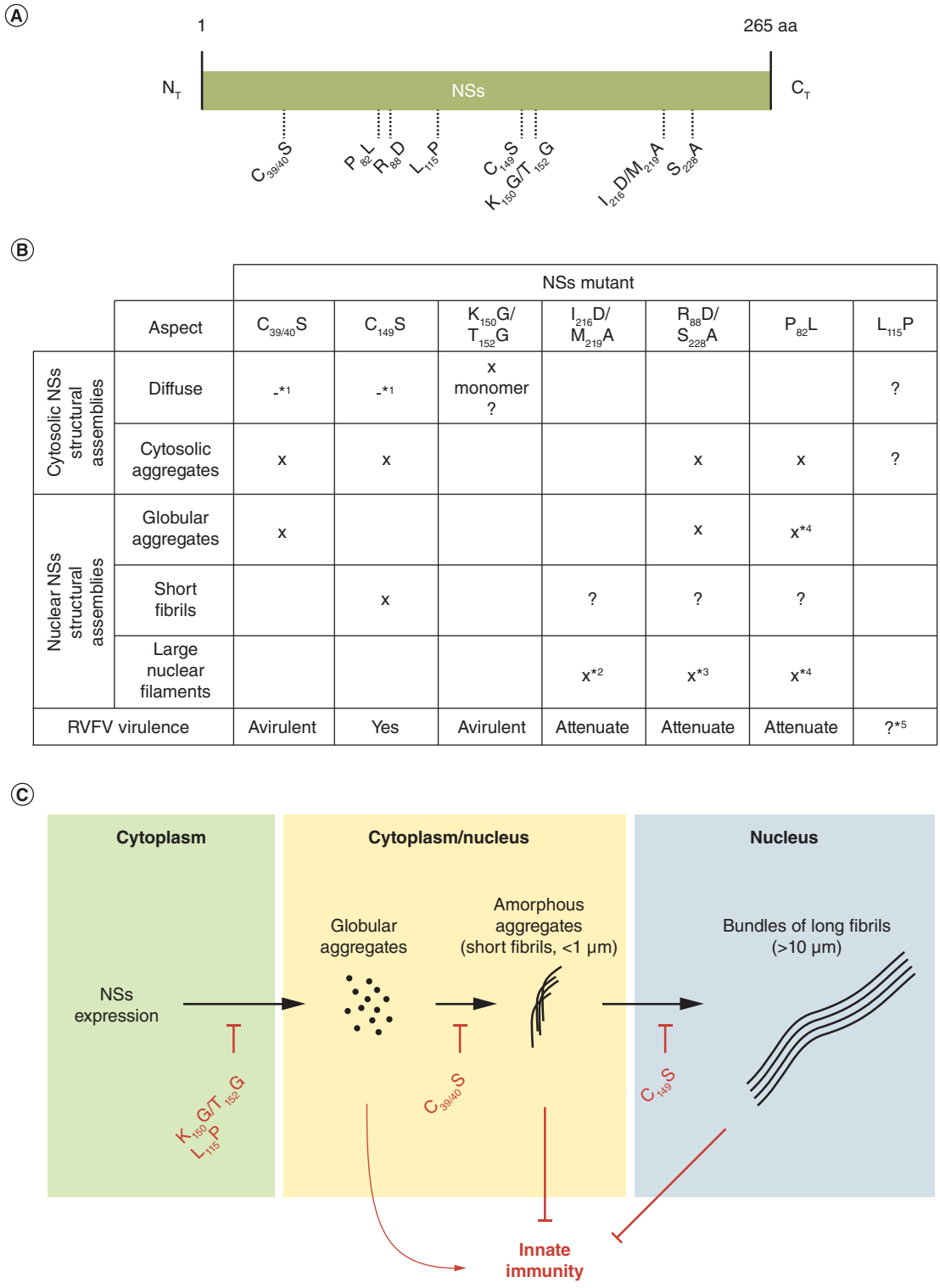
Ultrastructure of nuclear NSs assemblies

RVFV infects a wide range of cell types and tissues. More than ten cell lines, each representative of different tissues and species, have been tested for their sensitivity to RVFV infection, and all show typical large nuclear filaments following infection [7]. The ectopic expression of NSs from DNA plasmids, in other words, in the absence of any other viral proteins, leads to the formation of large nuclear assemblies. Transmission electron microscopy analysis allowed the visualization of the filament ultrastructure, which is easily found in the nuclei of infected cells, with an average diameter of 600 nm and a length greater than 10 µm. The filaments appeared, in fact, to be bundles of thin, roughly parallel fibrils 12 nm in width, a structural organization similar to that of amyloid deposits (Figure 3B). Amyloid aggregates are in general resistant to solubilization by strong detergents (e.g., sodium dodecyl sulfate). This is also the case for NSs aggregates, the analysis of which requires specific biochemical methods developed for the study of large protein assemblies, such as the semidenaturing detergent agarose gel electrophoresis (SDD-AGE) technique [7]. The amyloid nature of NSs fibrils was further confirmed using thioflavin-S (ThS), an amyloid-binding dye broadly used to identify and classify amyloid aggregates [43].

Biogenesis of NSs aggregates

Amyloid aggregates develop preferentially through the continuous incorporation of amyloid protein monomers rather than by increasing the number of structures. Similarly, the number of nuclear NSs filaments remains stable throughout infection, in other words, half a dozen per nucleus. On the other hand, the volume of filaments increases with increasing expression of NSs during infection. In the later stages of infection, NSs filaments can reach 40 µm in length. Live-cell imaging analysis has made it possible to monitor and confirm the dynamics of NSs filament formation in real time [7].

The aggregation of some amyloid proteins depends on oxidation–reduction mechanisms. NSs exhibits six cysteine residues, and the large NSs aggregates were shown to be sensitive to dithiothreitol treatments (DTT). Together, these observations suggest that NSs cysteine residues can form disulfide bonds through their thiol function. The replacement of two cysteines, at positions 39 and 40, by serine residues free of thiol function was shown to abolish the virulence of RVFV in mice (Figure 4) [44]. This double mutant of NSs is no longer able to assemble into large



	Aspect	NSs mutant						
		$C_{39/40}S$	$C_{149}S$	$K_{150}G/T_{152}G$	$I_{216}D/M_{219}A$	$R_{88}D/S_{228}A$	$P_{82}L$	$L_{115}P$
Cytosolic NSs structural assemblies	Diffuse	-*1	-*1	x monomer ?				?
	Cytosolic aggregates	x	x			x	x	?
Nuclear NSs structural assemblies	Globular aggregates	x				x	x*4	
	Short fibrils		x		?	?	?	
	Large nuclear filaments				x*2	x*3	x*4	
RVFV virulence		Avirulent	Yes	Avirulent	Attenuate	Attenuate	Attenuate	?*5

Figure 4. The nonstructural protein NSs. (A) Schematic representation of NSs mutations known to play a role in the assembly of NSs into filaments. aa, amino acids. **(B)** Table correlating the mutations shown in (A) with the structure of NSs assemblies in infected cells. **(C)** Schematic representation of NSs structural assemblies.
*1 not determined; *2 NSs filaments look less numerous but thicker than those formed from the wt protein; *3 Nuclear NSs filaments appear fragmented; *4 Nuclear NSs filaments appear thin and nuclear globular aggregates are predominant; and *5 IFN-β gene is not repressed in infected cell lines [7,34,45,53,54].
NS: Nonstructural protein.

filaments but forms amorphous clusters [7,44], suggesting a correlation between nuclear filamentous structures and RVFV virulence.

An NSs mutant lacking the cysteine at position 149 (C149S) was also shown to be unable to form large filaments but to form amorphous aggregates instead (Figure 4) [7]. Interestingly, this mutant remains virulent in mice [44]. Léger and colleagues later revealed that this mutant, though forming amorphous aggregates, still assembles into fibrils much shorter than the typical long nuclear fibrils, in other words, 1 μm versus 20–40 μm, respectively [7]. The mutant C149S of NSs and these shorter fibrils most likely reflect structural intermediates in the formation of the longer assemblies. The oxidation–reduction process thus seems to play a central role in the formation of large NSs filaments. Nevertheless, the small intermediate fibrils appear to be sufficient for RVFV virulence.

While NSs assemble into large nuclear filaments, aggregates are also found in the cytosol of infected cells at later stages of infection [7]. The cytosolic aggregates have a globular aspect when imaged by fluorescence microscopy, ranging from 10- to 20-times shorter than the filaments observed in the nuclei of infected cells. Correlative light electron microscopy showed that their ultrastructure is reminiscent of the fibrils of the NSs mutant C149S, a few hundred nanometers in length. It is likely that the redox potential of the cytosol, unlike that of the nucleus, does not allow the further assembly of NSs fibrillar intermediates into large filamentous structures. Overall, although NSs forms aggregates with two distinct morphologies when examined by fluorescence microscopy, filamentous aggregates in the nucleus and globular aggregates in the cytosol all consist of fibrils and resemble amyloid deposits in their ultrastructure.

Biological functions of NSs fibrils

How the different NSs structural arrangements contribute to subverting or blocking the cellular machinery and innate immune responses against RVFV remains elusive. The L115P point mutation in NSs has been shown to abrogate both the formation of large nuclear filamentous structures and the ability of RVFV to suppress the expression of IFN-β gene [45]. The NSs mutant C149S, which assembles into short fibrils but not into large filaments (Figure 4), is able to silence IFN-β gene expression and to induce PKR degradation as efficiently as the wt viral protein [7]. In contrast, the double cysteine mutant of NSs, which forms globular aggregates and no filaments or fibrils, is unable to do so. The formation of fibrils, but not necessarily the formation of large nuclear filaments, therefore, appears to be essential for the ability of NSs to hamper the innate immune response. More experimental investigations will be required to determine whether amyloid-like fibrils are required for the other biological functions of NSs.

NSs amyloid formation & neurovirulence

Neuropathy is one major clinical sign of RVFV infection in humans and other mammalian hosts [6], and it is tempting to postulate that RVFV is responsible for PMDs in vertebrates. BALB/c mice represent an interesting model to investigate RVFV-induced pathologies. They mimic quite closely the development and outcome of the disease generally observed in the most severe human cases, in other words, acute hepatitis and delayed-onset encephalitis. The presence of NSs amyloid deposits has been confirmed in the brains of infected BALB/c mice, accumulating mainly in the brain stem [7]. The virus strain clone 13 did not spread to the brains of the animals, probably due to the inability of this natural mutant, devoid of NSs, to counteract the innate immune response. Together, the data indicate that NSs is essential not only for virulence but also for neurotropism of RVFV.

To bypass the need for NSs for viruses to reach the brain, the wt virus or the clone 13 strain was inoculated into mice by intracranial injection. Infection was followed by efficient replication of the virus and then by the production of infectious viral progenies. As expected, only mice exposed to the wt virus showed large nuclear NSs filamentous structures. That only these mice developed paralysis and convulsions indicates that NSs can be considered the main viral factor responsible for the neurological disorders associated with RVFV infection.

Conclusion

PMDs are associated with the aggregation of specific proteins into amyloid fibrils and deposits, which have so far been thought to be exclusively encoded by the host cell, in both prokaryotes and eukaryotes. Prion diseases, the only PMDs acquired through infection, were originally believed to be slow viral diseases. This has since been refuted by Stanley Prusiner and others showing that they were solely caused by amyloid aggregation of the host-encoded prion protein. Interestingly, recent works have shown that infection by herpes simplex virus type 1 catalyzes the aggregation of the host-encoded amyloid β-peptide [46], which is believed to have implications in Alzheimer's

disease [47]. Other works established that some viruses can subvert host-encoded amyloidogenic proteins to ensure their propagation. Semen-derived amyloids dramatically enhance infection with human immunodeficiency virus [48] and cytomegalovirus [49]. The observations that NSs forms amyloid assemblies by itself and that NSs amyloid formation seems to be linked to RVFV pathology now close the circle.

Future perspective

It is obvious that future research is required to improve our understanding of the mechanisms underlying amyloid formation and the processes causing the toxicity associated with viral amyloids. NSs assembles into distinct structures and aggregates, depending mainly on the cellular location of the protein, in other words, the nucleus versus cytosol. It will be important to revisit the biological functions of NSs in the light of the different structural assemblies of the protein. One can easily imagine that the information obtained in such investigations could shed new light on the biological functions of amyloid aggregates in general. There is a growing body of evidence supporting the view that amyloids are not only toxic but can also have beneficial biological functions [50].

Histopathological investigations revealed the presence of filamentous aggregates in the liver of mice infected with RVFV [51]. Although not identified in this study at the time, these filamentous structures were reminiscent of NSs filaments. Typically, acute hepatitis is not fatal, and the liver recovers, which addresses the question how the organ gets rid of the virus and the NSs filaments. Similar questions can apply to infected arthropod vectors. Mosquitoes carrying RVFV do not present obvious clinical signs, and RVFV infection is persistent in mosquitoes [2]. Nuclear filamentous structures were also observed in infected mosquito cells, but interestingly, they disappeared in the later stages of infection, followed by the extinction of NSs expression [52]. RNA interference was likely responsible for the silencing of NSs expression, consistent with the asymptomatic and persistent RVFV infection in mosquitoes [52]. However, how mosquito cells cleared already assembled NSs filaments remains unknown, which may help to understand why mammalian cells are unable to do so.

A recent study involving genetically engineering approaches showed that some NSs mutants still form nuclear filaments in infected cell monolayers, but RVFV virulence was greatly attenuated when the virus encoded the corresponding NSs mutants [53]. Similarly, an RVFV variant selected in cell culture, with one substitution (P82L) in NSs, has recently been shown to be highly attenuated *in vivo*, though it still formed filamentous aggregates [54]. However, the pattern of NSs accumulation significantly differed whether cells were infected with the wt strain or the RVFV variant P82L. While more information on the ultrastructure, number and kinetics and dynamics of filament formation of these NSs mutants in the nucleus and cytosol of infected cells is needed, these results highlight a complex interplay between the RVFV strain, the amount of virus transmitted, the level of viral replication and NSs expression and the diverse NSs structural assemblies in RVFV virulence. The high levels of viral replication observed in infected cells, regardless the presence of NSs, likely contributes to RVFV virulence. Other viral proteins such as NSm certainly contribute to virulence too, by themselves or through coordinated actions with other viral proteins, including NSs. Further research on these important topics will be paramount to clarify the link between NSs, its different structural assemblies, and the virus-induced disease outcome.

Many human amyloidoses are becoming increasingly prevalent worldwide with the aging of populations globally. NSs thus appears to be a first-choice model to improve our understanding of amyloid aggregation and toxicity. The structure of NSs deposits was similar in aspect and dimensions to the filaments reported for the amyloid fibrils formed by amyloid-β and tau [55,56], the aggregation of which is associated with Alzheimer's disease. NSs relies on redox reactions for fibrilization, and one could speculate that NSs fibrils are built from globular subunits such as β2-microglobulin and MLKL, which both form amyloid fibrils stabilized by disulfide bonds [57,58]. Overall, data indicate that the aggregation dynamics of NSs resembles that of the highly aggregation-prone amyloidogenic proteins, such as the mutant Huntingtin protein and other proteins containing expanded polyglutamine (polyQ) stretches [59]. However, the majority of host-encoded amyloid fibrils usually require artificial activation, take days to form, and are difficult to visualize [60,61] whereas viral fibrils assemble rapidly, in only 4 h, and are easy to detect. To better appreciate the utility of NSs as a model system to study amyloid aggregation and functions, we recommend the following excellent reviews on the structure, assembly/disassembly and detection of amyloid proteins [37,61,62].

NSs filaments are a signature of RVFV infection, but more than one hundred other viruses belong to the *Bunyavirales*, and most of them encode an NSs protein. Only a few have been studied so far, the main reason being the lack of tools. However, both Schmallenberg and Dabie bunyaviruses have been found to have a NSs protein that forms, not filaments, but large amorphous aggregates in the nuclei and cytoplasm of infected cells, respectively [63,64]. The ultrastructure of these aggregates remains to be elucidated. Unrelated viruses, such as polyomaviruses and

adenoviruses, also code for proteins forming filamentous structures [65]. Some recent biochemical characterizations suggest that these assemblies are of an amyloid nature [41,42,66]. Others found that herpes viruses have proteins that form amyloid assemblies with functions related to the control of necroptosis and cell death [67,68]. It is therefore highly likely that many other viruses produce amyloid deposits *in vivo*. The exact role of amyloid formation in the pathology of these viruses thus remains a challenge for future work.

Executive summary

Rift Valley fever virus

- Rift Valley fever virus (RVFV) is an emerging and reemerging mosquito-borne pathogen that represents a serious threat to humans and livestock globally.

The RVFV nonstructural protein

- The nonstructural proteins NSs forms amyloidogenic fibrils responsible for the suppression of type I interferon responses.

NSs amyloid formation & neurovirulence

- NSs by itself causes neurological disorders in infected animals.

Challenges & future directions

- NSs appears to be a promising model to shed new light on amyloid aggregation and toxicity in general.

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