Mini review

# RIG-I-like receptors and negative-strand RNA viruses: RLRly bird catches some worms

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ARTICLE INFO

ABSTRACT

Negative strand RNA viruses with a nonsegmented genome (ns-NSVs) or a segmented genome (s-NSVs) are an important source of human and animal diseases. Survival of the host from those infections is critically dependent on rapidly reacting innate immune responses. Two cytoplasmic RNA helicases, RIG-I and MDA5 (collectively termed RIG-I-like receptors, RLRs), are essential for recognizing virus-specific RNA structures to initiate a signalling cascade, resulting in the production of the antiviral type I interferons. Here, we will review the current knowledge and views on RLR agonists, RLR signalling, and the wide variety of countermeasures ns-NSVs and s-NSVs have evolved. Specific aspects include the consequences of genome segmentation for RLR activation and a discussion on the physiological ligands of RLRs.

## 1. Introduction

Viruses with a single-stranded (ss) RNA genome of negative polarity (negative-strand RNA viruses, NSVs) are responsible for a wide range of diseases. Members of the NSV group cause respiratory problems (e.g. influenza, hantavirus pulmonary syndrome, respiratory syncytial virus), severe childhood diseases (measles, mumps), neuronal infection (La Crosse virus, Toscana virus, Borna disease virus), even with up to 100% mortality (Rabies), as well as multi-organ failure and haemorrhagic fevers (Rift Valley fever, Severe fever with Thrombocytopenia, Nipah, Ebola, Lassa, Hanta, Crimean Congo haemorrhagic fever).

NSVs encode highly active polymerases, producing in a typical case 100,000 RNA copies per cell within 10 h of infection [1]. Quite expectedly, such numbers of non-self RNAs in the cytoplasm are highly immunogenic and could trigger massive antiviral responses. To avoid being defeated by the innate immune system, NSVs have evolved strategies against recognition, antiviral signalling, and the launched antiviral mechanisms. In this review article, we will attempt to provide an update on the principles of cytoplasmic virus recognition and viral escape. For deeper insights into specific aspects and into the extracellular virus recognition which is not covered here, we refer to the wealth of recent, excellent reviews by others [2–14].

## 2. Negative-strand RNA viruses

Taxonomically, the group of NSVs is divided into those having one, continuous strand of genomic RNA (the *Mononegavirales*) and those whose genome is divided into several segments. The *Mononegavirales*, also called nonsegmented NSVs (ns-NSVs) have all genes lined up along the ssRNA genome, separated by regulatory intergenic regions acting as transcriptional promoters, and flanked by the regulatory leader and trailer sequences (Fig. 1A). For segmented NSVs (s-NSVs), by contrast, each RNA segment contains one promoter (formed by annealing of the 5′ and 3′ ends) driving expression of one gene (Fig. 1B). The number of segments differs between s-NSV families. Members of the *Orthomyxoviridae* possess mostly 8 segments, whereas *Bunyaviridae* have 3 and *Arenaviridae* have 2 segments (Table 1). Some bunyaviruses and all arenaviruses enlarge the genetic capacity of their segments by transcribing an additional gene from the copy of the genome RNA (the cRNA), the so-called ambisense strategy.

## 3. Intracellular virus recognition by RIG-I like receptors

A rapid and efficient antiviral response is essential to limit virus replication and ensure survival of the host. The cytoplasmic RIG-I-like receptors (RLRs) are able to detect viral RNA species and to trigger an intracellular signalling cascade that eventually results in the release of specific cytokines and chemokines [2,10]. Type I interferons (IFN-α/β) represent the most important antiviral cytokines, inducing expression of more than 300 IFN-stimulated

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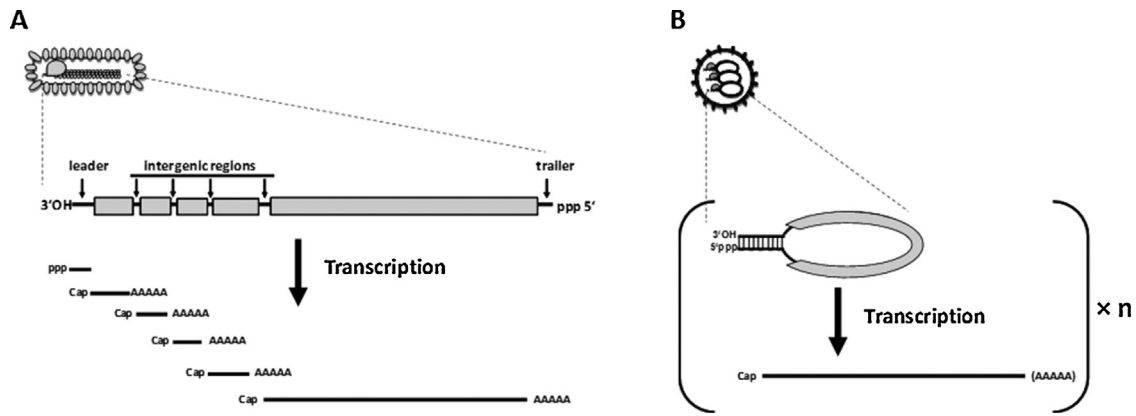*E-mail address:* friedemann.weber@staff.uni-marburg.de (F. Weber).

**Fig. 1.** Differing coding strategies of nonsegmented and segmented NSVs. Symbolized virus particles are shown on top, followed by a sketch of the unencapsidated viral genome with the individual genes as grey boxes. By convention the ssRNA genomes of NSVs are drawn from 3′–5′. (A) The genome of ns-NSVs consists of one continuous stretch of ssRNA from which all mRNAs are synthesized. Intergenic regions contain stop/start signals regulating termination and initiation of transcription. Note the uncapped 5′ppp RNA transcribed from the leader region. Basic set-up of a Rhabdovirus is shown as example. (B) s-NSVs transcribe mostly one mRNA per genome segment (with the exception of ambisense segments which express another gene transcribed from the opposite direction). $n$ = number of segments. Not all s-NSVs have mRNAs with a polyA, which is therefore shown in brackets.

genes (ISGs) [12]. ISGs act in a variety of ways to inhibit virus replication, to elevate antiviral alertness in the infected and surrounding cells, and to modulate the adaptive immune system.

### 3.1. RLR structure

The group of RLRs consists of RIG-I (retinoic acid inducible gene 1), MDA5 (melanoma differentiation association factor 5) and LGP2 (laboratory of genetics and physiology 2) [10]. RLRs are members of the DExD/H box RNA helicase family and consist of a carboxy-terminal domain (CTD), a central DExD/H box RNA helicase domain (composed of Hel1, Hel2 with the insertion domain Hel2i in between) and, in the case of RIG-I and MDA5, two additional amino-terminal caspase recruitment domains (CARDs) (Fig. 2) [15,16]. For RIG-I, the CTD and the helicase domain are conducting ligand recognition, whereas in case of MDA5 this function seems to

be performed by the helicase domain. The terminal CARDs are required for signal transduction. LGP2 does not signal by itself but can bind double-stranded RNA (dsRNA) and acts as a positive regulator of MDA5 [17,18].

### 3.2. RLR signalling

RIG-I has in non-infected cells an auto-repressed conformation in which the CARDs are masking the RNA binding site of the helicase domain [16]. The CTD is exposed to the cytosol and connected by a flexible linker. When the CTD binds to a specific ligand, RIG-I switches its conformation to wrap around the RNA ligand (via CTD and helicase domain) and expose the CARDs instead of the CTD [9]. Stabilization of the active conformation and homo-oligomerization of RIG-I are facilitated by post-translational modifications like dephosphorylation, covalent K63 ubiquitinylation via TRIM25, and association of unanchored ubiquitin chains [4,19–23]. Activated RIG-I can form helical tetramers, but – similar to MDA5 – also longer oligomers along dsRNA [21,24,25].

The oligomers of RIG-I and MDA5 serve as platforms to recruit the adaptor protein MAVS (mitochondrial antiviral signalling) via multiple CARD-CARD interactions [2]. Activated MAVS associates with TRAF3/6 to promote activation of the kinases TBK1/IKKε which are responsible for the phosphorylation of the transcription factors IRF3 and IRF7 [2]. In parallel, the kinases IKKα and IKKβ can activate NF-κB. IRF3/7 and NF-κB translocate into the nucleus to initiate expression of IFN-α/β and proinflammatory cytokines. The basics of RLR signalling are outlined in Fig. 3 (middle panel).

**Table 1**
Negative-strand RNA viruses.

| Family | Genome organization | Representative members[a] |
|---|---|---|
| *Filoviridae* | Nonsegmented | Ebola virus (EBOV), Marburg virus (MARV) |
| *Paramyxoviridae* | Nonsegmented | Nipah virus (NiV), measles virus (MeV), Parainfluenza virus (PIV), Respiratory syndrome virus (RSV), Sendai virus (SeV) |
| *Rhabdoviridae* | Nonsegmented | Rabies virus, vesicular stomatitis virus (VSV) |
| *Bornaviridae* | Nonsegmented | Borna disease virus (BDV) |
| *Arenaviridae* | 2 Segments | Lassa virus (LASV) |
| *Bunyaviridae* | 3 Segments | Bunyamwera virus (BUNV; Orthobunyavirus) La Crosse virus (LACV; Orthobunyavirus) Rift Valley fever virus (RVFV; Phlebovirus) Toscana virus (TOSV; Phlebovirus) Severe fever with thrombocytopenia virus (SFTSV; Phlebovirus) Hantaan virus (HTNV; Hantavirus) Crimean-Congo haemorrhagic fever virus (CCHFV; Nairovirus) |
| *Orthomyxoviridae* | 6–8 Segments | Influenza A virus (FLUAV), Thogoto virus (THOV) |

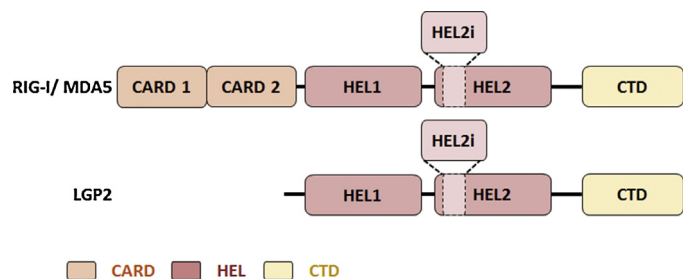[a] Virus acronyms and genera shown in brackets



**Fig. 2.** RIG-I like receptors and their domains. Domain structure of RIG-I, MDA5 and LGP2. CARD, caspase activation recruitment domains; HEL, DExD/H-box helicase domain; HEL2i, insertion domain of helicase domain 2; CTD, carboxy-terminal domain.
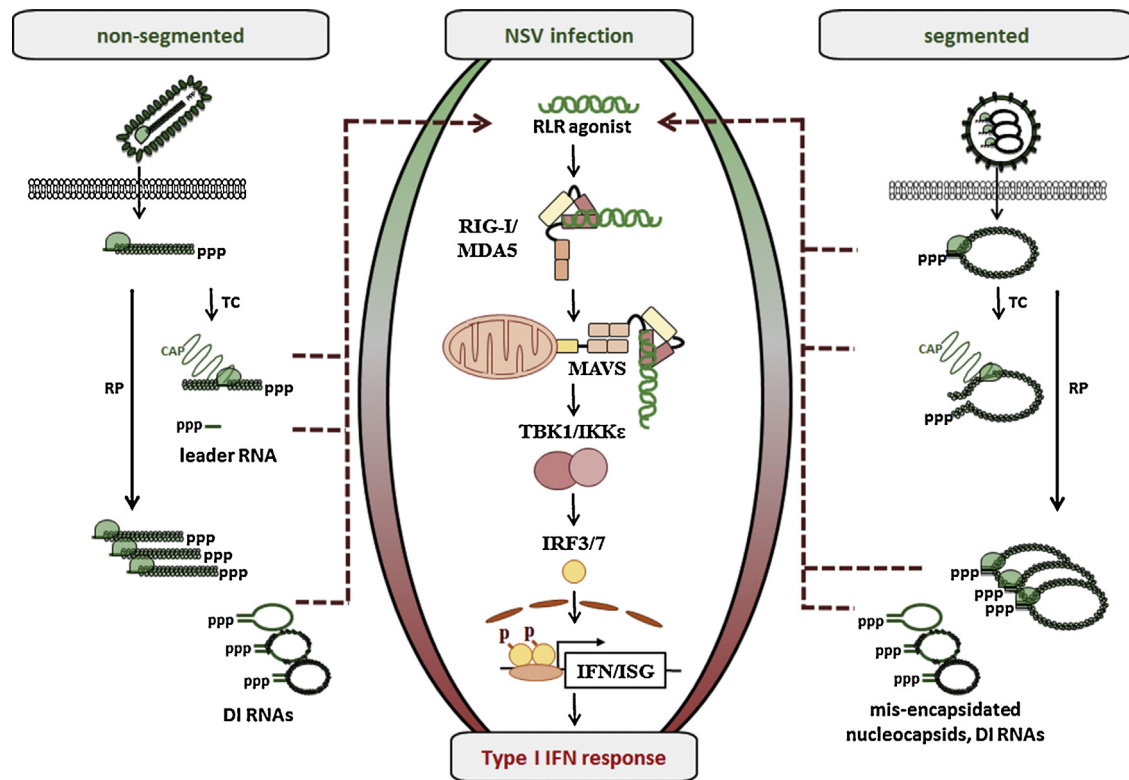
**Fig. 3.** Agonists of RIG-I like receptors produced by NSV infection. Left panel: Upon infection with ns-NSVs, RNAs and DI particles are produced which activate RIG-I and MDA5. Right panel: RNA synthesis by s-NSVs similarly generates regular and aberrant RLR agonists. In contrast to ns-NSVs, the incoming nucleocapsids of s-NSVs can directly activate RIG-I independent of RNA synthesis. Middle panel: Ligand-bound RLRs switch conformation and homo-oligomerize (not depicted) to recruit the signalling adapter MAVS on mitochondria. MAVS triggers antiviral type I IFN responses through a signalling cascade involving the kinases TBK1/IKKε and their substrates IRF3 and IRF7. TC, transcription; RP, replication.

### 3.3. RLR agonists

Despite their structural and functional similarity, RIG-I and MDA5 recognize different RNA ligands (see below), and are activated by distinct but overlapping subsets of viruses [13]. Ligand-wise, RIG-I is strongly activated by blunt-ended dsRNAs of at least 10 base pairs length bearing a 5′triphosphate (5′ppp) group [15,26,27], by long dsRNA molecules of more than 200 base pairs (irrespective of the 5′ end) [24,28], but also by 3′-monophosphorylated ssRNAs [29] and poly U/UC rich stretches of ssRNA [30]. MDA5 detects long dsRNA molecules, ideally with higher order RNA structures [31], and can be activated by particular stretches of NSV RNAs [32,33]. Both RLRs also show preference for AU-rich ssRNAs which seems independent of structure [33,34].

While these activating molecules had mostly been identified and characterized by transfections and manipulations of naked RNAs, they nonetheless allowed conclusions on the nature of the physiological RLR agonists present in infected cells (summarized in Fig. 3, left and right panels). NSVs encapsidate their ssRNA genome and its template (and copy), the antigenome, with nucleocapsid proteins [35]. Therefore, NSVs do not produce detectable amounts of RLR-critical dsRNA [36]. However, synthesis of the NSV genome is initiated with an unprimed nucleoside triphosphate, resulting in a 5′ppp terminus. For ns-NSVs, nucleocapsids are linear [35], suggesting that the 5′ppp ssRNA end does not meet the 3′ counterpart and hence cannot activate RIG-I [13]. For s-NSVs, by contrast, the promoter structure which is formed by annealing of the genome termini ("panhandle", see Fig. 1B) bears hallmarks of a RIG-I agonist. Indeed, we recently showed that the 5′ppp dsRNA

panhandle structures packaged into nucleocapsids of two members of the *Bunyaviridae* (La Crosse virus (LACV) and Rift Valley Fever virus (RVFV)) act as RIG-I agonists in the natural context of infection [37]. Therefore, RIG-I can recognize the nucleocapsids of s-NSVs directly after their entry into the cytoplasm. In the case of ns-NSVs, only RNA synthesis products (e.g. 5′ppp leader RNA) can act as triggers of RIG-I [6,20,33,38–41]. Moreover, particular stretches of viral mRNA can activate MDA5 [32,33]. During replication of the RNA genome, the viral polymerase occasionally jumps template, resulting in RNA products with internal deletions, the defective interfering (DI) RNAs, named so because they strongly compete with the full-length genomes for resources [42]. One particular class, the copy-back DI RNAs, contain complementary 5′ and 3′ ends. It is thought that a percentage of DI RNAs is incompletely encapsidated [43]. For the ns-NSV Sendai virus (SeV), it was shown that copyback DI RNAs form panhandle-like dsRNA structures which can accommodate RIG-I oligomers and activate IFN induction [20,43]. Also for s-NSVs, products of viral RNA synthesis (including internally deleted genomes) can activate RIG-I and innate immune signalling [34,44–46]. Thus, taken together, for s-NSVs incoming nucleocapsids can be detected by RIG-I, and also later RNA synthesis products trigger innate immunity. For ns-NSVs, by contrast, the RLRs have to await the appearance of regular and irregular products of viral RNA synthesis.

## 4. Pros and cons of genome segmentation

s-NSVs are schlepping a bundle of genome segments, each with a RIG-I-sensitive 5′ppp dsRNA promoter. This clearly represents a

disadvantage with respect of immune activation. Moreover, segmentation requires sophisticated packaging mechanisms to ensure each virion contains the full set of genes. On the other hand, the segments can easily be exchanged between different virus variants (reassortment), allowing rapid emergence of new viral strains with altered antigenic or replicatory properties [5,47]. Dividing the genome in separate units is also considered an insurance against sequence degeneration in systems with high error rates, such as RNA-dependent polymerases [48], and packaging several shorter segments instead of one large genome was recently shown to increase the physical stability of virus particles [49]. One evolutionary trade-off of genome segmentation, however, is the elevated number of RLR agonists presented to the innate immune system. It is therefore conceivable that s-NSVs have to express early-hitting IFN evasion strategies which may also be stronger than those of ns-NSVs.

## 5. RLR evasion strategies of NSVs

To our knowledge, all NSVs investigated so far have evolved countermechanisms of RLR signalling, acting to prevent recognition by RLRs, to interfere with RLR signalling, and/or to suppress

**Table 2**
Mechanisms of NSVs to counteract RLR signalling.

| Interference with | Viral protein or function | Virus | Mechanism | References |
|---|---|---|---|---|
| RLR sensing | ssRNA encapsidation | all NSVs | Prevents dsRNA formation | [35,36] |
| | dsRNA unwinding by cellular helicases UAP56 and URH49 | FLUAV, VSV | Prevents dsRNA formation | [50,51] |
| | Recruitment of La | ns-NSVs | Prevents RIG-I recognition of viral leader RNA | [39] |
| | Regulation of RNA synthesis by viral proteins or promoter sequences | ns-NSVs, FLUAV | Prevents formation of aberrant RNAs | [45,52–57] |
| | Nuclear replication | FLUAV | Hiding from cytoplasmic RLRs | [44] |
| | VP35 | EBOV, MARV | dsRNA binding | [60,61] |
| | NS1 | FLUAV | dsRNA binding | [59,62] |
| | Cleavage of the 5′ppp RNA end to 5′p | Bornaviridae, Hantaviruses, CCHFV | Prevents RIG-I activation | [67,68] |
| | Genome RNA 5′overhang | Arenaviridae | Disturbs RIG-I function | [71] |
| | dsRNA degradation by nucleocapsid protein | Lassa virus | Removes dsRNA | [72,73] |
| RLR signalling | NS2 | RSV | Interacts with RIG-I to prevent association with MAVS | [75] |
| | Z | New World Arenaviruses | Interacts with RIG-I to prevent association with MAVS | [74] |
| | OTU domain | Nairoviruses (Bunyaviridae) | De-ubiquitinylates RIG-I | [76,77] |
| | V | Paramyxoviruses | Wedges into MDA5 structure to prevent formation of signalling-competent filaments | [78,79] |
| | VP35 | EBOV | Sequesters the RIG-I cofactor PACT | [64] |
| | V | Paramyxoviruses | Assemble RIG-I and LGP2 into a refractory complex | [80] |
| | NS1 | FLUAV | Interacts with TRIM25 to counteract ubiquitinylation of RIG-I | [63] |
| | Upregulation of Siglec-G by unknown mechanism | VSV, SeV (NSVs in general?) | Ubiquitin-mediated RIG-I degradation | [81] |
| | NSs | TOSV | Ubiquitin-mediated RIG-I degradation | [82] |
| | N and P | RSV | Recruitment of MDA5, MAVS, and RIG-I into inclusion bodies | [83] |
| | NSs | SFTSV | Relocalization of RIG-I, TRIM25, TBK1, IKKε and IRF3 into inclusion bodies | [84,85] |
| | NS1 and NS2 | RSV | Formation of degradosome to destroy MAVS and IRFs | [86] |
| | PB1, PB2, PA, PB1-F2 | FLUAV | Impairment of MAVS signalling | [87–91] |
| | Nucleocapsid protein | Arenaviruses, hantaviruses | Prevention of TBK1 or IKKε activation | [93,96] |
| | Gn | Hantaviruses | Prevention of TBK1 action | [95] |
| | P | BDV and Rabies | Prevention of TBK1 action | [92,97] |
| | V | Paramyxoviruses | Prevention of TBK1 activation | [94] |
| | VP35 | EBOV | Prevents interactions of TBK1 and IKKε with IRFs | [66] |
| | VP35 | EBOV | Inhibits IRF7 function by enhancing its SUMOylation via the cellular E3 ligase PIAS1 | [98] |
| | ML | THOV | Blocks IRF3 and IRF7 dimerization and association with CBP, TRAF6 and the general transcription factor IIB | [99,100] |
| | V | Paramyxoviruses | Interact with IRF3 and impair nuclear translocation. | [101] |
| | W | NiV | Inhibits activation of the IFN-β promoter | [102] |
| | NSs | RVFV | Recruits repression factor SAP30 to inhibit IFN-β transcription | [103] |
| Host cell gene expression | Cap-snatching | s-NSVs | Destruction of host cell mRNAs by viral endonuclease function | [108] |
| | PA-X | FLUAV | Separate endonuclease domain, suppresses antiviral host cell responses | [109] |
| | NS1 | FLUAV | Interferes with processing, nuclear export and translation of host mRNAs | [8] |
| | M | VSV | Interferes with nuclear export of host mRNAs | [110] |
| | NSs | RVFV | Disturbs assembly of TFIIH | [111] |
| | NSs | RVFV | Promotes degradation of the TFIIH subunit p62 via the E3 ubiquitin ligase FBXO3. | [112,113] |
| | NSs | BUNV | Inhibits phosphorylation of the RNA polymerase II subunit RPB1 | [114] |
| | NSs | LACV | Drives proteasomal degradation of RPB1 | [115] |

induction of the type I IFN response. The molecular mechanisms involved range from a selective interference with key components of the IFN system to a broad shut-off of the host cell transcription. Since it has become virtually impossible to summarize all known mechanisms and factors of viral IFN antagonism, we can only describe prominent examples to highlight the underlying principles (Table 2).

## 5.1. Prevention of RLR sensing

An efficient way of innate immune escape is to avoid the initiation of RLR signalling directly at its root, i.e. at the level of detection. As mentioned, NSVs do normally not produce long dsRNAs [36], first of all because of the packaging of genome and antigenome RNAs into separate nucleocapsids [35]. For the s-NSV influenza A virus (FLUAV), depletion of the cellular helicases UAP56 and URH49, which are known to be involved in RNA encapsidation [50], resulted in the appearance of long dsRNA [51]. Also for an ns-NSV (vesicular stomatitis virus, VSV) UAP56 and URH49 are necessary for prevention of dsRNA formation, suggesting a general mechanism [51]. In addition, ns-NSVs are recruiting the cellular RNA binding protein La to shield the leader RNA from RIG-I recognition [39]. The generation of immuno-active RNAs products can also be avoided by tightly controlling the processivity of genome transcription and replication. For several paramyxoviruses (i.e. ns-NSVs) it was described that a loss of regulatory proteins (dependent on the virus either P, C, V) or a promoter mutation enhanced viral RNA synthesis but also facilitated appearance of innate immunity-inducing dsRNA, DI particles, or other aberrant RNAs [52–57]. A similar control of RNA synthesis and DI particle formation to avoid IFN induction was recently reported for FLUAV, suggesting that this is a general mechanism of NSVs [45].

RLR sensing could also be a reason why members of the *Orthomyxoviridae* are replicating in the nucleus. This virus family contain 6 (Thogoto virus, THOV) to 8 (FLUAV) genome segments, i.e. the virions contain at least 2 times as many RLR ligands as those of other s-NSVs. Since RLRs are localized in the cytoplasm, they are only transiently in contact with orthomyxoviral nucleocapsids, namely during the initial transport from the endosome to the nucleus, and during the late step of particle egress. Hiding in the nucleus could be thus a way by which heavily-segmented NSVs can minimize RLR sensing. Indeed, innate immunity activation by FLUAV requires RNA synthesis and RNA nuclear export [44,58]. Thus, the early cytoplasmic passage of nucleocapsids can occur largely unnoticed by the cell.

On top of the strategies to prevent production or exposure of RLR-relevant RNAs, both ns-NSVs and s-NSVs exhibit a range of active mechanisms. The proteins VP35 of Ebola (EBOV) and Marburg virus (MARV) and NS1 of FLUAV directly bind dsRNA [59–62], although it seems that additional host factor interactions are necessary to prevent IFN induction [63–66]. Moreover, members of the *Bornaviridae* (ns-NSVs) and *Bunyaviridae* (s-NSVs) remove the 5′ppp group from their genome to avoid RIG-I activation [67,68], although some residual activating potential seems to remain [37,69]. Similarly, for the *Arenaviridae* (s-NSVs) a fraction of genome ends [70] can exhibit a single 5′overhanging nucleotide with the potential to disturb RIG-I function [71]. Moreover, Lassa virus (LASV, family *Arenaviridae*) encodes a nucleoprotein with 3′–5′ exoribonuclease activity to degrade dsRNA [72,73].

## 5.2. Specific interference with RLR signalling

In addition to masking, processing or degrading RLR agonists, NSVs can interfere with key components of the RLR signalling

pathway. Several factors of ns-NSVs and s-NSVs are known to act on the level of RLRs. NS2 of respiratory syncytial virus (RSV) and the Z protein of New World Arenaviruses directly interact with RIG-I to prevent the association with MAVS [74,75]. The ovarian tumour (OTU) domain of Nairoviruses (family *Bunyaviridae*) supresses IFN induction and de-ubiquitinylates RIG-I [76,77]. MDA5 was originally discovered as a host interactor and inhibitor of the paramyxovirus IFN antagonist V [78]. The V proteins are wedging into the MDA5 structure in a manner that prevents assembly into signalling-competent filaments [79]. VP35 of EBOV sequesters the RIG-I activating protein PACT to interfere with RIG-I activation [64], V proteins of paramyxoviruses assemble RIG-I and LGP2 into a complex which becomes refractory to activation by RIG-I ligands [80], and NS1 of FLUAV interacts with TRIM25 to counteract ubiquitinylation of RIG-I [63]. Interestingly, infections with VSV or SeV were shown to upregulate the lectin Siglec-G, resulting in RIG-I degradation via K48-linked ubiquitinylation [81]. A strategy of ubiquitin-dependent RIG-I degradation is also conducted by Toscana virus (TOSV) via its NSs protein [82].

Some viral factors also sequester key components of RLR signalling in cellular compartments. RSV N and P proteins recruit MDA5, MAVS, and less efficiently RIG-I, into virus induced inclusion bodies [83]. Also Severe fever with thrombocytopenia syndrome virus (SFTSV) NSs prevents RLR signalling by promoting relocalization of RIG-I, TRIM25, TBK1, IKKε and IRF3 into virus-induced cytoplasmic structures [84,85]. NS1 and NS2 of RSV induce the formation of the so-called degradosome to target MAVS and IRFs for degradation [86]. MAVS signalling is impaired by FLUAV polymerase subunits (PB1, PB2 and PA) [87,88] and the accessory protein PB1-F2 [89–91]. Further downstream, TBK1 activation or action are prevented by N of arenaviruses and hantaviruses, and Gn proteins of hantaviruses, P proteins of BDV and Rabies virus, V proteins of paramyxoviruses, and also by EBOV VP35 [66,92–97].

Finally, at the level of IFN transcription, several viral factors are known to interfere with IRF or NF-κB activity. EBOV VP35 (a multifunctional IFN antagonist like FLUAV NS1) enhances SUMOylation of IRF7 via the cellular E3 ligase PIAS1, thereby inhibiting its function as an IFN transcription factor [98]. ML of THOV (family *Orthomyxoviridae*), acts by blocking IRF3 and IRF7 dimerization and association with CBP, TRAF6 and the general transcription factor IIB [99,100]. V proteins of several (but not all) paramyxoviruses interact with IRF3 and impair its nuclear translocation [101]. In case of the highly pathogenic Nipah virus (NiV) (family *Paramyxoviridae*), the W protein translocates into the nucleus to inhibit activation of the IFN-β promoter [102]. Also the NSs protein of RVFV specifically inhibits IFN-β mRNA transcription by forming a promoter-bound complex with the repression factor SAP30 [103].

## 5.3. Unspecific interference with RLR signalling

In contrast to the above-described specific mechanisms aiming at antiviral signalling pathways, some representatives of the NSVs interfere with the cellular transcription machinery in total. It is thereby important to distinguish between a primary host cell shut-off imposed by specific viral mechanisms from the secondary shut-off caused by translation inhibition due to the dsRNA-activated host cell kinase PKR. While the primary, "intended" shut-off seems to be mostly (but not exclusively) the domain of s-NSVs, the secondary shut-off is in fact an efficient antiviral host reaction. PKR activation is thus avoided by both s-NSVs and sn-NSVs, either by tight regulation of RNA synthesis (see above), or by specific anti-PKR mechanisms [11,104].

A virus-induced, general block of gene expression provides an efficient mechanism to prevent synthesis of IFN and ISGs, but could

bear the disadvantage of cutting off from renewable resources. However, viruses undergoing rapid and lytic infection cycles seem to care little for such considerations. That the viral host shut-off, a long-known phenomenon, is often an IFN antagonism in disguise was revealed by genetic complementation studies showing that the particular viral shut-off factor is only necessary if the host organism possesses a functional IFN system [105–107]. In other words, in an IFN-free host environment the shut-off factors are dispensable for viral success. Interestingly, all s-NVs (but not ns-NSVs) commit cap-snatching, an endonuclease-mediated mechanism to degrade host mRNAs by depriving them of their 5′ cap structure. Cap-snatching spares the s-NSVs to encode their own mRNA capping enzyme, and could have the convenient side effect of reducing host cell gene expression, including that of upregulated components of the RLR and IFN signalling pathways [108]. FLUAV expresses its endonuclease even as a separate domain, called PA-X, which was actually shown to suppress antiviral host cell responses [109]. FLUAV impairs host gene expression also by other mechanisms. The multifunctional protein NS1 interferes with processing, nuclear export and translation of host mRNAs [8]. Inhibition of nuclear-cytoplasmic RNA transport is a strategy shared by the matrix protein M of VSV [110]. Broad disruption of RNA polymerase II activity is achieved by representatives of the *Bunyaviridae*. The NSs of RVFV disturbs assembly of the general transcriptional factor TFIIH [111] and promotes degradation of the TFIIH subunit p62 via the E3 ubiquitin ligase FBXO3 [112,113]. The NSs protein of Bunyamwera virus and LACV impact on the RNA polymerase II subunit RPB1, disturbing its phosphorylation (and hence processivity) or driving its proteasomal degradation [114,115].

## 6. Conclusions and outlook

NSV-infected cells are flooded with foreign RNAs which are – compared to the pool of host cell RNAs – rather homogeneous in sequence and structure. Unusual features like double-strandedness or triphosphorylated 5′ends are extremely efficient markers of infection. However, also structures with less prominent features are conceivable to act as triggers of antiviral responses if they appear in high enough amounts.

Since the discoveries of RIG-I and MDA5 [78,116], the research field of intracellular RNA recognition and viral countermechanisms has virtually exploded. There are ongoing discussions on the physiological ligands of RLRs and on major RLRs engaged during a particular infection, questions which are touching the very core of the immune reponse, the distinction between self and non-self. In our review, we wanted to highlight that NSVs are a quite heterogeneous taxonomic group, with different genome and nucleocapsid structures, subcellular localizations, replication strategies, tendencies to produce DI particles, and a multitude of IFN antagonists which can obscure RLR–ligand interactions. Moreover, different infection phases may produce different RLR triggers. To make things even more complicated, it is meanwhile established that RLRs can get help from accessory factors such as e.g. other RNA-binding proteins, helicases or RNases, further expanding the range of in vivo RLR triggers [17,32,39,117–119], which may not work as such in vitro. So, again, the question which RLR recognizes which virus and viral structure under physiological conditions may return different answers which depend on the infection phase, the DI particle content of virus stocks, the specific activity of virus regulators and IFN antagonists, and on host cell cofactors.

## Conflict of interest

No conflicts of interest declared.

## Acknowledgments

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