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How hantaviruses modulate cellular pathways for efficient replication?

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1. ABSTRACT

Hantaviruses are zoonotic category-A pathogens that cause highly fatal diseases in humans. The hantaviral genome encodes three viral proteins: RNA-dependent RNA polymerase (RdRp or L protein), nucleocapsid protein (N), and a glycoprotein precursor (GPC), which is post-translationally cleaved into two surface glycoproteins Gn and Gc. The cytoplasmic tail of Gn interferes with interferon signaling pathways. N is a multifunctional molecule that was shown to be involved in the transcription and translation of viral proteins. N binds to the host mRNA caps and protects the degradation of mRNA 5' termini, which are later snatched and used as primers by the viral RdRp during transcription initiation. N also seems to lure the host translation machinery for the preferential translation of viral transcripts. Moreover, N was shown to delay the induction of cellular apoptosis and facilitate the transport and localization of viral ribonucleoproteins (RNPs) by exploiting the cellular cytoskeleton and SUMOlyation machinery. Therefore, with their limited protein coding capacity, hantaviruses have evolved several strategies to modulate cellular pathways for their efficient replication.

2. INTRODUCTION

Hantaviruses are zoonotic emerging viruses that are classified as category A pathogens by the Centers for Disease Control and Prevention (CDC). They belong to the family *Bunyaviridae*, which contains five genera: *Hantavirus*, *Nairovirus*, *Orthobunyavirus*, *Phlebovirus* and *Tospovirus* (1). Unlike all other members of the family, hantaviruses are not transmitted by biting insects. Human infection occurs by inhalation of dust contaminated by droppings, urine or saliva from infected rodents (2). These viruses establish persistent asymptomatic infection in the rodent host reservoir (3). Human-to-human transmission is very rare and has been reported in a single outbreak in Argentina (4).

Hantaviruses cause two serious disease conditions in humans: hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS), with mortality rates of up to 15% and 50%, respectively. Both syndromes are characterized by capillary leakage and increased permeability of vascular endothelial cells, which is believed to be the result of an immense uncontrolled immune response to infection rather

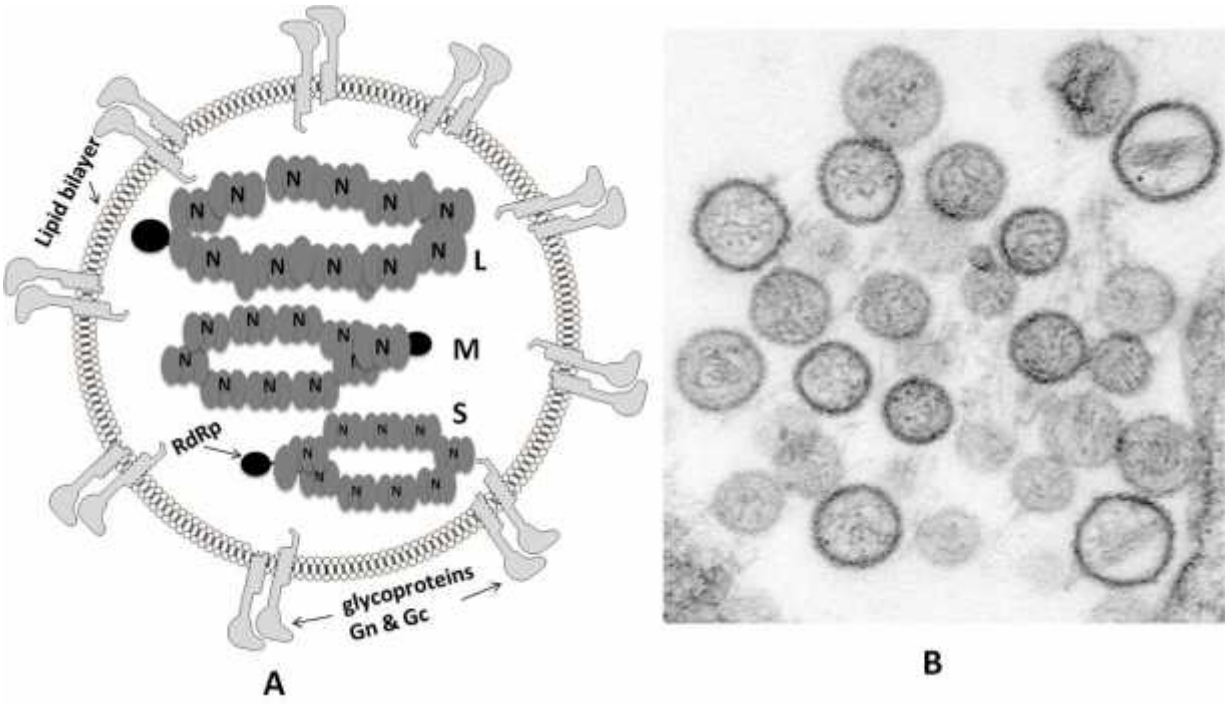

Figure 1. (A) Schematic representation of hantavirus particle, showing three nucleocapsids (L, M and S) and the viral RNA-dependent RNA polymerase (RdRp) eclosed in a lipid bilayer envelope carrying the viral glycoproteins Gn and Gc. (B) Thin-section electron micrograph of an SNV isolate from the outbreak of HCPS that occurred in the southwestern United States in 1993. Electron micrograph was obtained from the CDC website with permission (http://www.cdc.gov/ncidod/diseases/hanta/hps/noframes/hpsem.htm). This electron micrograph was also reported in our previous publication (14).

than direct cytopathic effects caused the virus replication in infected cells. Patients develop flu-like symptoms followed by hemorrhage, pulmonary edema, shortness of breath and death from respiratory failure and cardiac insufficiency (5). Depending on their potential to cause a disease in humans, hantaviruses are classified into pathogenic or non-pathogenic. Examples of non-pathogenic hanatviruses include Prospect Hill (PHV) and Tula virus (TULV). According to their geographic distribution, pathogenic hantaviruses are further classified into two groups Old and New World. Old World hantaviruses, such as Hantaan virus (HTNV), Seoul virus (SEOV), Dobrava virus (DOBV) and Puumala virus (PUUV), are widely distributed in Europe and Asia. On the other hand, New World hantaviruses, such as Sin Nombre virus (SNV), New York-1 virus (NYV) and Andes virus (ANDV) are confined to North and South America. SNV infects deer mouse and is the major cause of HCPS in North America (6). It was first discovered during a major outbreak that occurred in 1993 in the Four Corners region of the US (the point where Colorado, Arizona, Utah, and New Mexico meet). This outbreak claimed the lives of many victims; about one in three people diagnosed with HCPS died (7) (8). At present, there is no effective vaccine or antiviral therapy available to treat hantavirus infections.

Hantaviruses have a negative-sense segmented RNA genome that consists of three segments: small (S), medium (M) and large (L) (Figure 1). All three segments have non-coding untranslated regions (UTR) at their 5' and 3' termini, which carry partially complementary sequences that fold into panhandle structures (9) (10). Panhandles have been reported to serve as promoters for transcription initiation by the viral L protein. The S segment encodes a nucleocapsid protein (N). In addition to N, the S segment of hantaviruses, carried by the Cricetidae family of rodents, have an overlapping open reading frame (ORF) that codes for the putative nonstructural protein (NSs) (11) (12). The M segment encodes a glycoprotein precursor (GPC) that is proteolytically cleaved after a highly conserved pentapeptide motif WAASA, into two envelope proteins: Gn and Gc. The L segment encodes the viral RdRp (L protein). N is the main structural protein and plays several important roles in viral assembly, transcription and translation. Gn and Gc mediate the attachment of virus particles to host cell receptors during viral entry into host cells. The cytoplasmic tail (CT) of Gn plays an important role in packaging and has been proposed to be an important viral virulence factor due to its role in blocking the host innate immune responses (13). L protein is the viral replicase and transcriptase, which uses the negative-sense genome as a template for the synthesis of positive sense complementary RNA (cRNA) and messenger RNA (mRNA) (14).

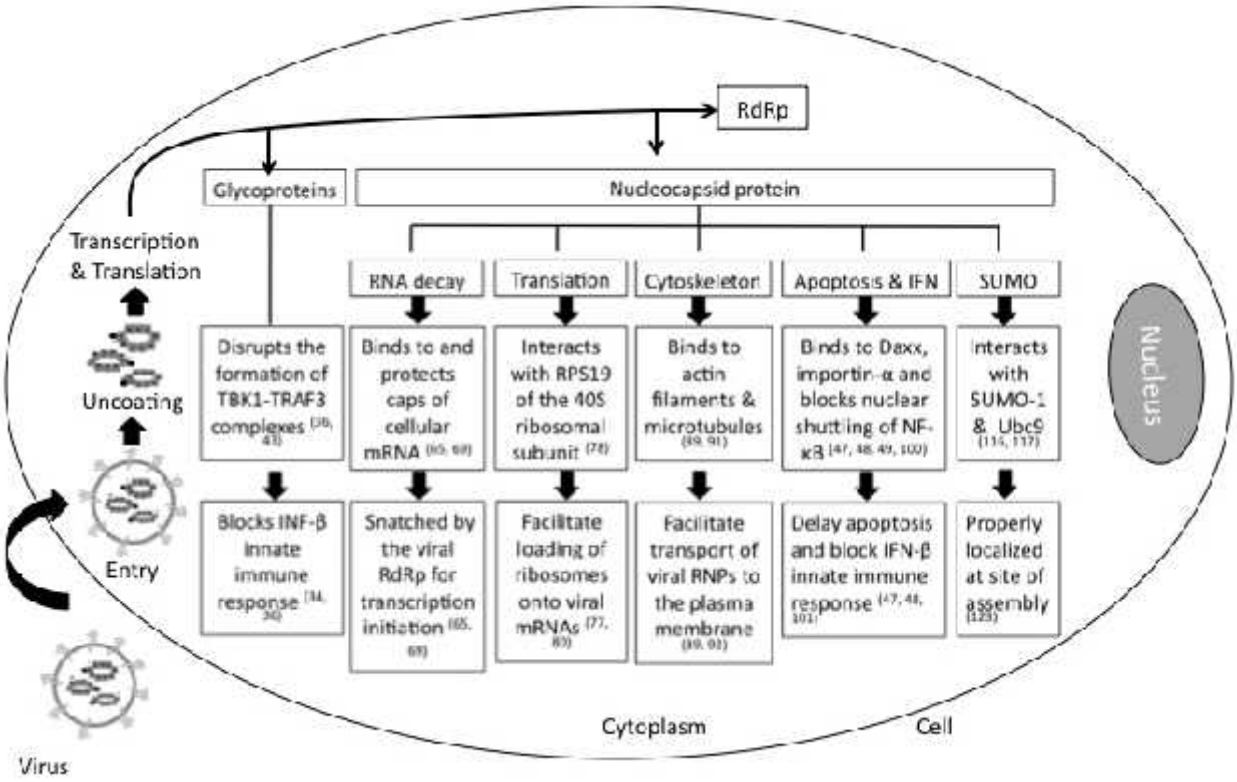

Figure 2. A summary of the various mechanisms used by the glycoproteins and N protein of hantaviruses to modulate cellular functions for efficient virus replication.

After attachment, hantaviruses are internalized into target endothelial cells by receptor-mediated endocytosis (15). Virions are then delivered to lysosomes, where the acidic environment facilitates fusion between viral and cellular lipid membranes, releasing viral nucleocapsids into the cytoplasm. The viral L protein generates three mRNAs from the three viral genome segments, which upon translation produce the four viral proteins. Later in the replication cycle, the virus switches to the genome-copying mode where cRNAs are used as templates for producing negative-sense genomic viral RNAs (12). N is an RNA-binding protein that undergoes trimerization and oligomerization. The trimeric form of N binds specifically to viral RNA panhandle (16). N-panhandle interaction has been proposed to play a role in selective encapsidation and packaging of the viral genome (17). N also interacts with the cytoplasmic tail domain of Gn, which has been proposed to mediate the selective packaging of virion RNPs (18). Newly formed virions bud off the cell and infect new cells. During this complex replication cycle, hantaviruses control important cellular functions for efficient replication in host cells. In this review, we cover the main elegant strategies that hantaviruses have evolved to modulate basic cellular pathways for their efficient replication (Figure 2). To the best of our knowledge, we have tried to cover all the published data in this review; any author's contribution that was left out is totally unintentional.

3. MODULATION OF THE INNATE IMMUNE RESPONSE

The innate immune response constitutes the first line of defense against invading viruses. It acts mainly to delay virus replication until the more specific adaptive immune response gets into action. Type I interferons (IFNs) (alpha/beta IFN) are key cytokines in this innate immune response; they are secreted in response to viral infection and function to create an antiviral state that protects uninfected cells and limit further virus replication and spread. Under normal conditions, IFN genes are transcriptionally silent. The signaling cascades that induce IFN production are activated upon recognition of foreign pathogen-associated molecular patterns (PAMPs) of viruses by cellular pattern recognition receptors (PRRs) expressed by many cells including endothelial and epithelial cells. Double-stranded RNA (dsRNA) produced as an intermediate product of ssRNA virus replication, ssRNA with tri-phosphorylated 5' end, unmethylated CpG DNA and envelope glycoproteins have been identified to interact with cellular PRRs (19).

The best-characterized PRRs include Toll-like receptors (TLRs) and retinoic acid-inducible gene I-like RNA helicases (RLHs). RLHs comprise retinoic acid-inducible gene I helicase (RIG-I) and melanoma differentiation-associated gene 5 helicase (MDA-5). TLRs are type I integral membrane glycoproteins that are mostly

present in endosomal membranes, while RLHs are cytoplasmic. Up to ten different TLRs have been identified in humans (20); they consist of leucine-rich repeats (LRRs), a transmembrane domain, and a cytoplasmic signaling domain known as the Toll/interleukin-1 receptor (IL-1R) homology (TIR) domain. RIG-I and MDA5 are comprised of two N-terminal caspase recruitment domains (CARDs) that are responsible for initiating downstream signaling cascades and a DExD/H box helicase domain, which interacts with dsRNA (21).

Induction of type I IFN-α/β by the binding of specific PAMPs to TLRs, RIG-I, and MDA-5 engage multiple signal transduction pathways that all converge at the activation of TNF receptor-associated factor 3 (TRAF3). TRAF3 connects the upstream sensory responses to downstream effector functions by forming a complex with and activating a group of kinases: TRAF family member-associated NF-κB activator binding kinase 1 (TBK-1) and IκB kinase ε (IKKε). Ultimately, these kinases phosphorylate and activate two key transcription factors: IFN-regulatory factor-3 (IRF-3) and nuclear factor κB (NFκB) that translocate to the nucleus, where they bind to IFN-stimulated response elements (ISREs) resulting in the expression of type I IFNs (22).

Secreted IFNs bind to cell surface receptors and activate cells in an autocrine and paracrine manner via Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathways. This leads to the expression of a diverse group of more than 300 IFN-stimulated genes (ISGs), such as 2',5'-oligoadenylate synthetase (OAS)/RNase L, dsRNA-activated protein kinase (PKR) and orthomyxovirus resistance gene 1 (MxA), which constitute an antiviral state by disrupting viral transcription and translation (23) (24). The OAS/RNase L is a powerful endoribonuclease system that inhibits broad range of RNA viruses by cleaving single-stranded RNA. PKR is a serine/threonine kinase that induces shut down of host cell translation by phosphorylating the protein synthesis initiation factor eIF2α. Mx proteins are large GTPases that were originally identified because they conferred resistance of the mouse strain A2G to influenza A viruses (25). In vitro, the MxA protein has been shown to inhibit the growth of hantaviruses and other members of the *Bunyaviridae* family (26) (27) (28).

Hantaviruses have been shown to induce IFN-β production in human endothelial cells, and antibodies to IFN-β inhibited the induction of ISGs and enhanced HTNV replication (29). However, the PRR responsible for hantavirus recognition remains elusive (30) (31) (32). Escaping the early innate IFN response is crucial for virus survival. Many viruses have evolved elegant strategies to block the activation of the IFN response allowing the virus to win some time to establish a productive infection in the host (33). Several reports have indicated that pathogenic hantaviruses delay the induction of IFN response and modulate the innate immune response differently than their non-pathogenic counterparts (13). DNA microarrays have shown that PHV infection of human umbilical vein endothelial cells (HUVEC) resulted in an early strong IFN response one day post-infection, which was absent following infection with HTNV or NYV (34). Also, the kinetics of expression of the antiviral protein MxA were different upon TULV and HTNV infection of HUVEC cells. TULV infection resulted in rapid induction of MxA (16 h post-infection), whereas HTNV induced MxA relatively late (48 h post-infection). Accordingly, TULV titers were much lower than HTNV in HUVEC cells. However, both viruses replicated equally well in African green monkey kidney (Vero E6) cells, which lack INF genes (35). In infected HUVEC cells, PHV RNA and protein synthesis were shown to be restricted to the first day post-infection, and then dropped dramatically 2 – 5 days post-infection. This was in direct contrast to HTNV and NYV, which continued to synthesize viral mRNA and protein 1 – 5 days post-infection (36). These findings have suggested that differences in INF responses may account for the pathogenic potential of hantaviruses. Pathogenic, but not non-pathogenic hantaviruses are able to delay early IFN responses to allow efficient viral replication. Moreover, these differential IFN responses have clearly indicated that some proteins encoded by pathogenic hantaviruses are capable of regulating the early innate immune responses. Indeed, IFN antagonists have been identified in several Old and New World hantaviruses; and the ability of hanatviruses to inhibit IFN induction has been mapped to three viral proteins: Gn, NSs and N.

3.1. Cytoplasmic tail of Gn protein

In contrast to N and Gc proteins, Gn is expressed very poorly in infected and transfected cells (37). Gn of hantaviruses contains a long cytoplasmic tail (CT) (142 amino acid residues) that has been proposed to be a virulence factor that contributes to hantavirus pathogenesis. Geimonen and colleagues identified key signaling elements termed immunoreceptor tyrosine-based activation motifs (ITAMs) within the Gn CT of all HPS-causing hantaviruses, but not HFRS or non-pathogenic hantaviruses (38). ITAM motifs consist of two tandem Yxx(L/I) sequences and were originally identified in the CTs of B- and T-cell receptors (BCR and TCR). Upon ligand binding, they recruit Src and Syk family kinases to convey signals to the intracellular signaling pathways (39). Two-hybrid analysis revealed that the tyrosine residues of NYV Gn ITAMs interacted with Src and Syk family kinases in a manner similar to the ITAMs of BCR and TCR. The fact that these ITAMs were conserved in all HPS-causing hantaviruses strongly suggested a direct role for the Gn tail in modulating immune and endothelial cell functions by altering normal cell signaling responses. It was also shown that the CT of NYV Gn is polyubiquitinated and targeted for proteosmal degradation. Mutational analysis revealed that the tyrosine residues of the ITAM motif were responsible for this interaction (40). The signal for degradation was later mapped to the C-terminal 30 residues of the pathogenic ANDV and HTNV Gn CTs. Moreover, it was reported that the CT of the non-pathogenic PHV is stable and not proteosomally degraded in Vero E6 and COS-7 cells (41). These findings have suggested that there is a direct link between CT degradation and pathogenesis. Later, Wang and colleagues showed that the Gn CTs of the

non-pathogenic TULV and PHV were proteosomally degraded in Human Embryonic Kidney (HEK-293) and Vero E6 cells (42). This conflicting evidence suggested that this degradation might not be necessarily related to viral pathogenesis. However, additional reports showed that the CTs of pathogenic, but not non-pathogenic hantaviruses regulate IFN activation signaling pathways. Expression of the NYV CT, but not the PHV CT, inhibited TBK-1-directed IFN induction by disrupting the formation of TBK1-TRAF3 complexes and the downstream signaling responses required for IFN-β transcription (36) (43).

3.2. Non-structural protein (NSs)

Three out of the five genera of the *Bunyaviridae* family (*Orthobunyavirus, Phlebovirus* and *Tospovirus*) are known to encode a nonstructural protein (NSs) in their S segment as an overlapping reading frame to the ORF of N (12). In *Phlebo-* and *Tospoviruses*, the NSs protein is encoded by the (+) strand of the S segment RNA, i.e., an ambi-sense coding strategy is employed. The presence of an overlapping NSs protein was described for at least 16 distinct hantaviruses (11). The NSs encoded by some of these viruses, such as Bunyamwera virus (BUNV) and Rift Valley Fever virus (RVFV), have been associated with IFN antagonizing functions (44) (45). Members of the other two genera (*Hantavirus* and *Nairovirus*) are generally not known to encode NSs. However, overlapping reading frames corresponding to the NSs have been identified in SNV, TULV, PUUV and PHV. More recently, a protein corresponding to the predicted size of TULV NSs was detected using coupled *in vitro* transcription and translation reactions, and a protein corresponding to the predicted size of PUUV NSs was also detected in infected cells by western blotting (46). In reporter-based systems, transiently expressed TULV and PUUV NSs in COS-7 cells had weak inhibitory effects on the activities of IFN-β promoter, and NF-kB and IRF-3 responsive promoters. These findings have suggested that hantavirus NSs is also a weak IFN antagonist, however the mechanism of this anti-IFN activity is not known (46).

3.3. Nucleocapsid protein (N)

Some studies have suggested that hantavirus N is a potential IFN-β antagonist. As will be explained later in this review, HTNV N was shown to interfere with NF-κB nuclear transport by binding to importin-α (47) (48). A similar activity was also reported for SEOV and DOBV (49). For ANDV, it was shown that co-expression of both GPC and N is required for inhibition of INF-β induction and signaling. Expression of ANDV N alone resulted in 50% inhibition of STAT-1 phosphorylation and of JAK/STAT-dependent promoter activity (50).

4. MODULATION OF THE HOST mRNA DEGRADATION PATHWAYS FOR CAP SNATCHING AND TRANSCRIPTION INITIATION:

The genome of segmented negative-stranded RNA viruses (*Orthomyxoviridae, Arenaviridae* and *Bunyaviridae*) is made up of negative-sense RNA strands. In order for this genome to be replicated, it has to be first converted into a positive-sense strand that can serve as a template for the production of viral RNA. The viral L protein cannot initiate this transcription process *de novo*, and therefore requires a primer (51). Mature cellular messenger RNAs (mRNAs) have a 5' methylated cap and a 3' poly (A) tail. Early studies on influenza virus have shown that viral mRNAs were polyadenylated and contained heterogenous non-viral cap sequences at the 5' end (52) (53). Further work revealed that those caps were derived from the 5' termini of cellular mRNAs and led to the development of the concept of "cap snatching". Cap snatching is a unique mechanism used by negative-stranded RNA viruses to generate short-capped RNA primers from host cell mRNAs that are used for transcription initiation by the viral L protein. This mechanism has been well characterized for influenza virus. In this process, the influenza virus polymerase binds to the m7G cap of nuclear pre-mRNAs and cleaves them 10 – 15 nucleotides downstream the terminal cap. The resulting RNA oligonucleotides are used as primers to initiate transcription from the viral RNAs, resulting in the generation of capped and polyadenylated mRNAs that resemble the host cell messages. Viral mRNAs are then exported from the nucleus to the cytoplasm for translation (54) (55). Influenza virus RdRp is made up of three viral proteins: polymerase acidic protein (PA), polymerase basic protein 1 (PB1), polymerase basic protein 2 (PB2) (56). The cap-binding domain of influenza virus was mapped to the central region of the PB2 subunit. The endonuclease activity was shown to reside in the N-terminal region of the PA subunit. PB1 has intrinsic polymerase activity and is responsible for mRNA elongation (57) (58) (59).

A similar cap snatching mechanism was proposed for the viruses of the *Bunyaviridae* and *Arenaviridae* families (60) (61) (62), although their RdRps are structurally different (14). Based on the precedent with influenza virus, it has been assumed that the endonuclease activity required for cap snatching resides in the RdRp of Bunyaviruses and Arenaviruses. Recent biochemical and structural data from the RdRp of La Crosse orthobunyavirus (LACV) showed that it has a functional, manganese-dependent N-terminal endonuclease domain (180 residues) that has very similar characteristics to that of influenza virus endonuclease (63). Similarly, the NL1 domain (N-terminal 196 residues) of the RdRp from the prototypic arenavirus, lymphocytic choriomeningitis virus (LCMV), was shown to bind and cleave RNA (64). Using a structure-based sequence alignments approach, (63) the existence of a similar endonuclease domain at the N-terminus of the RdRps of all known segmented negative-stranded RNA viruses was predicted.

In contrast to influenza virus, which replicates in the nucleus, bunya- and arenavirus replication is exclusively cytoplasmic, and therefore caps are derived from cellular mRNAs rather than pre-mRNA (51). Since cellular mRNA degradation also occurs in the cytoplasm, this raised the question of the relationship between mRNA decay pathways and the bunya- and arenavirus cap-snatching process. Indeed, we have shown that Sin Nombre hantavirus N colocalized with the discrete cytoplasmic processing bodies (P-bodies), where cellular mRNA decay is known to occur (65). Each P body can be envisioned as

an extensive network of RNA and RNA binding proteins, comprised of decapping enzymes associated with the mRNA degradation machinery and translation repression. In addition to their role in mRNA degradation, P bodies can also serve as cellular temporal storage sites for mRNAs that can later return and resume translation (66) (67). In eukaryotic cells, two mRNA decay pathways have been identified, both of which begin with removing the 3′ poly (A) tail in a process referred to as deadenylation. Following deadenylation, mRNAs can be degraded in a 3′ to 5′ direction by an exonuclease complex termed the exosome. Alternatively, the mRNA can be decapped by the DCP1/DCP2 decapping enzyme, rendering the mRNA susceptible to 5′ to 3′ degradation by the exonuclease XRN1 (68).

Interestingly, we have demonstrated an important role for SNV N in cap snatching (65). N was shown to bind and protect the 5' caps of cellular mRNAs from degradation and store them in the P-bodies. This pool of sequestered capped oligoribonucleotides in P-bodies is later used as primers by the viral L protein during transcription initiation. Moreover, it was shown that SNV N has distinct cap- and RNA-binding sites (69). N undergoes a conformational change upon binding of the capped RNA primer at the cap-binding site. It was suggested that the conformationally altered N with a capped primer loaded at the cap-binding site binds specifically to the conserved 3′ nine nucleotides of viral RNA and assists the bound primer to anneal at the 3′ terminus, therefore facilitating transcription initiation (69).

5. MODULATION OF THE HOST CELL TRANSLATION MACHINERY

The mRNAs in eukaryotic cells carry 7-methylguanosine (m7G) cap at their 5' ends. Translation of these capped mRNAs is a complex process and is initiated with the recruitment of eIF4F cap binding complex at the 5' cap, followed by loading of the 43S-preinitiation ribosome complex at the cap with the assistance of the eIF4F complex. The eIF4F complex is an amalgam of three initiation factors: eIF4E (cap binding protein), eIF4A (DEAD box RNA helicase that dissociates the secondary structures at the 5' UTR of mRNA) and eIF4G (linking peptide that stabilizes the ribosome at the 5' mRNA cap via interaction with eIF3 (70) (71). The 43S pre-initiation complex contains the eukaryotic initiation factors 3, 1, 1A, 5 and a ternary complex composed of methionine-loaded initiator tRNA and eIF2 coupled with GTP. Recruitment of the 43S complex to capped mRNA by the cap-binding complex enables subsequent scanning of the mRNA from the 5' end, and when a start AUG codon is encountered, the 60S large ribosomal subunit is recruited and translation begins (72).

Viruses rely on the host cell translation machinery for the translation of their own mRNAs. Confronted by the tough competition with cellular mRNAs for the same translation apparatus, viruses have evolved several strategies to hijack the cellular translation machinery (73). Some viruses, such as picornaviruses and flaviviruses, shut down host but not viral mRNA translation. These viruses have evolved a cap-independent mechanism of translation that is mediated by an internal ribosome entry site (IRES), which is a cis-acting RNA high-order structural elements in the 5′ UTR of the viral mRNA, and is capable of recruiting ribosomes without the need for the eIF4F cap-binding complex or some of its components (74) (75) (76).

Hantaviruses have evolved a unique translation mechanism operated by the viral N protein, that lures the host translation machinery for the preferential translation of viral transcripts. N is a surrogate of the entire eIF4F cap-binding complex, and therefore functions as a multifunctional translation initiation factor. N also binds to the ribosomal protein S19 (RPS19), located at the head region of the 40S ribosomal subunit, and subsequently helps recruiting the 43S pre-initiation complex at the mRNA cap (77) (78) (79). We have shown that N augments translation of both viral and non-viral mRNA. However, competitive translation reactions containing both viral and non-viral mRNA revealed that the translation of mRNA containing the viral mRNA 5' UTR was more robust compared to mRNA containing non-viral leader sequence. Mutational analysis of the viral mRNA 5'UTR revealed that the sequence "GUAGUAG" of the triplet repeat motif was sufficient for preferential N-mediated translation initiation and for high-affinity binding of N to the UTR (80).

6. MODULATION OF THE CYTOSKELETAL PROTEINS FOR PROPER TRAFFICKING AND ASSEMBLY

The cellular cytoskeleton is composed of a complex network of three types of filaments: actin microfilaments, microtubules and intermediate filaments. The cytoskeleton serves many key structural functions in the biology of the cell, including: providing mechanical strength, motility, organelle anchoring and intracellular transport (81). The host cytoskeleton also plays important roles in the life cycle of many viruses. Due to the complexity of the cellular interior, the movement of virions, subviral particles and viral proteins within the cell cannot simply rely on passive diffusion. Therefore, cytoplasmic transport of viral cargos is an active process that is mediated by interactions between the cytoskeletal filaments and motor proteins. Three classes of cytoskeletal motor proteins are known: myosin, which interacts with actin microfilaments, and two types of microtubule-interacting motors, kinesin and dynein. In order for these motors to perform their mechanical function, which is binding to cytoskeletal filaments and moving cargos along them, they use energy derived from ATP hydrolysis (82) (83). Viruses exploit the host cytoskeleton and their motors to move on microtubules toward the cell interior during entry and to move assembling virus particles toward the cells membrane during egress. Many viruses interact with actin at different stages during their replication cycle, both disrupting and rearranging the actin cytoskeleton to their own advantages (84) (85) (86).

In contrast to the majority of enveloped viruses that mature by budding from the plasma membrane, many members of the *Bunyaviridae* family, especially Old World hantaviruses, were shown to bud into the lumen of the Golgi complex. Progeny virions are then released by fusion of secretory vesicles with the plasma membrane (87) (12). However, it was suggested that the assembly of New World hantaviruses [e.g. SNV and Black Creek Canal virus (BCCV)] might occur at the plasma membrane (88). Several reports have pointed out that the components of the microtubule and actin cytoskeleton are essential for the replication and morphongenesis of both Old and New World hantaviruses (89) (90) (91) (92). Ravkov et al have shown that N of BCCV binds and colocalizes with actin filaments (89). Moreover, disruption of actin filaments with cytochalasin D (Cyt D), an actin microfilament-depolymerizing drug, led to inhibition of virus release. This finding has indicated that actin filaments play an important role in the transport of viral ribonucleoproteins (RNPs) to the plasma membrane where the assembly and release of this hantavirus occurs.

Using antibodies to various subcellular compartments, Ramanathan et al showed that the N of the Old World HTNV colocalized with the ER-Golgi intermediate compartment (ERGIC), but not with the ER, Golgi compartment, endosomes or actin markers (91). The ERGIC constitutes an independent structure that is not continuous with the ER or the Golgi compartment. ERGIC is maintained by a continuous flow of membranes mediated by motor proteins, dynein and kinesin, which transport cargo bi-directionally to the ER and to the Golgi compartment (93). Treatment of HTNV-infected cells with nocodazole (NOC), a microtubule-depolymerizing agent, caused rapid distribution of N and decreased levels of HTNV S segment vRNA. Over-expression of dynamitin, a dominant-negative form of dynien, which blocks transport on microtubules reduced N accumulation in the perinuclear region. These results suggested that microtubules are essential for the intracellular N transport and viral RNA replication of HTNV. These results also implied that N traffics to the ERGIC prior to its movement to the Golgi compartment, and an intact ERGIC is essential for virus replication. Ramanathan and Jonsson have further extended those studies to another Old World hantavirus, SEOV, and two additional New World hantaviruses, ANDV and BCCV (92). The N protein of all of these viruses also colocalized with the ERGIC. However, distinct differences in cytoskeletal requirements for replication were detected. Immunofluorescence studies showed clear association of ANDV N protein with actin in the perinuclear region and ANDV replication was found to be sensitive to Cyt D. HTNV and SEOV were sensitive to NOC. However, BCCV's replication was affected by both Cyt D and NOC. These findings indicated that Old and New World hantaviruses share common features, but they have evolved differences in their interaction with host cytoskeletal machinery.

7. MODULATION OF THE APOPTOSIS SIGNALING PATHWAYS

Hantaviruses replicate in the cytoplasm of endothelial cells and several mammalian cell lines without causing any pronounced cytopathic effect. However, several studies have suggested that Old and New World hantaviruses (HTNV, ANDV, SEOV, TULV and PUUV) induce apoptosis (94) (95) (96) (97) (98). The role of apoptosis in the hantavirus life cycle and the viral protein responsible for its induction are poorly understood. The ability of viruses to regulate the induction of cellular apoptosis during infection is critical for viral survival. Several studies have suggested that hantaviruses use some unique approaches to block apoptosis, and therefore enhance its ability to replicate in infected cells.

Daxx is a well-known Fas-mediated apoptosis enhancer, which transduces death signals through the Jun N-terminal kinase (JNK) pathway (99). Yeast two-hybrid screening of a cDNA library from Hela cells has revealed that PUUV N interacts with Daxx (100). This interaction was further confirmed by GST pull-down assay, co-immunoprecipitation and co-localization studies. It was suggested that binding of N to Daxx might interfere with its functions, and therefore delay apoptosis (101).

Another strategy used by hantaviruses to block apoptosis is via sequestering the NF-κB in the cytoplasm, and thus inhibiting its activity. NF-κB is a transcription factor that plays a key role in initiating the host antiviral signaling and apoptotic response. It is activated by inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), through the TNF receptor family. Normally, NF-κB is sequestered in the cytoplasm by IκB, which masks its nuclear localization signal (NLS). Upon its activation, NF-κB translocates to the nucleus and serves as a transcription factor for a number of genes that are mainly associated with triggering both the host innate and adaptive immune responses (102) (103) (104). NF-κB is too large to diffuse through the nuclear pore, therefore it requires a nuclear import system consisting of importin-α and importin-β, which bind to the NLS (105). Upon TNF receptor stimulation, NF-κB was sequestered in the cytoplasm of HTNV N protein-expressing cells, which showed inhibition of caspase activation. However, cells expressing HTNV N protein truncation mutants lacking the region from amino acids 270 – 330, were unable to inhibit the nuclear import of NF-κB. Those mutants has also lost their ability to down-regulate apoptosis. Therefore, it was concluded that HTNV suppresses host apoptotic responses via blocking the trafficking of NF-κB into the nucleus either by binding to it directly or by binding to importin-α molecules responsible for its nuclear shuttling (47) (48).

8. MODULATION OF THE SUMOYLATION MACHINERY FOR PROPER N SUBCELLULAR LOCALIZATION AND ASSEMBLY

SUMOylation is a post-translational modification that occurs to many cellular and viral proteins. It involves the conjugation of a small protein, known as SUMO (small ubiquitin-related modifier), to the lysine side chain of the target protein via isopeptide bonding. It is a multi-step process that involves four enzymatic reactions. First, SUMO proteins are post-translationally processed by SUMO proteases to expose a C-terminal diglycine motif,

which can then form a thioester bond with the catalytic cysteine residue of the E1-activating enzyme, the SAE1/SAE2 (SUMO activating enzyme) heterodimer. This activation step is then followed by conjugation of the activated SUMO to the E2-conjugating enzyme Ubc9 (ubiquitin-conjugating enzyme) through the formation of another thioester bond. Finally, an isopeptide bond is formed between SUMO proteins and the target protein through the mutual action of Ubc9 and E3 SUMO protein ligases (106) (107) (108). The functional consequences of SUMOylation seem to be substrate-specific, but have generally been implicated in regulating stability, protein–protein interactions and subcellular localization of target proteins (109).

Viruses have evolved to utilize or subvert the SUMOylation system to their own benefit (110) (111). Some viruses, such as human cytomegalovirus, influenza, hepatitis delta virus and human papilloma virus, can have their proteins SUMOylated (112) (113) (114) (115). Other viruses such as dengue virus and hantaviruses express proteins that can interact with components of the SUMOylation machinery (116) (117) (118). Yet, some other viruses have evolved strategies to counteract SUMOylation such as adenoviruses and SARS coronavirus (119) (120). The outcome of all of these types of interactions is to modulate the cellular environment to become more favorable for viral replication. Regulation of viral protein subcellular localization and assembly by interaction with components of the SUMOylation machinery has been also reported for other viruses. The E1 protein of papillomavirus is a SUMOylated nuclear protein that is essential for viral genome replication. SUMOylation-deficient E1 mutants were shown to accumulate in the cytoplasm and perinuclear region rather than the nucleus. These results indicate that sumoylation is critical for nuclear accumulation of E1 protein (121) (122). More recently, it was reported that influenza A virus matrix protein (M1) is SUMOylated (115). Abolishment of M1 SUMOylation resulted in dramatic reduction of the virus titer. Further analysis revealed that the lack of M1 SUMOylation prevented the nuclear export of vRNP, and the subsequent virus assembly and release.

Yeast two-hybrid screening of human cDNA libraries has revealed that HTNV and SEOV N proteins interact not only with SUMO-1 conjugating enzyme (Ubc9), but also with the SUMO-1-interacting proteins PIAS1, PIASxβ, HIPK2, CHD3, and TTRAP (117) (123). Analysis of the interaction between truncated N proteins and Ubc9 revealed that the amino acid residues 101 – 238 of HTNV and 100 – 125 of SEOV N proteins were responsible for this interaction. Further yeast two-hybrid screening of host proteins revealed that TULV N protein also interacts with SUMO-1 and SUMO-1 conjugating enzyme, Ubc9 (116). In these studies, the N and SUMO-1 were shown to colocalize at the perinuclear area. The N itself was not found to be SUMOylated both *in vivo* and *in vitro*. However, it was possible to correlate between the degree of interaction of truncated N proteins with SUMO-1 or Ubc9 and differences in the localization patterns of the mutants in the cytoplasm. Therefore, it was concluded that the interaction of the N with Ubc9 or SUMO-1 is necessary for the proper subcellular localization of N at the perinuclear region where virus assembly is believed to occur.

9. CONCLUSION AND FUTURE PERSPECTIVES

We have witnessed over the past few decades the emergence of several viral disease epidemics of zoonotic origins. Hantaviruses have firstly emerged as causes of serious human illness in the early 1950s during the Korean War. However, it was not until 1981 when the causative agent was identified. The second major outbreak, which occurred in the southwestern region of the US in 1993, with its high fatality rate, has alarmed the world's population to the highly pathogenic potential of this group of viruses. Despite several efforts to develop a vaccine or antiviral drug to protect or treat hantavirus infection, an FDA-approved control strategy is still lacking. Progress in hantavirus research was hindered by two major roadblocks, namely the lack of a reliable animal model and reverse genetics system. Understanding the molecular details of hantavirus-replication cycle is of paramount significance for identifying novel targets for inhibiting virus replication. As outlined in this review, although several mechanisms for how hantaviruses exploit cellular machineries and signaling pathways for their own advantage have been identified, more work is needed to define steps that can be interrupted with chemical inhibitors without interrupting major cellular functions. One newly identified promising target for inhibiting hantavirus replication is the N-mediated mechanism of translation initiation. Chemical compounds that can selectively inhibit N-mediated translation mechanism will likely interfere in the translation efficiency of viral mRNAs and consequently inhibit virus replication in infected cells. High-throughput screening of chemical libraries is currently underway to identify positive leads that would set the stage for the development of specific anti-hantavirus drugs.

10. REFERENCES

1. J. Hooper and C. S. Schmaljohn: Hantavirus. In: *The Springer Index of Viruses*. Ed C. A. Tidona&G. Darai. Springer-Verlag, Berlin Heidelberg New York (2001)

2. C. A. Hart and M. Bennett: Hantavirus infections: epidemiology and pathogenesis. *Microbes Infect*, 1(14), 1229-37 (1999)

3. J. D. Easterbrook and S. L. Klein: Immunological mechanisms mediating hantavirus persistence in rodent reservoirs. *PLoS Pathog*, 4(11), e1000172 (2008)

4. P. J. Padula, A. Edelstein, S. D. Miguel, N. M. Lopez, C. M. Rossi and R. D. Rabinovich: Hantavirus pulmonary syndrome outbreak in Argentina: molecular evidence for person-to-person transmission of Andes virus. *Virology*, 241(2), 323-30 (1998)

5. S. Q. Simpson, L. Spikes, S. Patel and I. Faruqi: Hantavirus pulmonary syndrome. *Infect Dis Clin North Am*, 24(1), 159-73 (2010)

6. C. B. Jonsson, L. T. Figueiredo and O. Vapalahti: A global perspective on hantavirus ecology, epidemiology, and disease. *Clin Microbiol Rev*, 23(2), 412-41 (2010)

7. S. T. Nichol, C. F. Spiropoulou, S. Morzunov, P. E. Rollin, T. G. Ksiazek, H. Feldmann, A. Sanchez, J. Childs, S. Zaki and C. J. Peters: Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. *Science*, 262(5135), 914-7 (1993)

8. B. Hjelle, S. Jenison, N. Torrez-Martinez, T. Yamada, K. Nolte, R. Zumwalt, K. MacInnes and G. Myers: A novel hantavirus associated with an outbreak of fatal respiratory disease in the southwestern United States: evolutionary relationships to known hantaviruses. *J Virol*, 68(2), 592-6 (1994)

9. V. E. Chizhikov, C. F. Spiropoulou, S. P. Morzunov, M. C. Monroe, C. J. Peters and S. T. Nichol: Complete genetic characterization and analysis of isolation of Sin Nombre virus. *J Virol*, 69(12), 8132-6 (1995)

10. S. K. Kukkonen, A. Vaheri and A. Plyusnin: Completion of the Tula hantavirus genome sequence: properties of the L segment and heterogeneity found in the 3' termini of S and L genome RNAs. *J Gen Virol*, 79 (Pt 11), 2615-22 (1998)

11. A. Plyusnin: Genetics of hantaviruses: implications to taxonomy. *Arch Virol*, 147(4), 665-82 (2002)

12. C. S. Schmaljohn and S. T. Nichol: Bunyaviridae. In: *Fields Virology*. Ed D. M. Knipe&P. M. Howley. Lippincott Williams & Wilkins, Philadelphia (2007)

13. A. Rang: Modulation of innate immune responses by hantaviruses. *Crit Rev Immunol*, 30(6), 515-27 (2010)

14. I. T. Hussein, A. Haseeb, A. Haque and M. A. Mir: Recent advances in hantavirus molecular biology and disease. *Adv Appl Microbiol*, 74, 35-75 (2011)

15. M. Jin, J. Park, S. Lee, B. Park, J. Shin, K. J. Song, T. I. Ahn, S. Y. Hwang, B. Y. Ahn and K. Ahn: Hantaan virus enters cells by clathrin-dependent receptor-mediated endocytosis. *Virology*, 294(1), 60-9 (2002)

16. M. A. Mir and A. T. Panganiban: Trimeric hantavirus nucleocapsid protein binds specifically to the viral RNA panhandle. *J Virol*, 78(15), 8281-8 (2004)

17. M. A. Mir and A. T. Panganiban: The hantavirus nucleocapsid protein recognizes specific features of the viral RNA panhandle and is altered in conformation upon RNA binding. *J Virol*, 79(3), 1824-35 (2005)

18. J. Hepojoki, T. Strandin, H. Wang, O. Vapalahti, A. Vaheri and H. Lankinen: Cytoplasmic tails of hantavirus glycoproteins interact with the nucleocapsid protein. *J Gen Virol*, 91(Pt 9), 2341-50 (2010)

19. S. Koyama, K. J. Ishii, C. Coban and S. Akira: Innate immune response to viral infection. *Cytokine*, 43(3), 336-41 (2008)

20. E. Meylan and J. Tschopp: Toll-like receptors and RNA helicases: two parallel ways to trigger antiviral responses. *Mol Cell*, 22(5), 561-9 (2006)

21. O. Takeuchi and S. Akira: Recognition of viruses by innate immunity. *Immunol Rev*, 220, 214-24 (2007)

22. J. Hiscott: Triggering the innate antiviral response through IRF-3 activation. *J Biol Chem*, 282(21), 15325-9 (2007)

23. E. C. Borden, G. C. Sen, G. Uze, R. H. Silverman, R. M. Ransohoff, G. R. Foster and G. R. Stark: Interferons at age 50: past, current and future impact on biomedicine. *Nat Rev Drug Discov*, 6(12), 975-90 (2007)

24. G. R. Stark: How cells respond to interferons revisited: from early history to current complexity. *Cytokine Growth Factor Rev*, 18(5-6), 419-23 (2007)

25. O. Haller, M. Frese and G. Kochs: Mx proteins: mediators of innate resistance to RNA viruses. *Rev Sci Tech*, 17(1), 220-30 (1998)

26. M. Frese, G. Kochs, H. Feldmann, C. Hertkorn and O. Haller: Inhibition of bunyaviruses, phleboviruses, and hantaviruses by human MxA protein. *J Virol*, 70(2), 915-23 (1996)

27. M. Kanerva, K. Melen, A. Vaheri and I. Julkunen: Inhibition of puumala and tula hantaviruses in Vero cells by MxA protein. *Virology*, 224(1), 55-62 (1996)

28. G. Kochs, C. Janzen, H. Hohenberg and O. Haller: Antivirally active MxA protein sequesters La Crosse virus nucleocapsid protein into perinuclear complexes. *Proc Natl Acad Sci U S A*, 99(5), 3153-8 (2002)

29. M. N. Pensiero, J. B. Sharefkin, C. W. Dieffenbach and J. Hay: Hantaan virus infection of human endothelial cells. *J Virol*, 66(10), 5929-36 (1992)

30. J. Prescott, C. Ye, G. Sen and B. Hjelle: Induction of innate immune response genes by Sin Nombre hantavirus does not require viral replication. *J Virol*, 79(24), 15007-15 (2005)

31. J. B. Prescott, P. R. Hall, V. S. Bondu-Hawkins, C. Ye and B. Hjelle: Early innate immune responses to Sin Nombre hantavirus occur independently of IFN regulatory factor 3, characterized pattern recognition receptors, and viral entry. *J Immunol*, 179(3), 1796-802 (2007)

32. W. Handke, R. Oelschlegel, R. Franke, D. H. Kruger and A. Rang: Hantaan virus triggers TLR3-dependent innate immune responses. *J Immunol*, 182(5), 2849-58 (2009)

33. G. A. Versteeg and A. Garcia-Sastre: Viral tricks to grid-lock the type I interferon system. *Curr Opin Microbiol*, 13(4), 508-16 (2010)

34. E. Geimonen, S. Neff, T. Raymond, S. S. Kocer, I. N. Gavrilovskaya and E. R. Mackow: Pathogenic and nonpathogenic hantaviruses differentially regulate endothelial cell responses. *Proc Natl Acad Sci U S A*, 99(21), 13837-42 (2002)

35. A. A. Kraus, M. J. Raftery, T. Giese, R. Ulrich, R. Zawatzky, S. Hippenstiel, N. Suttorp, D. H. Kruger and G. Schonrich: Differential antiviral response of endothelial cells after infection with pathogenic and nonpathogenic hantaviruses. *J Virol*, 78(12), 6143-50 (2004)

36. P. J. Alff, I. N. Gavrilovskaya, E. Gorbunova, K. Endriss, Y. Chong, E. Geimonen, N. Sen, N. C. Reich and E. R. Mackow: The pathogenic NY-1 hantavirus G1 cytoplasmic tail inhibits RIG-I- and TBK-1-directed interferon responses. *J Virol*, 80(19), 9676-86 (2006)

37. C. F. Spiropoulou, C. S. Goldsmith, T. R. Shoemaker, C. J. Peters and R. W. Compans: Sin Nombre virus glycoprotein trafficking. *Virology*, 308(1), 48-63 (2003)

38. E. Geimonen, R. LaMonica, K. Springer, Y. Farooqui, I. N. Gavrilovskaya and E. R. Mackow: Hantavirus pulmonary syndrome-associated hantaviruses contain conserved and functional ITAM signaling elements. *J Virol*, 77(2), 1638-43 (2003)

39. L. B. Ivashkiv: Cross-regulation of signaling by ITAM-associated receptors. *Nat Immunol*, 10(4), 340-7 (2009)

40. E. Geimonen, I. Fernandez, I. N. Gavrilovskaya and E. R. Mackow: Tyrosine residues direct the ubiquitination and degradation of the NY-1 hantavirus G1 cytoplasmic tail. *J Virol*, 77(20), 10760-868 (2003)

41. N. Sen, A. Sen and E. R. Mackow: Degrons at the C terminus of the pathogenic but not the nonpathogenic hantavirus G1 tail direct proteasomal degradation. *J Virol*, 81(8), 4323-30 (2007)

42. H. Wang, T. Strandin, J. Hepojoki, H. Lankinen and A. Vaheri: Degradation and aggresome formation of the Gn tail of the apathogenic Tula hantavirus. *J Gen Virol*, 90(Pt 12), 2995-3001 (2009)

43. P. J. Alff, N. Sen, E. Gorbunova, I. N. Gavrilovskaya and E. R. Mackow: The NY-1 hantavirus Gn cytoplasmic tail coprecipitates TRAF3 and inhibits cellular interferon responses by disrupting TBK1-TRAF3 complex formation. *J Virol*, 82(18), 9115-22 (2008)

44. M. Bouloy, C. Janzen, P. Vialat, H. Khun, J. Pavlovic, M. Huerre and O. Haller: Genetic evidence for an interferon-antagonistic function of rift valley fever virus nonstructural protein NSs. *J Virol*, 75(3), 1371-7 (2001)

45. A. Bridgen, F. Weber, J. K. Fazakerley and R. M. Elliott: Bunyamwera bunyavirus nonstructural protein NSs is a nonessential gene product that contributes to viral pathogenesis. *Proc Natl Acad Sci U S A*, 98(2), 664-9 (2001)

46. K. M. Jaaskelainen, P. Kaukinen, E. S. Minskaya, A. Plyusnina, O. Vapalahti, R. M. Elliott, F. Weber, A. Vaheri and A. Plyusnin: Tula and Puumala hantavirus NSs ORFs are functional and the products inhibit activation of the interferon-beta promoter. *J Med Virol*, 79(10), 1527-36 (2007)

47. S. L. Taylor, N. Frias-Staheli, A. Garcia-Sastre and C. S. Schmaljohn: Hantaan virus nucleocapsid protein binds to importin alpha proteins and inhibits tumor necrosis factor alpha-induced activation of nuclear factor kappa B. *J Virol*, 83(3), 1271-9 (2009)

48. S. J. Ontiveros, Q. Li and C. B. Jonsson: Modulation of apoptosis and immune signaling pathways by the Hantaan virus nucleocapsid protein. *Virology*, 401(2), 165-78 (2010)

49. S. L. Taylor, R. L. Krempel and C. S. Schmaljohn: Inhibition of TNF-alpha-induced activation of NF-kappaB by hantavirus nucleocapsid proteins. *Ann N Y Acad Sci*, 1171 Suppl 1, E86-93 (2009)

50. J. R. Levine, J. Prescott, K. S. Brown, S. M. Best, H. Ebihara and H. Feldmann: Antagonism of type I interferon responses by new world hantaviruses. *J Virol*, 84(22), 11790-801 (2010)

51. R. M. Elliott: Negative-Strand RNA Virus Replication. In: *Topley and Wilson's Microbiology and Microbial Infections*. Ed B. H. Mahy&V. Meulen. Edward Arnold Ltd., (2007)

52. R. M. Krug, M. A. Morgan and A. J. Shatkin: Influenza viral mRNA contains internal N6-methyladenosine and 5'-terminal 7-methylguanosine in cap structures. *J Virol*, 20(1), 45-53 (1976)

53. S. J. Plotch, J. Tomasz and R. M. Krug: Absence of detectable capping and methylating enzymes in influenza virions. *J Virol*, 28(1), 75-83 (1978)

54. R. Dhar, R. M. Chanock and C. J. Lai: Nonviral oligonucleotides at the 5' terminus of cytoplasmic influenza viral mRNA deduced from cloned complete genomic sequences. *Cell*, 21(2), 495-500 (1980)

55. S. J. Plotch, M. Bouloy, I. Ulmanen and R. M. Krug: A unique cap(m7GpppXm)-dependent influenza virion endonuclease cleaves capped RNAs to generate the primers that initiate viral RNA transcription. *Cell*, 23(3), 847-58 (1981)

56. T. Samji: Influenza A: understanding the viral life cycle. *Yale J Biol Med*, 82(4), 153-9 (2009)

57. D. Guilligay, F. Tarendeau, P. Resa-Infante, R. Coloma, T. Crepin, P. Sehr, J. Lewis, R. W. Ruigrok, J. Ortin, D. J. Hart and S. Cusack: The structural basis for cap binding by influenza virus polymerase subunit PB2. *Nat Struct Mol Biol*, 15(5), 500-6 (2008)

58. A. Dias, D. Bouvier, T. Crepin, A. A. McCarthy, D. J. Hart, F. Baudin, S. Cusack and R. W. Ruigrok: The cap-snatching endonuclease of influenza virus polymerase resides in the PA subunit. *Nature*, 458(7240), 914-8 (2009)

59. P. Yuan, M. Bartlam, Z. Lou, S. Chen, J. Zhou, X. He, Z. Lv, R. Ge, X. Li, T. Deng, E. Fodor, Z. Rao and Y. Liu: Crystal structure of an avian influenza polymerase PA(N) reveals an endonuclease active site. *Nature*, 458(7240), 909-13 (2009)

60. J. L. Patterson, B. Holloway and D. Kolakofsky: La Crosse virions contain a primer-stimulated RNA polymerase and a methylated cap-dependent endonuclease. *J Virol*, 52(1), 215-22 (1984)

61. R. Raju, L. Raju, D. Hacker, D. Garcin, R. Compans and D. Kolakofsky: Nontemplated bases at the 5' ends of Tacaribe virus mRNAs. *Virology*, 174(1), 53-9 (1990)

62. D. Garcin, M. Lezzi, M. Dobbs, R. M. Elliott, C. Schmaljohn, C. Y. Kang and D. Kolakofsky: The 5' ends of Hantaan virus (Bunyaviridae) RNAs suggest a prime-and-realign mechanism for the initiation of RNA synthesis. *J Virol*, 69(9), 5754-62 (1995)

63. J. Reguera, F. Weber and S. Cusack: Bunyaviridae RNA polymerases (L-protein) have an N-terminal, influenza-like endonuclease domain, essential for viral cap-dependent transcription. *PLoS Pathog*, 6(9) (2010)

64. B. Morin, B. Coutard, M. Lelke, F. Ferron, R. Kerber, S. Jamal, A. Frangeul, C. Baronti, R. Charrel, X. de Lamballerie, C. Vonrhein, J. Lescar, G. Bricogne, S. Gunther and B. Canard: The N-terminal domain of the arenavirus L protein is an RNA endonuclease essential in mRNA transcription. *PLoS Pathog*, 6(9) (2010)

65. M. A. Mir, W. A. Duran, B. L. Hjelle, C. Ye and A. T. Panganiban: Storage of cellular 5' mRNA caps in P bodies for viral cap-snatching. *Proc Natl Acad Sci U S A*, 105(49), 19294-9 (2008)

66. A. Eulalio, I. Behm-Ansmant and E. Izaurralde: P bodies: at the crossroads of post-transcriptional pathways. *Nat Rev Mol Cell Biol*, 8(1), 9-22 (2007)

67. C. J. Beckham and R. Parker: P bodies, stress granules, and viral life cycles. *Cell Host Microbe*, 3(4), 206-12 (2008)

68. R. Parker and U. Sheth: P bodies and the control of mRNA translation and degradation. *Mol Cell*, 25(5), 635-46 (2007)

69. M. A. Mir, S. Sheema, A. Haseeb and A. Haque: Hantavirus nucleocapsid protein has distinct m7G cap- and RNA-binding sites. *J Biol Chem*, 285(15), 11357-68 (2010)

70. T. E. Dever: Translation initiation: adept at adapting. *Trends Biochem Sci*, 24(10), 398-403 (1999)

71. A. C. Gingras, B. Raught and N. Sonenberg: eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. *Annu Rev Biochem*, 68, 913-63 (1999)

72. R. J. Jackson, C. U. Hellen and T. V. Pestova: The mechanism of eukaryotic translation initiation and principles of its regulation. *Nat Rev Mol Cell Biol*, 11(2), 113-27 (2010)

73. M. Bushell and P. Sarnow: Hijacking the translation apparatus by RNA viruses. *J Cell Biol*, 158(3), 395-9 (2002)

74. D. Etchison, S. C. Milburn, I. Edery, N. Sonenberg and J. W. Hershey: Inhibition of HeLa cell protein synthesis following poliovirus infection correlates with the proteolysis of a 220,000-dalton polypeptide associated with eucaryotic initiation factor 3 and a cap binding protein complex. *J Biol Chem*, 257(24), 14806-10 (1982)

75. J. Pelletier and N. Sonenberg: Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. *Nature*, 334(6180), 320-5 (1988)

76. E. Martinez-Salas, A. Pacheco, P. Serrano and N. Fernandez: New insights into internal ribosome entry site elements relevant for viral gene expression. *J Gen Virol*, 89(Pt 3), 611-26 (2008)

77. M. A. Mir and A. T. Panganiban: A protein that replaces the entire cellular eIF4F complex. *EMBO J*, 27(23), 3129-39 (2008)

78. A. Haque and M. A. Mir: Interaction of hantavirus nucleocapsid protein with ribosomal protein S19. *J Virol*, 84(23), 12450-3 (2010)

79. E. Cheng, A. Haque, M. A. Rimmer, I. T. Hussein, S. Sheema, A. Little and M. A. Mir: Characterization of the Interaction between Hantavirus Nucleocapsid Protein (N) and Ribosomal Protein S19 (RPS19). *J Biol Chem*, 286(13), 11814-24 (2011)

80. M. A. Mir and A. T. Panganiban: The triplet repeats of the Sin Nombre hantavirus 5' untranslated region are sufficient in cis for nucleocapsid-mediated translation initiation. *J Virol*, 84(17), 8937-44 (2010)

81. D. A. Fletcher and R. D. Mullins: Cell mechanics and the cytoskeleton. *Nature*, 463(7280), 485-92 (2010)

82. L. S. Goldstein and Z. Yang: Microtubule-based transport systems in neurons: the roles of kinesins and dyneins. *Annu Rev Neurosci*, 23, 39-71 (2000)

83. B. Sodeik: Mechanisms of viral transport in the cytoplasm. *Trends Microbiol*, 8(10), 465-72 (2000)

84. G. A. Smith and L. W. Enquist: Break ins and break outs: viral interactions with the cytoskeleton of Mammalian cells. *Annu Rev Cell Dev Biol*, 18, 135-61 (2002)

85. K. Radtke, K. Dohner and B. Sodeik: Viral interactions with the cytoskeleton: a hitchhiker's guide to the cell. *Cell Microbiol*, 8(3), 387-400 (2006)

86. M. P. Taylor, O. O. Koyuncu and L. W. Enquist: Subversion of the actin cytoskeleton during viral infection. *Nat Rev Microbiol*, 9(6), 427-39 (2011)

87. E. Kuismanen, K. Hedman, J. Saraste and R. F. Pettersson: Uukuniemi virus maturation: accumulation of virus particles and viral antigens in the Golgi complex. *Mol Cell Biol*, 2(11), 1444-58 (1982)

88. E. V. Ravkov, S. T. Nichol and R. W. Compans: Polarized entry and release in epithelial cells of Black Creek Canal virus, a New World hantavirus. *J Virol*, 71(2), 1147-54 (1997)

89. E. V. Ravkov, S. T. Nichol, C. J. Peters and R. W. Compans: Role of actin microfilaments in Black Creek Canal virus morphogenesis. *J Virol*, 72(4), 2865-70 (1998)

90. I. Andersson, M. Simon, A. Lundkvist, M. Nilsson, A. Holmstrom, F. Elgh and A. Mirazimi: Role of actin filaments in targeting of Crimean Congo hemorrhagic fever virus nucleocapsid protein to perinuclear regions of mammalian cells. *J Med Virol*, 72(1), 83-93 (2004)

91. H. N. Ramanathan, D. H. Chung, S. J. Plane, E. Sztul, Y. K. Chu, M. C. Guttieri, M. McDowell, G. Ali and C. B. Jonsson: Dynein-dependent transport of the hantaan virus nucleocapsid protein to the endoplasmic reticulum-Golgi intermediate compartment. *J Virol*, 81(16), 8634-47 (2007)

92. H. N. Ramanathan and C. B. Jonsson: New and Old World hantaviruses differentially utilize host cytoskeletal components during their life cycles. *Virology*, 374(1), 138-50 (2008)

93. J. Klumperman, A. Schweizer, H. Clausen, B. L. Tang, W. Hong, V. Oorschot and H. P. Hauri: The recycling pathway of protein ERGIC-53 and dynamics of the ER-Golgi intermediate compartment. *J Cell Sci*, 111 (Pt 22), 3411-25 (1998)

94. J. I. Kang, S. H. Park, P. W. Lee and B. Y. Ahn: Apoptosis is induced by hantaviruses in cultured cells. *Virology*, 264(1), 99-105 (1999)

95. A. Markotic, L. Hensley, T. Geisbert, K. Spik and C. Schmaljohn: Hantaviruses induce cytopathic effects and apoptosis in continuous human embryonic kidney cells. *J Gen Virol*, 84(Pt 8), 2197-202 (2003)

96. X. D. Li, S. Kukkonen, O. Vapalahti, A. Plyusnin, H. Lankinen and A. Vaheri: Tula hantavirus infection of Vero E6 cells induces apoptosis involving caspase 8 activation. *J Gen Virol*, 85(Pt 11), 3261-8 (2004)

97. X. D. Li, H. Lankinen, N. Putkuri, O. Vapalahti and A. Vaheri: Tula hantavirus triggers pro-apoptotic signals of ER stress in Vero E6 cells. *Virology*, 333(1), 180-9 (2005)

98. J. Klingstrom, J. Hardestam, M. Stoltz, B. Zuber, A. Lundkvist, S. Linder and C. Ahlm: Loss of cell membrane integrity in puumala hantavirus-infected patients correlates with levels of epithelial cell apoptosis and perforin. *J Virol*, 80(16), 8279-82 (2006)

99. X. Yang, R. Khosravi-Far, H. Y. Chang and D. Baltimore: Daxx, a novel Fas-binding protein that activates JNK and apoptosis. *Cell*, 89(7), 1067-76 (1997)

100. X. D. Li, T. P. Makela, D. Guo, R. Soliymani, V. Koistinen, O. Vapalahti, A. Vaheri and H. Lankinen: Hantavirus nucleocapsid protein interacts with the Fas-mediated apoptosis enhancer Daxx. *J Gen Virol*, 83(Pt 4), 759-66 (2002)

101. P. Kaukinen, A. Vaheri and A. Plyusnin: Hantavirus nucleocapsid protein: a multifunctional molecule with both housekeeping and ambassadorial duties. *Arch Virol*, 150(9), 1693-713 (2005)

102. A. A. Beg, S. M. Ruben, R. I. Scheinman, S. Haskill, C. A. Rosen and A. S. Baldwin, Jr.: I kappa B interacts with the nuclear localization sequences of the subunits of NF-kappa B: a mechanism for cytoplasmic retention. *Genes Dev*, 6(10), 1899-913 (1992)

103. H. Wajant, K. Pfizenmaier and P. Scheurich: Tumor necrosis factor signaling. *Cell Death Differ*, 10(1), 45-65 (2003)

104. M. S. Hayden and S. Ghosh: Signaling to NF-kappaB. *Genes Dev*, 18(18), 2195-224 (2004)

105. A. Lange, R. E. Mills, C. J. Lange, M. Stewart, S. E. Devine and A. H. Corbett: Classical nuclear localization signals: definition, function, and interaction with importin alpha. *J Biol Chem*, 282(8), 5101-5 (2007)

106. F. Melchior: SUMO--nonclassical ubiquitin. *Annu Rev Cell Dev Biol*, 16, 591-626 (2000)

107. R. T. Hay: SUMO: a history of modification. *Mol Cell*, 18(1), 1-12 (2005)

108. R. Geiss-Friedlander and F. Melchior: Concepts in sumoylation: a decade on. *Nat Rev Mol Cell Biol*, 8(12), 947-56 (2007)

109. V. G. Wilson and D. Rangasamy: Intracellular targeting of proteins by sumoylation. *Exp Cell Res*, 271(1), 57-65 (2001)

110. V. G. Wilson and D. Rangasamy: Viral interaction with the host cell sumoylation system. *Virus Res*, 81(1-2), 17-27 (2001)

111. R. Boggio and S. Chiocca: Viruses and sumoylation: recent highlights. *Curr Opin Microbiol*, 9(4), 430-6 (2006)

112. C. H. Tseng, T. S. Cheng, C. Y. Shu, K. S. Jeng and M. M. Lai: Modification of small hepatitis delta virus antigen by SUMO protein. *J Virol*, 84(2), 918-27 (2009)

113. E. T. Kim, Y. E. Kim, Y. H. Huh and J. H. Ahn: Role of noncovalent SUMO binding by the human cytomegalovirus IE2 transactivator in lytic growth. *J Virol*, 84(16), 8111-23 (2010)

114. M. B. Marusic, N. Mencin, M. Licen, L. Banks and H. S. Grm: Modification of human papillomavirus minor capsid protein L2 by sumoylation. *J Virol*, 84(21), 11585-9 (2010)

115. C. Y. Wu, K. S. Jeng and M. M. Lai: The SUMOylation of Matrix M1 Protein Modulates the Assembly and Morphogenesis of Influenza A Virus. *J Virol* (2011)

116. P. Kaukinen, A. Vaheri and A. Plyusnin: Non-covalent interaction between nucleocapsid protein of Tula hantavirus and small ubiquitin-related modifier-1, SUMO-1. *Virus Res*, 92(1), 37-45 (2003)

117. A. Maeda, B. H. Lee, K. Yoshimatsu, M. Saijo, I. Kurane, J. Arikawa and S. Morikawa: The intracellular association of the nucleocapsid protein (NP) of hantaan virus (HTNV) with small ubiquitin-like modifier-1 (SUMO-1) conjugating enzyme 9 (Ubc9). *Virology*, 305(2), 288-97 (2003)

118. M. W. Chiu, H. M. Shih, T. H. Yang and Y. L. Yang: The type 2 dengue virus envelope protein interacts with small ubiquitin-like modifier-1 (SUMO-1) conjugating enzyme 9 (Ubc9). *J Biomed Sci*, 14(3), 429-44 (2007)

119. R. Boggio, A. Passafaro and S. Chiocca: Targeting SUMO E1 to ubiquitin ligases: a viral strategy to counteract sumoylation. *J Biol Chem*, 282(21), 15376-82 (2007)

120. Z. Fan, Y. Zhuo, X. Tan, Z. Zhou, J. Yuan, B. Qiang, J. Yan, X. Peng and G. F. Gao: SARS-CoV nucleocapsid protein binds to hUbc9, a ubiquitin conjugating enzyme of the sumoylation system. *J Med Virol*, 78(11), 1365-73 (2006)

121. D. Rangasamy, K. Woytek, S. A. Khan and V. G. Wilson: SUMO-1 modification of bovine papillomavirus E1 protein is required for intranuclear accumulation. *J Biol Chem*, 275(48), 37999-8004 (2000)

122. D. Rangasamy and V. G. Wilson: Bovine papillomavirus E1 protein is sumoylated by the host cell Ubc9 protein. *J Biol Chem*, 275(39), 30487-95 (2000)

123. B. H. Lee, K. Yoshimatsu, A. Maeda, K. Ochiai, M. Morimatsu, K. Araki, M. Ogino, S. Morikawa and J. Arikawa: Association of the nucleocapsid protein of the Seoul and Hantaan hantaviruses with small ubiquitin-like modifier-1-related molecules. *Virus Res*, 98(1), 83-91 (2003)

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