2016

Host Cell Functions In Vesicular Stomatitis Virus Replication

Phat X. Dinh
University of Nebraska - Lincoln

Anshuman Das
University of Nebraska-Lincoln

Asit K. Pattnaik
University of Nebraska - Lincoln, apattnaik2@unl.edu

Published in Biology and Pathogenesis of Rhabdo and Filoviruses

HOST CELL FUNCTIONS IN VESICULAR STOMATITIS VIRUS REPLICATION

Phat X. Dinh, Anshuman Das, and Asit K. Pattnaik

School of Veterinary Medicine and Biomedical Sciences and the Nebraska Center for Virology, University of Nebraska-Lincoln*, 109 Morrison Life Science Research Center, 4240 Fair Street, Lincoln, Nebraska 68583, USA E-mail: apattnaik2@unl.edu

Summary

Vesicular stomatitis virus (VSV), the prototypic rhabdovirus, has been used as an excellent paradigm for understanding the mechanisms of virus replication, pathogenesis, host response to virus infection and also for myriads of studies on cellular and molecular biology. Biochemical studies as well as high-throughput genomics, proteomics, and chemical approaches have revealed a plethora of cellular factors and pathways that regulate replication of VSV. These factors include those that support virus replication and also those that restrict its replication. This chapter discusses the role(s) of many of these host cell factors and pathways involved in VSV replication. Although mechanistic understanding of the roles of some of these factors in VSV replication has been obtained, the roles of many others need to be investigated for a better understanding of the virus-host cell interactions.

Introduction

Vesicular stomatitis virus (VSV) is an enveloped, non-segmented negative stranded (NNS) RNA virus in the family *Rhabdoviridae* and order *Mononegavirales*. Members of the family have been classified into eleven genera primarily based on antigenicity and phylogenetic analyses.[1] Among them, the vesiculoviruses (prototype: VSV) and the lyssaviruses [prototype: rabies virus (RABV)] are the best characterized. Novel and emerging rhabdoviruses are continuously being identified and characterized (see chapter 13), many of which are yet to be classified due to lack of genome sequence information and low number of identified representative viruses in serogroups.

The two known serotypes of VSV [Indiana (VSV_I) and New Jersey (VSV_{NJ})] share many similar properties although differences have been reported.[2] VSV_I is the most well characterized serotype. Its negative- sense RNA genome encodes 5 major proteins: the nucleocapsid protein (N), the phosphoprotein (P), the matrix (M), the glycoprotein (G) and the large polymerase protein (L). Inside the virion, the viral genome is tightly encapsidated with the N protein to form ribonucleocapsid complex (RNP or NC) with which the viral RNA dependent RNA polymerase (RdRp) complex of P-L proteins is associated. The G protein forms spikes on the surface of VSV virions and is responsible for attachment to host cells. Both M and G proteins are required for assembly and budding of infectious particles. Following entry by endocytosis, the G protein mediates fusion of the viral envelope with the endosomal membrane, thereby releasing the viral RNP into the cytoplasm. The RNP undergoes primary transcription by the associated RdRp to produce five capped and

polyadenylated mRNAs, which are translated by the cellular translation machinery to generate the viral proteins for further steps in the infection process including genome replication, secondary transcription, virus assembly, and budding.[3]

VSV has been used as a workhorse for studying virus replication mechanisms, pathogenesis, evolution, and virus-host interactions as well as understanding many aspects of cellular and molecular biology. With the development of the reverse genetics system in the early 1990's using a defective interfering (DI) particle of VSV,[4] recovery of infectious RABV and VSV soon followed.[5-7] Manipulation of full-length viral genomes and recovery of infectious viruses encoding a variety of foreign proteins have resulted in the use of these "designer viruses" as vaccine vectors (see chapter 9), as cancer therapeutics (chapter 10), and for myriads of studies on molecular and cellular biology of virus replication (chapters 3, 5, and 8) and pathogenic mechanisms (chapters 7 and 15).

Since VSV has been widely recognized as a valuable research tool with promising applications, recent efforts have focused on understanding its interaction with host cells by identification of crucial host factors using high throughput genomics, proteomics, and chemical approaches. This chapter describes our current understanding of the role(s) of the host functions in VSV replication, with a focus on recent findings on the cellular factors and pathways that regulate various steps in the viral life cycle.

Host Functions Involved in Early Stages of VSV Infection

Attachment

Although VSV has been studied for over four decades, the key cellular factor(s) driving its pantropism has not been unequivocally identified. The low density lipoproteins (LDLs)[8] and the phospholipids,[9-11] in particular, phosphatidylserine (PS)[10] were described as molecules responsible for VSV attachment to host cells. Subsequent studies revealed that PS is unlikely to serve such a role,[12] although it may be involved in a post-binding steps of virus entry. Since the extent of G protein conformational change depends on the presence of PS on the target membrane and the rate of fusion reaction was remarkably increased when the PS content was increased,[13] the function of PS in VSV infection process may likely be to support a fusion step during the uncoating of viral RNPs. Other membrane lipids, like sialoglycolipids (gangliosides) but not asialoglycolipids (cerebrosides) were also implicated in the virus attachment to host cells.[14,15] However, a recent study has identified the LDL receptor (LDLR) as the major receptor and other LDLR family members, as the alternative receptors for VSV entry.[16] LDLR family members are ubiquitously expressed in all cells and tissues across the animal kingdom and their specific interaction with the G protein may account for the pantropism of VSV.[16]

In addition, VSV binding to the target membrane may further depend on electrostatic interactions between positive charges on the G protein and negatively charged phospholipids in the cellular membranes.[13,17] The observation that removal of negatively charged polar head groups from these lipid molecules significantly reduces VSV attachment and fusion while the presence of polycation DEAE-dextran enhances VSV entry[18,19] further strengthens this contention. The observed requirement for the endoplasmic reticulum (ER) chaperone gp96, a component of the multiprotein complex required for protein folding in the ER, in VSV binding to cells[20] is likely to be indirect, as it is involved in glycosylation of LDLR.[16]

Entry and NC Release

The attached VSV virion is internalized by receptor-mediated endocytosis in clathrin coated vesicles[21-23] in a dynamin-2-dependent manner.[24] Since the average size of VSV (200nm long) is significantly larger than the dimensions of typical clathrin-coated pits, the vesicles used for clathrin-mediated endocytosis (CME) of VSV are not fully coated with clathrin and require actin polymerization for efficient internalization.[22] However, endocytosis of smaller VSV particles, such as the defective interfering (DI-T) particles (75nm long) does not necessitate actin assembly.[23] VSV G protein is responsible for both virus attachment and uncoating of viral RNP into the cytosol in a RAB5- dependent manner.[25] RhoB and RhoC members of the Rho GTPase family which regulate many cellular aspects such as actin dynamics, cell proliferation, apoptosis, and gene expression[26] also modulate VSV entry.[27] Phosphorylation by cellular kinases is necessary for VSV entry as has been demonstrated by the use of kinase inhibitors[28] and kinome siRNA screen.[29] Okadaic acid, a serine/threonine phosphatase inhibitor, inhibits viral penetration and uncoating.[28] Elegant studies to identify kinases involved in endocytic pathways revealed at least 48 kinases that act at different stages of endocytic pathway to be required for VSV entry by CME.[29] Among them, kinases involved in cytoskeleton-dependent transport, G-protein receptor-linked kinases and kinases that regulate the mammalian target of rapamycin (mTOR)-dependent signaling and the Wnt/β-catenin signaling were identified.[29] Mechanistic understanding of the role(s) of these kinases and pathways in VSV entry warrants further investigation.

Following endocytic entry, VSV uncoating involves low pH dependent conformational change in G protein to induce fusion of viral envelope with the membrane of internal vesicles present in the multivesicular bodies (MVBs) inside the endosomes followed by a back- fusion of these internal vesicles with the limiting membrane of the late endosomes to release viral RNP into the cytoplasm[30] (see chapter 3 for further details). Using fluorescent VSVs, studies show that the release of the viral RNPs into the cytoplasm occurs within 20-30 min after binding of the virus to the host cell plasma membrane.[25,31] The RNP release depends on the lipid lysobisphosphatidic acid (LBPA), which is only found in the late endosomes and its putative effector, ALG-2-interacting protein 1 (AIP1/ALIX), and are regulated by phosphatidylinositol-3- phosphate.[30] Recent studies show that interaction of LBPA with ALIX is required for RNP release.[32] LBPA was also recently shown to preferentially influence the ability of VSV G to mediate lipid mixing during membrane fusion.[33,34] Depletion of several subunits of the endosomal sorting complex required for transport (ESCRT) pathway results in inhibition of VSV RNA synthesis during infection,[30,32] suggesting that components of the ESCRT machinery are also required for the release of the viral RNPs into the cytoplasm from the endosomes.

Host Functions in Viral Macromolecular Synthesis

Viral mRNA Transcription

After release into the cytoplasm, the RNP template-associated viral RdRp complex performs primary transcription to produce the viral mRNAs from the input template. The mRNAs are translated to generate the viral proteins including N, P, and L proteins, which then carry out replication of the input viral genome to generate multiple progeny genomes. The newly synthesized negative-sense RNPs then undergo secondary transcription which amplifies the amount of viral mRNAs and proteins for further rounds of replication. Cyclophilin A (CypA), a chaperone protein possessing peptidyl *cis-trans* prolyl-isomerase activity, is required for primary transcription of VSV_{NJ} genome but not the VSV_{I} genome.

The enzymatic activity of CypA appears to be important, since its inhibition by cyclosporine A results in inhibition of primary transcription of VSV_{NJ} by 85-90%.[35] Biochemical and mutational studies have shown that the viral RdRp involved in transcription is distinct from that involved in genome replication. Two distinct RdRp complexes have been isolated from infected cells and characterized. One complex, comprising of the viral L and P proteins along with the cellular guanylyltransferase, translation elongation factor 1α, and heat shock protein 60, was found to exclusively synthesize mRNAs and is thus called the viral transcriptase. The other complex, comprising of the viral L, P, and N proteins, synthesizes only the full- length encapsidated replication products and is termed the viral replicase.[36] Although the exact role of the cellular proteins in viral transcriptase complex is not known, they may facilitate viral transcription. Further studies are required to understand their role in VSV transcription.

VSV mRNAs, like most eukaryotic mRNAs, are capped, methylated, and polyadenylated and these modifications are carried out by the viral RdRp (see chapter 5 for further details). Methylation of VSV mRNAs to form 5'-terminal structures is required for efficient translation in infected cells. Capping of the nascent transcripts is also important for transcription elongation and failure of correct 5′ capping results in abortive transcription.[37] VSV RdRp executes the capping reaction differently by adding GDP instead of GMP (as seen in eukaryotic capping) to the viral mRNAs but not to the Le RNA.[38] Whether this unique mechanism of capping of the mRNAs by the viral RdRp is regulated by cellular and/or viral factors is not known but is of interest for identifying specific targets for therapeutic development.

Phosphorylation by cellular protein kinases plays important roles in VSV infection not only during virus entry but also for viral gene expression. This was demonstrated by the effects on viral growth when the overall levels of protein phosphorylation were either enhanced or inhibited.[28,39] The VSV P protein is a multifunctional protein that is phosphorylated by cellular kinase(s) for its function in viral genome transcription and replication.[40-43] Although casein kinase II (CKII), a serine/threonine kinase, has been identified as one of the kinases involved in phosphorylation of P protein,[44-46] other kinases may also be involved as demonstrated by the use of inhibitors that do not affect CKII but block VSV RNA synthesis significantly.[39] With the recent identification of a tyrosine phosphorylation site in the P protein,[47] it is possible that other cellular kinase(s) may also mediate P phosphorylation for transcription and replication.

Viral Protein Synthesis

VSV mRNAs are translated in a cap-dependent manner by the host cells translation machinery. In the face of ongoing cellular mRNA translation, VSV usurps the translation machinery to preferentially translate its mRNAs via multiple mechanisms. The cellular eIF4F is a multisubunit complex involved in cap-dependent translation of host mRNAs. eIF4F regulates host translation through phosphorylation of cap-binding protein eIF4E, a subunit of the eIF4F complex. VSV infection leads to dephosphorylation of eIF4E and the translation inhibitor 4E-BP1 (eIF4E- binding protein 1), resulting in lower levels of eIF4F complex. Although the mechanism(s) by which VSV mediates these modifications is unknown at this time, this strategy allows the virus to subvert the cellular translation machinery for preferential translation of the viral mRNAs.[48] In addition, it appears that the cellular site of the transcription also contribute to the fate of the transcripts. Nascent viral mRNAs made in the cytoplasm are preferentially translated while translation of the host mRNAs is inhibited during VSV infection.[49,50]

VSV mRNA synthesis occurs throughout the host cell cytoplasm[51,52] in inclusion bodies that contain the viral RNA synthesis machinery.[52] The mRNAs are transported from the inclusion bodies in a microtubule- dependent manner to the sites of translation.[52] VSV mRNAs do not contain *cis*-acting sequences that influence translation but they do contain *cis*-acting sequence-independent structural elements, which enhance translation efficiency of the viral mRNAs.[50] These structural elements may be utilized by cellular large ribosomal subunit protein rpL40 to facilitate formation of 80S ribosome on VSV mRNAs for translation.[53] rpL40 is required for cap-dependent translation of VSV, measles, and rabies virus mRNAs but not for cellular cap-dependent or internal ribosome entry site-driven mRNA translation. Thus, rpL40 is a host ribosomal protein that facilitates translation of mRNAs of several members of *Mononegavirales*, including VSV.

Another mechanism by which VSV mRNAs are preferentially translated is by global shutdown of host transcription and translation through the viral M protein. The M protein interacts with the nuclear export pathway proteins Rae1 and Nup98 and thereby blocks the export of nascent cellular transcripts from the nucleus to the cytoplasm, resulting in shutoff of host protein synthesis.[54,55] Recent studies support a model in which the VSV M-Rae1 complexes allow interaction of the M protein with factors involved in transcription,[56] resulting in inhibition of cellular transcription. During VSV infection, inactivation of the basal transcription initiation factor TFIID leading to downregulation of the host RNA polymerase II activity has been observed,[57] although detailed mechanisms are yet to be uncovered. The possibility that the M protein dismantles TFIID by targeting the phosphorylation or the function of TATA-binding protein of TFIID complex has been implicated.[58]

Viral Genome Replication

VSV genome replication is carried out by the viral replicase complex, which is primarily composed of the P and L proteins. Recent studies have identified various host factors that positively influence viral genome replication. The chaperone protein HSP90AB1[59] was found to enhance the stability of VSV RdRp polymerase while cytoskeleton protein tubulin[60] may associate with VSV L protein thereby facilitating viral genome replication. Studies have also implicated a role for the cellular La protein in VSV RNA replication. The La protein, which is normally associated with the pol III transcripts, was also shown to be specifically associated with the plus-strand and longest minus-strand (54 nt) leader RNAs in VSV-infected cells.[61,62] The association correlated with enhancement in genome replication but the exact role of La protein, if any, remains to be deciphered.

Host Functions in VSV Assembly and Budding

VSV assembly occurs at the plasma membrane and is mediated by the viral M and G proteins. Current evidences indicate that VSV RNA and the individual proteins are transported to the plasma membrane via different routes. The G protein is synthesized in association with the ER where it is glycosylated and transported to the Golgi apparatus for processing of the glycan moieties and is finally transported to the plasma membrane. The cellular secretory pathway is used for transport of G protein to the plasma membrane,[63] where it forms membrane microdomains[64] important for VSV budding. In addition to the role played by coatomer protein complex II (COPII) and COPI in VSV G protein transport[65] (see section 5.1 below), the maturation and transport of VSV G protein also requires the presence of the trafficking protein particle complex subunit 3 (Bet3),[66] which functions

downstream of COPII and upstream of Rab1, alpha-SNAP and the EGTA-sensitive stage during the ER-Golgi transport. The vesicle-trafficking protein SEC22b, which functions at the level of the intermediate compartment (IC) in ER- Golgi transport, is also required.[67] VSV M is a soluble protein and is brought to the plasma membrane by an unknown mechanism, but the association of M protein with the plasma membrane results in formation of microdomains, which is independent of the G protein.[68] The viral NC, with the associated polymerase complex is assembled in the cytoplasm and is then transported to the plasma membrane in a microtubule dependent manner[51] where it associates with M protein.[69]

After assembly, virus budding and release continue to depend on the function of the M protein and its cellular interacting partners. The N terminal region, especially the amino acid 17-33 of M protein contains a PPxY motif that interacts with WW (tryptophan) domains of cellular proteins such as Yes-kinase associated protein (YAP) to recruit them to the site of virus budding.[70,71] Additionally, the interaction between M protein and dynamin 1 and 2 also promotes viral assembly and budding.[72] Since enveloped virus budding and release is considered to be topologically equivalent to MVB biogenesis,[73,74] several of the factors including the ESCRT machinery are also required for VSV budding and release. Although the tumor susceptibility gene 101 protein (TSG101), a member of the ESCRT-I machinery appears to be dispensable for VSV budding,[75] the role of vacuolar protein sorting-associated protein 4A (VPS4A), an AAA+ ATPase required for disassembly and recycling of ESCRT components,[76] remains somewhat unresolved. In one study, the use of dominant negative VPS4A mutant showed that VPS4A is not essential for VSV budding;[75] however in a separate study, dominant negative forms of both VPS4A and 4B were shown to inhibit VSV budding.[77] Nevertheless, the ESCRT machinery is required for scission of the viral membrane from the plasma membrane during budding (see chapter 8 for further details). In addition, the proteasomal pathway has been found to be indispensable for the virus budding.[77-79] The lipid composition of the membrane bilayer and lipid biogenesis may also play certain roles in the binding of M protein to the host cell membrane, since it was shown that triacylglycerol synthesis might interfere with virus maturation.[80] A recent mass-spec analysis of virion-associated proteins revealed a number of cellular proteins including integrin β1, HSP90, HSP cognate 71, annexin 2, EEF1A1 to have been packaged in the VSV virions.[81] These cellular proteins may potentially be involved in virus assembly, budding, and/or in other stages of virus infection processes. Functional studies of these proteins will be required to understand and appreciate their role in VSV life cycle.

Cellular Pathways that Regulate VSV Infection

In the previous sections, we described the role(s) of many individual host factors that facilitate or restrict VSV replication at various stages. Many of these factors appear to be involved in multiple steps of the viral life cycle. Cross-talk between various host factors and the pathways they represent also seems to be important for efficient multiplication or efficient restriction of replication of the virus. In several chapters of this book, the role(s) of many of these pathways in VSV infection have been described and the reader is directed to those chapters for further details. Although the list of factors and pathways that regulate VSV infection is by no means complete and will likely grow in the future, we describe below several of the major pathways and the associated factors that influence VSV replication.

Endocytic and Secretory Pathways

As described above (section 2.2), factors associated in CME are required for entry of VSV. The host proteins in the endocytic pathway and their roles in viral entry and nucleocapsid uncoating have been described in greater detail in chapter 3.

The secretory pathway plays a critical role in VSV replication cycle. This pathway involves anterograde and retrograde transport of proteins and lipids, which are mediated by vesicles containing distinct sets of cytosolic coat protein complexes, the (COPII) and (COPI), respectively.[82,83] Early studies revealed that the transport of VSV G from its site of synthesis in the ER to the Golgi and finally to the plasma membrane requires both COPII and COPI.[65] In these studies, the newly synthesized G protein was found to accumulate in COPII-containing vesicles at the ER, which then fuse to form larger transport complexes or ICs. In these ICs, the COPII is then replaced by COPI and the vesicles are then transported to the Golgi complex in a microtubule-dependent manner, thus suggesting a sequential mode of action of these two coatomer protein complexes in VSV G protein transport.[65]
In addition to its role in G protein transport, the secretory pathway and, in particular, the COPI, also appears to play an important role in VSV gene expression.[84] The observations that (i)VSV gene expression is inhibited in COPI-depleted cells that are independent of the viral G protein processing and transport, (ii) pharmacologic inhibitors of COPI inhibit VSV gene expression as well as reduce viral RNA levels, (iii) viral gene expression requires the ADP ribosylation factor 1 (ARF1) and the Golgi-associated brefeldin A resistant factor 1 (GBF1), the two upstream effectors of COPI function, and (iv) VSV gene expression is reduced in nucleocapsid transfected cells that have been depleted of COPI strongly suggest a role for COPI function in viral RNA synthesis.[84] Additional evidence for the involvement of COPI in VSV RNA synthesis comes from studies using cells having a temperature-sensitive _-COP, a subunit of the heptameric COPI complex.[85] Mechanistically, whether the COPI complex is directly involved in regulating the viral polymerase functions or whether it may signal through downstream effectors essential for viral gene expression is not known at this time. VSV replicates throughout the cytoplasm of infected cells,[51,52] although the existence of specific replication organelles in the cytoplasm whose assembly requires COPI function cannot be ruled out. The requirement of COPI for genome replication has been well documented for positive- strand RNA viruses, which replicate in association with cytoplasmic membranous structures.[86] Further biochemical and ultrastructural studies in cells infected with VSV will likely illuminate the nature of the replication organelles, if any, for these viruses.

Innate Immune Signaling Pathways

Host cells survey invasive RNA viruses including VSV by the viral ssRNA, dsRNA or glycoproteins using either membrane-associated or cytosolic pattern recognition receptors (PRRs) to mount a well- orchestrated innate immune defense (see chapter 15 for further details). The membranous PRRs like toll-like receptor 4 (TLR4) present at the plasma membrane as well as TLR3, TLR7 and TLR8 in the endosomes, recognize the VSV glycoprotein (VSV-G) or RNA and transduce downstream antiviral signaling that culminates in the activation of transcription factors such as IFN regulatory factors 3 (IRF3), IRF7, IRF5 and/or nuclear factor κB (NF-κB),[87,88] leading to the production of IFN and proinflammatory cytokines by the infected cells. Recently, TLR13, a novel member of the mammalian TLR family was reported to activate type I IFN production via activation of NF-κB and IRF7.[89] TLR13 seems to detect VSV at the surface of dendritic cells, macrophages or cells of the spleen where it is predominantly expressed. VSV RNP contributes to the early

recognition of infection by the cytoplasmic PRRs consisting of the retinoic-acid-inducible gene I (RIG-I) and the melanoma differentiation-associated protein 5 (MDA5). RIG-I plays a major role in sensing VSV infection, since type I IFN production was found to be severely impaired in VSV infected *RIG-I*$^{-/-}$ MEFs compared to wild-type cells whereas MDA5 was dispensable.[90]

PRR signaling results in production and secretion of IFN-α/β, which in autocrine or paracrine fashion, activate the JAK-STAT signaling pathway leading to synthesis of hundreds of interferon stimulated gene (ISG) products. Many ISGs, such as, phospholipase C (PLC) and phospholipase A2 (PLA2),[91] guanylate binding protein-1 (GBP-1),[92] and Mx1[93] are well-known for their antiviral effects against VSV, but the mechanism(s) of their action is poorly understood. A recent functional ISG screen documented 34 ISGs showing antiviral activity against VSV; included among them are novel ISGs such as ISG20, IFITM3, OAS1, MGAT1, GPR146, PARP12, LY6E, APOBEC proteins.[94] ISGs antagonize various stages of the VSV life cycle. The enzyme, cholesterol-25-hydroxylase, generates 25-hydroxycholesterol (25HC) which then suppresses VSV and several other enveloped virus infections by blocking the membrane fusion step.[95] IFITM3 disrupts an early event after endocytosis but before primary transcription,[96] whereas the viral primary transcription step is blocked by poly (C) binding proteins 2 (PCBP2).[97] Viral genome transcription and replication are efficiently blocked by 2',5'-oligoadenylate (2-5As) and 2-5As synthetases (OASs). One of the well-characterized inhibitors of VSV translation is the double- stranded RNA-activated protein kinase (PKR).[98] PKR modulates the inhibitory effect through multiple mechanisms, the most prominent being the phosphorylation of the eukaryotic translation initiation factor 2α (eIF2α) and subsequent shutoff of host and viral translation.[99] The other mechanism involves phosphorylation of nuclear factor associated with dsRNA 1 and 2 (NFAR1/2) protein, which is then retained on the ribosomes, associates with viral transcripts and thereby sequesters viral mRNA from being translated.[100] Other ISGs suppress VSV growth at translation and replication steps. The promyelocytic leukemia (PML) protein interferes with VSV mRNA synthesis, protein synthesis, and contribute to the antiviral state induced in mice infected with VSV.[101, 102] This antiviral effect requires PML SUMOylation.[103] The final step in the VSV life cycle, virus budding, is inhibited by tetherin, which blocks virion particle release from infected cells.[96]

Contrary to the classical function of ISGs as antiviral effectors, many ISGs also play a negative regulatory role to prevent excessive IFN production. A number of ISGs such as ISG56 and gC1qR negatively regulate the VSV antiviral response by interfering with interactions of the STING/MITA, IPS-1/VISA, and TBK1,[104,105] whereas other ISGs such as TRIAD3a, TRAF induce degradation of TRAF3 and TBK1, respectively.[106,107] Recently, studies from our laboratory identified the interferon inducible protein IFI35 as a negative regulator of RIG-I antiviral responses during VSV infection.[108] IFI35 was found to interact with RIG-I and the RIG-I activity was attenuated through maintenance of its inactive, phosphorylated form and also through degradation of RIG-I by K-48-linked ubiquitination.[108] It appears that an intricate balance between the activities of various ISGs during VSV infection may modulate the outcome of the infection, although additional factors may also be involved.

In recent years, autophagy has emerged as an important pathway complementing the innate immune pathways. Autophagy is an evolutionarily conserved mechanism in eukaryotes for degradation of unwanted intracellular cargo. Studies have shown autophagy to play an antiviral or proviral role depending on the virus and the host cell type.[109] For VSV, autophagy plays an antiviral role in the *Drosophila* model.[110] Recent

studies have demonstrated that VSV infection induces autophagy in *Drosophila* cells via TLR-7-mediated recognition of VSV G protein from replication-competent VSV , UV inactivated (replication- incompetent) VSV or VSV-G harboring virus like particles (VLPs), suggesting the VSV G protein as the pathogen associated molecular pattern (PAMP) that stimulates the autophagy pathway. Interestingly, the AKT-mTOR axis involved in nutrient signaling also plays a role in activating the antiviral autophagy program.[111] In contrast, many positive sense RNA viruses like Dengue, Hepatitis C virus (HCV) and some DNA viruses like Kaposi sarcoma herpesvirus (KSHV) have been found to utilize autophagy as a proviral mechanism, which makes this pathway a necessary evil for the host in these cases.[109]

Apoptotic and Cell Survival Pathways

VSV infection initiates both intrinsic (mitochondrial) apoptosis through caspase-9[112] and extrinsic apoptosis through PKR, DAXX, and partly via Fas and caspase-8[113] (see chapter 7 for further details). Apoptosis can occur at an early stage of VSV life cycle without a requirement for RNA replication.[114] The apoptotic consequence is thought to be primarily due to viral M protein and leader RNA[115,116] in addition to the alteration of levels of proteins in apoptotic pathways.[117,118] The molecular mechanism for IFN to regulate VSV induced apoptosis is not clear yet but several signaling factors including STAT,[119,120] the ISGs such as PKR and OAS[121,122] and the apoptosis-inducing factor p53[123] appear to be involved. In addition, cross-talk between apoptosis and innate immune response in VSV infection is responsible for induction of the intrinsic apoptotic pathway involving Bax-Caspase-9, which triggers phosphorylation of IRF3 and downstream type I IFN production.[124]

Initiation of apoptosis by VSV M protein requires cellular factors and is cell-type dependent. Wt VSV induces faster apoptosis in HeLa cells than the mutant VSVM51R virus but it is the reverse in BHK-21 cells.[125] Consistent with this observation, we recently demonstrated a role for hnRNP K in supporting VSV infection through suppressing apoptosis induction and promoting survival of infected cells.[117] The studies revealed that hnRNP K helps maintain the low levels of expression of proapoptotic proteins such as BiK and Bcl-X_S and high levels of expression of antiapoptotic proteins such as Bcl-X_L, Bag1, and Bcl-2.[117] Additionally, hnRNP K supports expression of several cellular factors required for VSV replication and negatively regulates expression of cellular factors known to inhibit VSV replication. In particular, hnRNP K suppresses overall levels of total T-cell intracellular antigen 1 (TIA1) as well as expression of the TIA1b isoform (a differentially spliced form of TIA1a); both isoforms of TIA1 suppress VSV replication.[117,126] Studies of the hnRNP K interactome revealed more than 100 interacting partners and determined that hnRNP K protein is present in the nucleus, cytoplasm, mitochondria, and the vicinity of the plasma membrane.[127] Thus, it is possible that hnRNP K exerts its positive impact on VSV growth through one or more of its interacting partners and may be involved in cross-talk between multiple pathways required for efficient VSV replication. The requirement of hnRNP K in VSV replication and the observation that hnRNP K levels in many cancerous cell types is upregulated may add to the understanding of why tumor cells provide an appropriate microenvironment (in addition to defective type I IFN response and signaling) for efficient VSV growth.

In contrast to the function of hnRNP K, hnRNPA1 plays a supporting role in VSV mediated apoptosis.[128] Studies have shown that VSV infection leads to a decrease of other antiapoptotic proteins such as keratinocyte transcription factor TP63[129] and myeloid cell leukemia 1 protein (Mcl-1),[130] while proapoptotic Bax was increased in the immortalized

HaCaT keratinocyte cell line.[129] miR-706 is upregulated in VSV infection and is able to inhibit VSV-induced apoptosis by decreasing caspase-3 and -9 activation, suggesting that induction of miR- 706 expression may be a novel strategy for survival of VSV, allowing it to escape the apoptosis response of the host.[131]

Akt/phosphatidylinositol-3-kinase (PI3K) pathway is a major cellular pathway controlling cell growth, proliferation, translation, and suppression of apoptosis. A variety of viruses are known to modulate this pathway. The observations that treatment of cells with Akt inhibitors significantly reduced VSV growth[132] and constitutively active Akt enhanced VSV replication[133] suggest that the Akt pathway is important in VSV replication. However, conflicting data also exist as to the role of this pathway in viral replication. Akt is activated in macrophages infected with VSV and this leads to the inhibition of virus replication.[134] Similarly, inactivation of Akt also favors VSV replication in BHK-21 cells.[135] On the other hand, downregulation of Akt in *Drosophila* suppresses VSV replication.[110]

RNA Interference (RNAi) Pathway

The cellular RNA interference (RNAi) pathway, which is an innate immune defense mechanism to control viral infections in plants and insects, is also involved in regulating VSV infection. This was first demonstrated in the worm *Caenorhabditis elegans*.[136,137] Cells derived from mutant worms defective in the RNAi response supported higher levels of VSV replication as compared to the cells from the wild-type counterpart indicating that the RNAi pathway controls replication of VSV.[136,137] In *Drosophila*, RNAi-mediated response also confers protection against infection by VSV.[138,139] Depletion of the arsenite resistance protein 2 (Ars2), which is required for siRNA- and miRNA- mediated silencing, results in increased replication of VSV in *Drosophila* cell cultures and adult flies.[139] The RNAi pathway has been shown to be inducible by IFN[140] and thus constitutes another arm of the innate host defense against a variety of virus infections *in vitro*[141] and *in vivo*.[142] Mice hypomorphic for Dicer, the endoribonuclease that catalyzes production of siRNAs and miRNAs, are more sensitive to VSV infection due to impairment in production of miR24 and miR93,[143] highlighting an important role for miRNAs in antiviral defense against VSV in mammalian systems. The miR93 and miR24 target the viral mRNAs for the P and L proteins, respectively, and thus suppress VSV replication. The cellular RNAi pathway also participates in controlling the appropriate response of innate immunity to VSV infection. Some miRNAs such as miR-146a and miR-466l negatively regulate type I IFN responses and support VSV infection.[144,145] Thus, the important role of the RNAi pathway in regulating VSV replication is being increasingly appreciated.

ESCRT Pathway

The ESCRT pathway is intimately involved in steps during virus entry as well in virus assembly and exit from the cells. The specific role(s) of various ESCRT subunits in the VSV life cycle has been detailed in chapters 3 and 8.

Stress Response Pathway

The crosstalk between stress induced responses and cellular innate immunity has been established in cellular responses to suppress VSV infection. It is known that VSV infection induces both oxidative stress and an anti-oxidative response in mammalian cells.[146] Stressful conditions such as viral infections, hypoxia, heat shock, UV irradiation, amino acid starvation or oxidative stresses induced by arsenite treatment can activate one or more of the five putative eIF2α kinases (PERK, GCN2, PKR, HRI and Z-DNA kinases). eIF2α

phosphorylation results in global shutoff of cap-dependent translation with the exception of proteins belonging to the stress response pathway, such as heat shock proteins.[147,148] Stress-induced Growth Arrest and DNA Damage- inducible protein 34 (GADD34) is induced by VSV infection.[133] It suppresses viral replication by inhibiting the mTOR pathway,[133] which is required for viral protein synthesis. These studies indicate that the stress- inducible response plays a critical role in antiviral defense against VSV. In addition, hypoxia-inducible factor (HIF) plays an antiviral role in VSV infection by upregulating type I IFN and ISGs production.[149]

A functional proteasome pathway also seems to be important for VSV protein synthesis since proteasome inhibitors significantly suppress viral protein synthesis through stimulation of stress-related responses such as phosphorylation of eIF2α.[150] The host protein, REgulated in Development and DNA Damage responses 1 (REDD1), which is usually induced in cells undergoing ER stress,[151] is initially induced at early stages of VSV infection but quickly decreases back to normal levels at 4 hpi due to the global suppression of translation by VSV M protein.[152] The demonstration of antiviral activity by the small molecules, nephthalimides, through upregulation of REDD1 and concomitant inhihition of mTORC1 suggest that mTORC1 plays an important role in regulation of VSV replication. The requirement for activation of mTORC1 for VSV infection appears to be for viral mRNA translation.[152] In addition to its role in translational control, mTORC1 is known to stimulate type I IFN production through phosphorylation of p70S6K and 4E-BP1.[153,154] These findings suggest that exploitation of mTORC1 pathway may be another mechanism that VSV employs to facilitate viral mRNAs translation. However, the apparent paradoxical effects of mTORC1 activation on stimulation of IFN production and upregulation of VSV replication needs further investigation to help clarify and determine the mechanistic involvement of mTORC1 in VSV replication.

Other Factors and Pathways

Post-translational modifications such as glycosylation, phosphorylation, ubiquitination, SUMOylation, ISGylation, etc, affect the maturation of viral proteins and thus regulate VSV replication. These modifications also influence activities of many host cell proteins that regulate VSV infection. Prostaglandin A1 (PGA1) and the delta12-Prostaglandin J2 (delta 12-PGJ2), a naturally occurring dehydration product of prostaglandin D2, inhibit VSV growth via suppression of synthesis and post-translational glycosylation of G protein.[155,156] The activity of VSV P and M proteins are also affected by type I IFN in neuronal cells, whereP is hypophosphorylated and M is hyperphosphorylated. Hypophosphorylated P may affect VSV replication and transcription[40-43] whereas hyperphosphorylated VSV M protein inhibits its association with VSV nucleocapsid.[157] These studies suggest a possible mechanism for type I IFN-mediated misassembly through disruption of the interactions between viral RNP and the M protein.[157] The VSV M is ubiquitinated by Rsp5 ubiquitin ligase and thus may play a role in virus assembly and budding.[78]

The hnRNPU, a protein involved in pre-mRNA processing, specifically interacts with the VSV leader RNA in the nuclei and colocalizes with the viral RNP in the cytoplasm. hnRNPU is also packaged within purified VSV virions[158] but its role in the viral life cycle remains to be examined. In contrast, PCBP1 and PCBP2, cellular regulators of splicing, transcription, translation, and mRNA stability, interact with VSV P and function as negative regulators of virus gene expression.[97] Further studies reveal that TIA1, a PCBP2-interacting partner and a critical factor for stress granule formation,[147,159] also functions as a negative regulator of VSV gene expression.[126]

The PI3K/Akt/NF-κB pathway requires the presence of Grb2- associated binder 1 (Gab1), a member of scaffolding/adaptor proteins, to protect macrophages from VSV infection.[160] Akt1 phosphorylates and inactivates the transcription repressor EMSY, resulting in its inability to bind to ISG promoters, leading to the expression of ISGs and inhibition of VSV growth.[161] The protein arginine N-methyltransferase (PRMT1) specifically binds to the intracytoplasmic domain of the type I IFN receptor to transduce the antiviral signaling mediated by IFN-β.[162] The involvement of PKC in the antiviral response to VSV infection mediated by hormone oxytoxin in human amnion cells[163] and by cAMP in macrophages[164] has been noted. On the other hand, the tyrosine receptor kinase (TRK)-fused gene (TFG) protein plays a supportive role in VSV infection by negatively regulating the RIG-I/MAVS/NF-κB axis of antiviral signaling.[165]

Other factors, such as, HSP70 facilitates type I IFN-dependent antiviral immunity against VSV in neurons,[166] whereas Cancer Upregulated Gene 2 (CUG2), a novel oncogene, makes cells resistant to VSV through the activation of STAT1-ISGs signaling.[167] In addition to type I IFN, type II and type III IFNs antagonize VSV infection and replication. Type III IFNs (IFN-λ1, -λ2, and -λ3) are induced during VSV infection[168] and mediate antiviral responses similar to type I IFN. IFN-λ1 is more effective than the other two members. IFN-γ inhibits VSV infection[169,170] through inhibition of VSV protein synthesis as a result of upregulation of NOS-1 and increased NO production in neuronal cells.[171] In cells of the reticuloendothelial system, IFN-γ treatment induces the expression of a set of low molecular weight MHC- encoded proteins (LMPs), which replace the beta-subunit of the proteasome complex during the proteasome neosynthesis, resulting in an antiviral complex termed the immunoproteasome in neuronal cells[171] that restricts VSV replication.

Concluding Remarks and Future Perspectives

VSV requires many cellular factors and pathways for efficient replication in host cells. Likewise, the host cells respond to VSV infection by expressing and/or activating factors and pathways to restrict virus replication. It is the net effect of these opposing factors/pathways that likely determines the outcome of the virus infection. As discussed above, mechanistic understanding of many factors that regulate VSV infection has been achieved; however, our current understanding of the role(s) of many others remains elusive. At this time, two genome-wide siRNA screens,[84,172] a kinome screen for the endocytic pathway[29] and at least two antiviral small molecule screens[152,173] have revealed many interesting cellular factors that regulate VSV infection at multiple stages in the viral life cycle (Fig. 1). Understanding of the role(s) of these proteins and cross-talk between them and the cellular pathways involved in the VSV life cycle is of paramount significance for a better understanding of VSV-host cell interactions. VSV is not only used as a model pathogen for understanding the mechanisms of replication and pathogenesis of this class of viruses, it is also used as a vaccine vector and an oncotherapeutic agent. Therefore, a complete understanding of VSV-host interactions will also be crucial for development of more efficacious vaccine vectors and oncolytic VSV.

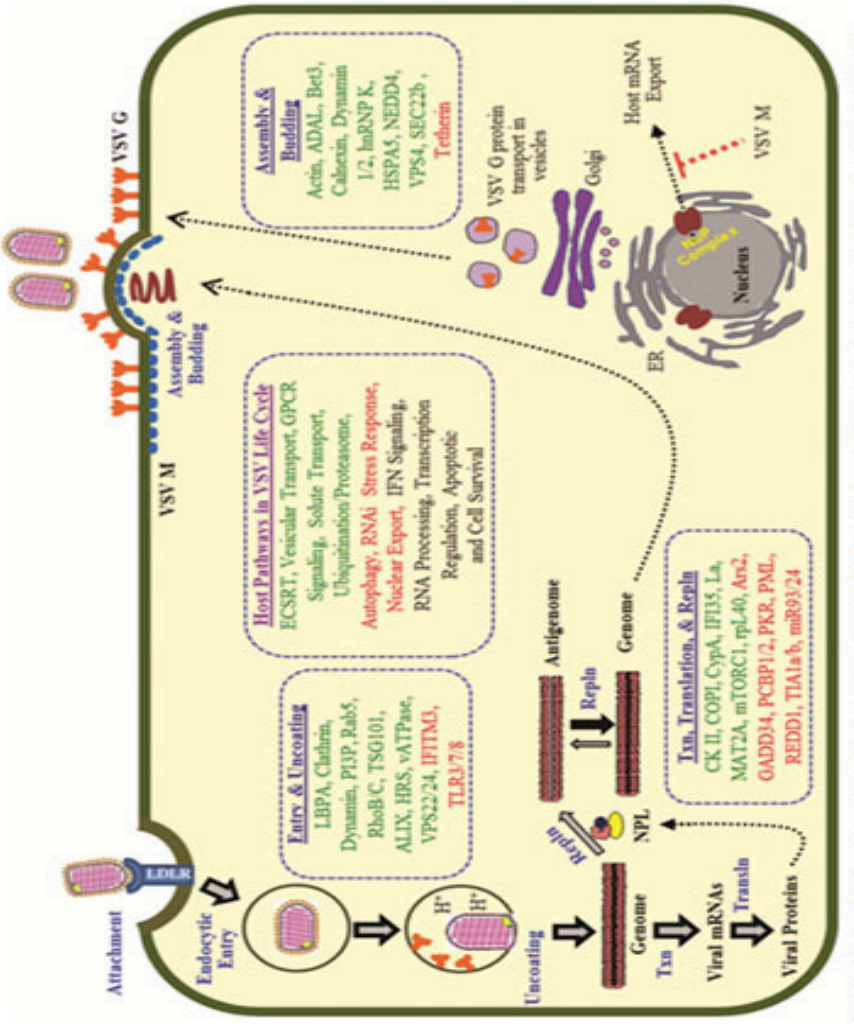

Fig. 1. Host cell factors and pathways involved in VSV replication. VSV-specific steps are shown in blue color text. Cellular factors that support VSV replication at various stages are shown in green, those that inhibit replication are in red. Cellular pathways (boxed) that positively and negatively regulate VSV infection are shown in green and red, respectively. Some cellular pathways with factors that support as well as inhibit VSV replication are shown in black. Txn, transcription; Repln, replication; Transln, translation. See text for additional details.

References

International Committee on Taxonomy of Viruses. Virus Taxonomy: July 2013 Release. EC 45, Edinburgh (2013).

Martinez, I. & Wertz, G.W. Biological differences between vesicular stomatitis virus Indiana and New Jersey serotype glycoproteins: identification of amino acid residues modulating pH-dependent infectivity. *J Virol* **79**, 3578-3585 (2005).

Lyles, D.S., I. V. Kuzmin, and C. E. Rupprecht. Rhabdoviridae. In D. M. Knipe and P. M. Howley (ed.), Fields virology, 6th ed., 885-922. Lippincott Williams & Wilkins,Philadelphia,PA. (2013).

Pattnaik, A.K., Ball, L.A., LeGrone, A.W. & Wertz, G.W. Infectious defective interfering particles of VSV from transcripts of a cDNA clone. *Cell* **69**, 1011-1020 (1992).

Schnell, M.J., Mebatsion, T. & Conzelmann, K.K. Infectious rabies viruses from cloned cDNA. *EMBO J* **13**, 4195-4203 (1994).

Lawson, N.D., Stillman, E.A., Whitt, M.A. & Rose, J.K. Recombinant vesicular stomatitis viruses from DNA. *Proc Natl Acad Sci USA* **92**, 4477-4481 (1995).

Whelan, S.P., Ball, L.A., Barr, J.N. & Wertz, G.T. Efficient recovery of infectious vesicular stomatitis virus entirely from cDNA clones. *Proc Natl Acad Sci USA* **92**, 8388-8392 (1995).

Seganti, L. et al. Activity of human serum lipoproteins on the infectivity of rhabdoviruses. *Microbiologica* **6**, 91-99 (1983).

Superti, F., Seganti, L., Tsiang, H. & Orsi, N. Role of phospholipids in rhabdovirus attachment to CER cells. Brief report. *Arch Virol* **81**, 321-328 (1984).

Schlegel, R., Tralka, T.S., Willingham, M.C. & Pastan, I. Inhibition of VSV binding and infectivity by phosphatidylserine: is phosphatidylserine a VSV-binding site? *Cell* **32**, 639-646 (1983).

Seganti, L., Superti, F., Girmenia, C., Melucci, L. & Orsi, N. Study of receptors for vesicular stomatitis virus in vertebrate and invertebrate cells. *Microbiologica* **9**, 259-267 (1986).

Coil, D.A. & Miller, A.D. Phosphatidylserine is not the cell surface receptor for vesicular stomatitis virus. *J Virol* **78**, 10920-10926 (2004).

Carneiro, F.A., Bianconi, M.L., Weissmuller, G., Stauffer, F. & Da Poian, A.T. Membrane recognition by vesicular stomatitis virus involves enthalpy-driven protein-lipid interactions. *J Virol* **76**, 3756-3764 (2002).

Superti, F., Girmenta, C., Seganti, L. & Orsi, N. Role of sialic acid in cell receptors for vesicular stomatitis virus. *Acta Virol* **30**, 10-18 (1986).

Sinibaldi, L. et al. Gangliosides in early interactions between vesicular stomatitis virus and CER cells. *Microbiologica* **8**, 355-365 (1985).

Finkelshtein, D., Werman, A., Novick, D., Barak, S. & Rubinstein, M. LDL receptor and its family members serve as the cellular receptors for vesicular stomatitis virus. *Proc Natl Acad Sci USA* **110**, 7306-7311 (2013).

Host Functions in VSV Replication 129

Conti, C., Mastromarino, P., Riccioli, A. & Orsi, N. Electrostatic interactions in the early events of VSV infection. *Res Virol* **142**, 17-24 (1991).

Mastromarino, P., Conti, C., Goldoni, P., Hauttecoeur, B. & Orsi, N. Characterization of membrane components of the erythrocyte involved in vesicular stomatitis virus attachment and fusion at acidic pH. *J Gen Virol* **68** (Pt 9), 2359- 2369 (1987).

Bailey, C.A., Miller, D.K. & Lenard, J. Effects of DEAE-dextran on infection and hemolysis by VSV. Evidence that nonspecific electrostatic interactions mediate effective binding of VSV to cells. *Virology* **133**, 111-118 (1984).

Bloor, S., Maelfait, J., Krumbach, R., Beyaert, R. & Randow, F. Endoplasmic reticulum chaperone gp96 is essential for infection with vesicular stomatitis virus. *Proc Natl Acad Sci USA* **107**, 6970-6975 (2010).

Sun, X., Yau, V.K., Briggs, B.J. & Whittaker, G.R. Role of clathrin-mediated endocytosis during vesicular stomatitis virus entry into host cells. *Virology* **338**, 53-60 (2005).

Cureton, D.K., Massol, R.H., Saffarian, S., Kirchhausen, T.L. & Whelan, S.P. Vesicular stomatitis virus enters cells through vesicles incompletely coated with clathrin that depend upon actin for internalization. *PLoS Pathog* **5**, e1000394 (2009).

Cureton, D.K., Massol, R.H., Whelan, S.P. & Kirchhausen, T. The length of vesicular stomatitis virus particles dictates a need for actin assembly during clathrin-dependent endocytosis. *PLoS Pathog* **6**, e1001127 (2010).
Johannsdottir, H.K., Mancini, R., Kartenbeck, J., Amato, L. & Helenius, A. Host cell factors and functions involved in vesicular stomatitis virus entry. *J Virol* **83**, 440-453 (2009).
Mire, C.E., White, J.M. & Whitt, M.A. A spatio-temporal analysis of matrix protein and nucleocapsid trafficking during vesicular stomatitis virus uncoating. *PLoS Pathog* **6**, e1000994 (2010).
Bustelo, X.R., Sauzeau, V. & Berenjeno, I.M. GTP-binding proteins of the Rho/Rac family: regulation, effectors and functions in vivo. *BioEssays* **29**, 356-370 (2007).
Quinn, K. et al. Rho GTPases modulate entry of Ebola virus and vesicular stomatitis virus pseudotyped vectors. *J Virol* **83**, 10176-10186 (2009).
Chang, T.L., Reiss, C.S. & Huang, A.S. Inhibition of vesicular stomatitis virus RNA synthesis by protein hyperphosphorylation. *J Virol* **68**, 4980-4987 (1994).
Pelkmans, L. et al. Genome-wide analysis of human kinases in clathrin- and caveolae/raft-mediated endocytosis. *Nature* **436**, 78-86 (2005).
Le Blanc, I. et al. Endosome-to-cytosol transport of viral nucleocapsids. *Nat Cell Biol* **7**, 653-664 (2005).
Das, S.C., Panda, D., Nayak, D. & Pattnaik, A.K. Biarsenical labeling of vesicular stomatitis virus encoding tetracysteine-tagged m protein allows dynamic imaging of m protein and virus uncoating in infected cells. *J Virol* **83**, 2611-2622 (2009).

Bissig, C. et al. Viral infection controlled by a calcium-dependent lipid-binding module in ALIX. *Dev Cell* **25**, 364-373 (2013).
Roth, S.L. & Whittaker, G.R. Promotion of vesicular stomatitis virus fusion by the endosome-specific phospholipid bis(monoacylglycero)phosphate (BMP). *FEBS Lett* **585**, 865-869 (2011).
Matos, P.M. et al. Anionic lipids are required for vesicular stomatitis virus G protein-mediated single particle fusion with supported lipid bilayers. *J Biol Chem* **288**, 12416-12425 (2013).
Bose, S., Mathur, M., Bates, P., Joshi, N. & Banerjee, A.K. Requirement for cyclophilin A for the replication of vesicular stomatitis virus New Jersey serotype. *J Gen Virol* **84**, 1687-1699 (2003).
Qanungo, K.R., Shaji, D., Mathur, M. & Banerjee, A.K. Two RNA polymerase complexes from vesicular stomatitis virus-infected cells that carry out transcription and replication of genome RNA. *Proc Natl Acad Sci USA* **101**, 5952-5957 (2004).
Stillman, E.A. & Whitt, M.A. Transcript initiation and 5'-end modifications are separable events during vesicular stomatitis virus transcription. *J Virol* **73**, 7199- 7209 (1999).
Ogino, T. & Banerjee, A.K. Unconventional mechanism of mRNA capping by the RNA-dependent RNA polymerase of vesicular stomatitis virus. *Mol Cell* **25**, 85-97 (2007).
Kim, Y.S., Sagara, J. & Kawai, A. Studies on the antiviral activity of protein kinase inhibitors against the replication of vesicular stomatitis virus. *Biol Pharm Bull* **18**, 895-899 (1995).
Das, S.C. & Pattnaik, A.K. Phosphorylation of vesicular stomatitis virus phosphoprotein P is indispensable for virus growth. *J Virol* **78**, 6420-6430 (2004).
Hwang, L.N., Englund, N., Das, T., Banerjee, A.K. & Pattnaik, A.K. Optimal replication activity of vesicular stomatitis virus RNA polymerase requires phosphorylation of a residue(s) at carboxy-terminal domain II of its accessory subunit, phosphoprotein P. *J Virol* **73**, 5613-5620 (1999).
Pattnaik, A.K. et al. Phosphorylation within the amino-terminal acidic domain I of the phosphoprotein of vesicular stomatitis virus is required for transcription but not for replication. *J Virol* **71**, 8167-8175 (1997).
Spadafora, D., Canter, D.M., Jackson, R.L. & Perrault, J. Constitutive phosphorylation of the vesicular stomatitis virus P protein modulates polymerase complex formation but is not essential for transcription or replication. *J Virol* **70**, 4538-4548 (1996).
Barik, S. & Banerjee, A.K. Phosphorylation by cellular casein kinase II is essential for transcriptional activity of vesicular stomatitis virus phosphoprotein P. *Proc Natl Acad Sci USA* **89**, 6570-6574 (1992).
Das, T. et al. Role of cellular casein kinase II in the function of the phosphoprotein (P) subunit of RNA polymerase of vesicular stomatitis virus. *J Biol Chem* **270**, 24100-24107 (1995).

Gupta, A.K., Das, T. & Banerjee, A.K. Casein kinase II is the P protein phosphorylating cellular kinase associated with the ribonucleoprotein complex of purified vesicular stomatitis virus. *J Gen Virol* **76** (Pt 2), 365-372 (1995).

Mondal, A., Victor, K.G., Pudupakam, R.S., Lyons, C.E. & Wertz, G.W. Newly identified phosphorylation site in the vesicular stomatitis virus P protein is required for viral RNA synthesis. *J Virol* **88**, 1461-1472 (2014).

Connor, J.H. & Lyles, D.S. Vesicular stomatitis virus infection alters the eIF4F translation initiation complex and causes dephosphorylation of the eIF4E binding protein 4E-BP1. *J Virol* **76**, 10177-10187 (2002).

Whitlow, Z.W., Connor, J.H. & Lyles, D.S. New mRNAs are preferentially translated during vesicular stomatitis virus infection. *J Virol* **82**, 2286-2294 (2008).

Whitlow, Z.W., Connor, J.H. & Lyles, D.S. Preferential translation of vesicular stomatitis virus mRNAs is conferred by transcription from the viral genome. *J Virol* **80**, 11733-11742 (2006).

Das, S.C., Nayak, D., Zhou, Y. & Pattnaik, A.K. Visualization of intracellular transport of vesicular stomatitis virus nucleocapsids in living cells. *J Virol* **80**, 6368-6377 (2006).

Heinrich, B.S., Cureton, D.K., Rahmeh, A.A. & Whelan, S.P. Protein expression redirects vesicular stomatitis virus RNA synthesis to cytoplasmic inclusions. *PLoS Pathog* **6**, e1000958 (2010).

Lee, A.S., Burdeinick-Kerr, R. & Whelan, S.P. A ribosome-specialized translation initiation pathway is required for cap-dependent translation of vesicular stomatitis virus mRNAs. *Proc Natl Acad Sci USA* **110**, 324-329 (2013).

Faria, P.A. et al. VSV disrupts the Rae1/mrnp41 mRNA nuclear export pathway. *Mol Cell* **17**, 93-102 (2005).

von Kobbe, C. et al. Vesicular stomatitis virus matrix protein inhibits host cell gene expression by targeting the nucleoporin Nup98. *Mol Cell* **6**, 1243-1252 (2000).

Rajani, K.R. et al. Complexes of vesicular stomatitis virus matrix protein with host Rae1 and Nup98 involved in inhibition of host transcription. *PLoS Pathog* **8**, e1002929 (2012).

Yuan, H., Yoza, B.K. & Lyles, D.S. Inhibition of host RNA polymerase II- dependent transcription by vesicular stomatitis virus results from inactivation of TFIID. *Virology* **251**, 383-392 (1998).

Yuan, H., Puckett, S. & Lyles, D.S. Inhibition of host transcription by vesicular stomatitis virus involves a novel mechanism that is independent of phosphorylation of TATA-binding protein (TBP) or association of TBP with TBP-associated factor subunits. *J Virol* **75**, 4453-4458 (2001).

Connor, J.H., McKenzie, M.O., Parks, G.D. & Lyles, D.S. Antiviral activity and RNA polymerase degradation following Hsp90 inhibition in a range of negative strand viruses. *Virology* **362**, 109-119 (2007).

132 *P. X. Dinh, A. Das, and A. K. Pattnaik*

Moyer, S.A., Baker, S.C. & Lessard, J.L. Tubulin: a factor necessary for the synthesis of both Sendai virus and vesicular stomatitis virus RNAs. *Proc Natl Acad Sci USA* **83**, 5405-5409 (1986).

Wilusz, J., Kurilla, M.G. & Keene, J.D. A host protein (La) binds to a unique species of minus-sense leader RNA during replication of vesicular stomatitis virus. *Proc Natl Acad Sci USA* **80**, 5827-5831 (1983).

Kurilla, M.G. & Keene, J.D. The leader RNA of vesicular stomatitis virus is bound by a cellular protein reactive with anti-La lupus antibodies. *Cell* **34**, 837-845 (1983).

Balch, W.E., McCaffery, J.M., Plutner, H. & Farquhar, M.G. Vesicular stomatitis virus glycoprotein is sorted and concentrated during export from the endoplasmic reticulum. *Cell* **76**, 841-852 (1994).

Brown, E.L. & Lyles, D.S. Organization of the vesicular stomatitis virus glycoprotein into membrane microdomains occurs independently of intracellular viral components. *J Virol* **77**, 3985-3992 (2003).

Scales, S.J., Pepperkok, R. & Kreis, T.E. Visualization of ER-to-Golgi transport in living cells reveals a sequential mode of action for COPII and COPI. *Cell* **90**, 1137- 1148 (1997).

Loh, E., Peter, F., Subramaniam, V.N. & Hong, W. Mammalian Bet3 functions as a cytosolic factor participating in transport from the ER to the Golgi apparatus. *J Cell Sci* **118**, 1209-1222 (2005).

Zhang, T., Wong, S.H., Tang, B.L., Xu, Y. & Hong, W. Morphological and functional association of Sec22b/ERS-24 with the pre-Golgi intermediate compartment. *Mol Biol Cell* **10**, 435-453 (1999).

Swinteck, B.D. & Lyles, D.S. Plasma membrane microdomains containing vesicular stomatitis virus

M protein are separate from microdomains containing G protein and nucleocapsids. *J Virol* **82**, 5536-5547 (2008).
Flood, E.A. & Lyles, D.S. Assembly of nucleocapsids with cytosolic and membrane-derived matrix proteins of vesicular stomatitis virus. *Virology* **261**, 295- 308 (1999).
Harty, R.N., Paragas, J., Sudol, M. & Palese, P. A proline-rich motif within the matrix protein of vesicular stomatitis virus and rabies virus interacts with WW domains of cellular proteins: implications for viral budding. *J Virol* **73**, 2921-2929 (1999).
Irie, T. et al. Functional analysis of late-budding domain activity associated with the PSAP motif within the vesicular stomatitis virus M protein. *J Virol* **78**, 7823- 7827 (2004).
Raux, H. et al. The matrix protein of vesicular stomatitis virus binds dynamin for efficient viral assembly. *J Virol* **84**, 12609-12618 (2010).
Gruenberg, J. & Stenmark, H. The biogenesis of multivesicular endosomes. *Nature reviews. Mol Cell Biol* **5**, 317-323 (2004).
Host Functions in VSV Replication 133
Carlton, J.G. & Martin-Serrano, J. The ESCRT machinery: new functions in viral and cellular biology. *Biochem Soc Trans* **37**, 195-199 (2009).
Irie, T., Licata, J.M., McGettigan, J.P., Schnell, M.J. & Harty, R.N. Budding of PPxY-containing rhabdoviruses is not dependent on host proteins TGS101 and VPS4A. *J Virol* **78**, 2657-2665 (2004).
Babst, M., Sato, T.K., Banta, L.M. & Emr, S.D. Endosomal transport function in yeast requires a novel AAA-type ATPase, Vps4p. *EMBO J* **16**, 1820-1831 (1997).
Taylor, G.M., Hanson, P.I. & Kielian, M. Ubiquitin depletion and dominant- negative VPS4 inhibit rhabdovirus budding without affecting alphavirus budding. *J Virol* **81**, 13631-13639 (2007).
Harty, R.N. et al. Rhabdoviruses and the cellular ubiquitin-proteasome system: a budding interaction. *J Virol* **75**, 10623-10629 (2001).
Jayakar, H.R., Murti, K.G. & Whitt, M.A. Mutations in the PPPY motif of vesicular stomatitis virus matrix protein reduce virus budding by inhibiting a late step in virion release. *J Virol* **74**, 9818-9827 (2000).
Hornung, B., Amtmann, E. & Sauer, G. Lauric acid inhibits the maturation of vesicular stomatitis virus. *J Gen Virol* **75**, 353-361 (1994).
Moerdyk-Schauwecker, M., Hwang, S.I. & Grdzelishvili, V.Z. Analysis of virion associated host proteins in vesicular stomatitis virus using a proteomics approach. *Virol J* **6**, 166 (2009).
Bonifacino, J.S. & Lippincott-Schwartz, J. Coat proteins: shaping membrane transport. *Nat Rev Mol Cell Biol* **4**, 409-414 (2003).
Bonifacino, J.S. & Glick, B.S. The mechanisms of vesicle budding and fusion. *Cell* **116**, 153-166 (2004).
Panda, D. et al. RNAi screening reveals requirement for host cell secretory pathway in infection by diverse families of negative-strand RNA viruses. *Proc Natl Acad Sci USA* **108**, 19036-19041 (2011).
Cureton, D.K., Burdeinick-Kerr, R. & Whelan, S.P. Genetic inactivation of COPI coatomer separately inhibits vesicular stomatitis virus entry and gene expression. *J Virol* **86**, 655-666 (2012).
Miller, S. & Krijnse-Locker, J. Modification of intracellular membrane structures for virus replication. *Nature Rev. Microbio* **6**, 363-374 (2008).
Randall, R.E. & Goodbourn, S. Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures. *J Gen Virol* **89**, 1-47 (2008).
Bowie, A.G. & Unterholzner, L. Viral evasion and subversion of pattern- recognition receptor signalling. *Nat Rev Immunol* **8**, 911-922 (2008).
Shi, Z. et al. A novel Toll-like receptor that recognizes vesicular stomatitis virus. *J Biol Chem* **286**, 4517-4524 (2011).
Kato, H. et al. Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. *Nature* **441**, 101-105 (2006).
134 *P. X. Dinh, A. Das, and A. K. Pattnaik*
Premecz, G., Markovits, A., Bagi, G., Farkas, T. & Foldes, I. Phospholipase C and phospholipase A2 are involved in the antiviral activity of human interferon-alpha. *FEBS Lett* **249**, 257-260 (1989).
Anderson, S.L., Carton, J.M., Lou, J., Xing, L. & Rubin, B.Y. Interferon-induced guanylate binding protein-1 (GBP-1) mediates an antiviral effect against vesicular stomatitis virus and

encephalomyocarditis virus. *Virology* **256**, 8-14 (1999).
Baise, E. et al. Conditional expression of type I interferon-induced bovine Mx1 GTPase in a stable transgenic vero cell line interferes with replication of vesicular stomatitis virus. *J Interferon Cytokine Res* **24**, 513-521 (2004).
Liu, S.Y., Sanchez, D.J., Aliyari, R., Lu, S. & Cheng, G. Systematic identification of type I and type II interferon-induced antiviral factors. *Proc Natl Acad Sci USA* **109**, 4239-4244 (2012).
Liu, S.Y. et al. Interferon-inducible cholesterol-25-hydroxylase broadly inhibits viral entry by production of 25-hydroxycholesterol. *Immunity* **38**, 92-105 (2013).
Weidner, J.M. et al. Interferon-induced cell membrane proteins, IFITM3 and tetherin, inhibit vesicular stomatitis virus infection via distinct mechanisms. *J Virol* **84**, 12646-12657 (2010).
Dinh, P.X., Beura, L.K., Panda, D., Das, A. & Pattnaik, A.K. Antagonistic effects of cellular poly(C) binding proteins on vesicular stomatitis virus gene expression. *J Virol* **85**, 9459-9471 (2011).
Stojdl, D.F. et al. The murine double-stranded RNA-dependent protein kinase PKR is required for resistance to vesicular stomatitis virus. *J Virol* **74**, 9580-9585 (2000).
Connor, J.H. & Lyles, D.S. Inhibition of host and viral translation during vesicular stomatitis virus infection. eIF2 is responsible for the inhibition of viral but not host translation. *J Biol Chem* **280**, 13512-13519 (2005).
Harashima, A., Guettouche, T. & Barber, G.N. Phosphorylation of the NFAR proteins by the dsRNA-dependent protein kinase PKR constitutes a novel mechanism of translational regulation and cellular defense. *Genes Dev* **24**, 2640- 2653 (2010).
Chelbi-Alix, M.K., Quignon, F., Pelicano, L., Koken, M.H. & de The, H. Resistance to virus infection conferred by the interferon-induced promyelocytic leukemia protein. *J Virol* **72**, 1043-1051 (1998).
Bonilla, W.V. et al. Effects of promyelocytic leukemia protein on virus-host balance. *J Virol* **76**, 3810-3818 (2002).
Blondel, D., Kheddache, S., Lahaye, X., Dianoux, L. & Chelbi-Alix, M.K. Resistance to rabies virus infection conferred by the PMLIV isoform. *J Virol* **84**, 10719-10726 (2010).
Li, Y. et al. ISG56 is a negative-feedback regulator of virus-triggered signaling and cellular antiviral response. *Proc Natl Acad Sci USA* **106**, 7945-7950 (2009).
Host Functions in VSV Replication 135
Xu, L., Xiao, N., Liu, F., Ren, H. & Gu, J. Inhibition of RIG-I and MDA5- dependent antiviral response by gC1qR at mitochondria. *Proc Natl Acad Sci USA* **106**, 1530-1535 (2009).
Nakhaei, P. et al. The E3 ubiquitin ligase Triad3A negatively regulates the RIG- I/MAVS signaling pathway by targeting TRAF3 for degradation. *PLoS Pathog* **5**, e1000650 (2009).
Zhang, M. et al. TRAF-interacting protein (TRIP) negatively regulates IFN-beta production and antiviral response by promoting proteasomal degradation of TANK-binding kinase 1. *J Exp Med* **209**, 1703-1711 (2012).
Das, A., Dinh, P.X., Panda, D. & Pattnaik, A.K. Interferon-inducible protein IFI35 negatively regulates RIG-I antiviral signaling and supports vesicular stomatitis virus replication. *J Virol* **88**, 3103-3113 (2014).
Dong, X. & Levine, B. Autophagy and viruses: adversaries or allies? *J Innate Imm* **5**, 480-493 (2013).
Shelly, S., Lukinova, N., Bambina, S., Berman, A. & Cherry, S. Autophagy is an essential component of Drosophila immunity against vesicular stomatitis virus. *Immunity* **30**, 588-598 (2009).
Nakamoto, M. et al. Virus recognition by Toll-7 activates antiviral autophagy in Drosophila. *Immunity* **36**, 658-667 (2012).
Kopecky, S.A. & Lyles, D.S. Contrasting effects of matrix protein on apoptosis in HeLa and BHK cells infected with vesicular stomatitis virus are due to inhibition of host gene expression. *J Virol* **77**, 4658-4669 (2003).
Gaddy, D.F. & Lyles, D.S. Vesicular stomatitis viruses expressing wild-type or mutant M proteins activate apoptosis through distinct pathways. *J Virol* **79**, 4170- 4179 (2005).
Gadaleta, P., Vacotto, M. & Coulombie, F. Vesicular stomatitis virus induces apoptosis at early stages in the viral cycle and does not depend on virus replication. *Virus Res* **86**, 87-92 (2002).
Remenick, J., Kenny, M.K. & McGowan, J.J. Inhibition of adenovirus DNA replication by vesicular stomatitis virus leader RNA. *J Virol* **62**, 1286-1292 (1988).

Chakraborty, P. et al. Vesicular stomatitis virus inhibits mitotic progression and triggers cell death. *EMBO Rep* **10**, 1154-1160 (2009).
Dinh, P.X., Das, A., Franco, R. & Pattnaik, A.K. Heterogeneous nuclear ribonucleoprotein k supports vesicular stomatitis virus replication by regulating cell survival and cellular gene expression. *J Virol* **87**, 10059-10069 (2013).
Munoz-Fontela, C. et al. Resistance to viral infection of super p53 mice. *Oncogene* **24**, 3059-3062 (2005).
Dai, C. & Krantz, S.B. Interferon gamma induces upregulation and activation of caspases 1, 3, and 8 to produce apoptosis in human erythroid progenitor cells. *Blood* **93**, 3309-3316 (1999).
136 *P. X. Dinh, A. Das, and A. K. Pattnaik*
Chin, Y.E., Kitagawa, M., Kuida, K., Flavell, R.A. & Fu, X.Y. Activation of the STAT signaling pathway can cause expression of caspase 1 and apoptosis. *Mol Cell Biol* **17**, 5328-5337 (1997).
Castelli, J.C. et al. The role of 2'-5' oligoadenylate-activated ribonuclease L in apoptosis. *Cell Death Diff* 5, 313-320 (1998).
Garcia, M.A. et al. Impact of protein kinase PKR in cell biology: from antiviral to antiproliferative action. *Microbiol Mol Biol Rev* **70**, 1032-1060 (2006).
Takaoka, A. et al. Integration of interferon-alpha/beta signalling to p53 responses in tumour suppression and antiviral defence. *Nature* **424**, 516-523 (2003).
Sharif-Askari, E. et al. Bax-dependent mitochondrial membrane permeabilization enhances IRF3-mediated innate immune response during VSV infection. *Virology* **365**, 20-33 (2007).
Kopecky, S.A. & Lyles, D.S. The cell-rounding activity of the vesicular stomatitis virus matrix protein is due to the induction of cell death. *J Virol* **77**, 5524-5528 (2003).
Dinh, P.X. et al. Induction of stress granule-like structures in vesicular stomatitis virus-infected cells. *J Virol* **87**, 372-383 (2013).
Mikula, M. et al. Landscape of the hnRNP K protein-protein interactome. *Proteomics* **6**, 2395-2406 (2006).
Pettit Kneller, E.L., Connor, J.H. & Lyles, D.S. hnRNPs Relocalize to the cytoplasm following infection with vesicular stomatitis virus. *J Virol* **83**, 770-780 (2009).
Megyeri, K., Orosz, L. & Kemeny, L. Vesicular stomatitis virus infection triggers apoptosis associated with decreased DeltaNp63alpha and increased Bax levels in the immortalized HaCaT keratinocyte cell line. *Biomed Pharmacother* **61**, 254-260 (2007).
Schache, P. et al. VSV virotherapy improves chemotherapy by triggering apoptosis due to proteasomal degradation of Mcl-1. *Gene Ther* **16**, 849-861 (2009).
Lian, H. et al. A laboratory-attenuated vesicular stomatitis virus induces apoptosis and alters the cellular microRNA expression profile in BHK cells. *Arch Virol* **155**, 1643-1653 (2010).
Sun, M. et al. Akt plays a critical role in replication of nonsegmented negative- stranded RNA viruses. *J Virol* **82**, 105-114 (2008).
Minami, K. et al. Suppression of viral replication by stress-inducible GADD34 protein via the mammalian serine/threonine protein kinase mTOR pathway. *J Virol* **81**, 11106-11115 (2007).
Schabbauer, G. et al. TLR4/CD14-mediated PI3K activation is an essential component of interferon-dependent VSV resistance in macrophages. *Mol Immunol* **45**, 2790-2796 (2008).
Dunn, E.F. & Connor, J.H. Dominant inhibition of Akt/protein kinase B signaling by the matrix protein of a negative-strand RNA virus. *J Virol* **85**, 422-431 (2011).
Host Functions in VSV Replication 137
Wilkins, C. et al. RNA interference is an antiviral defence mechanism in Caenorhabditis elegans. *Nature* **436**, 1044-1047 (2005).
Schott, D.H., Cureton, D.K., Whelan, S.P. & Hunter, C.P. An antiviral role for the RNA interference machinery in Caenorhabditis elegans. *Proc Natl Acad Sci USA* **102**, 18420-18424 (2005).
Mueller, S. et al. RNAi-mediated immunity provides strong protection against the negative-strand RNA vesicular stomatitis virus in Drosophila. *Proc Natl Acad Sci USA* **107**, 19390-19395 (2010).
Sabin, L.R. et al. Ars2 regulates both miRNA- and siRNA- dependent silencing and suppresses RNA virus infection in Drosophila. *Cell* **138**, 340-351 (2009).
Pedersen, I.M. et al. Interferon modulation of cellular microRNAs as an antiviral mechanism. *Nature*

449, 919-922 (2007).
van Mierlo, J.T. et al. Convergent evolution of argonaute-2 slicer antagonism in two distinct insect RNA viruses. *PLoS Pathog* **8**, e1002872 (2012).
Huang, T. & Zhang, X. Host defense against DNA virus infection in shrimp is mediated by the siRNA pathway. *Eur J Immunol* **43**, 137-146 (2013).
Otsuka, M. et al. Hypersusceptibility to vesicular stomatitis virus infection in Dicer1-deficient mice is due to impaired miR24 and miR93 expression. *Immunity* **27**, 123-134 (2007).
Hou, J. et al. MicroRNA-146a feedback inhibits RIG-I-dependent Type I IFN production in macrophages by targeting TRAF6, IRAK1, and IRAK2. *J Immunol* **183**, 2150-2158 (2009).
Li, Y. et al. MicroRNA-466l inhibits antiviral innate immune response by targeting interferon-alpha. *Cell Mol Immunol* **9**, 497-502 (2012).
Riva, D.A. et al. Oxidative stress in vero cells infected with vesicular stomatitis virus. *Intervirol* **49**, 294-298 (2006).
Anderson, P. & Kedersha, N. Stress granules: the Tao of RNA triage. *Trends Biochem Sci* **33**, 141-150 (2008).
Buchan, J.R. & Parker, R. Eukaryotic stress granules: the ins and outs of translation. *Mol Cell* **36**, 932-941 (2009).
Hwang, II, Watson, I.R., Der, S.D. & Ohh, M. Loss of VHL confers hypoxia- inducible factor (HIF)-dependent resistance to vesicular stomatitis virus: role of HIF in antiviral response. *J Virol* **80**, 10712-10723 (2006).
Neznanov, N. et al. Different effect of proteasome inhibition on vesicular stomatitis virus and poliovirus replication. *PloS ONE* **3**, e1887 (2008).
Kimball, S.R. & Jefferson, L.S. Induction of REDD1 gene expression in the liver in response to endoplasmic reticulum stress is mediated through a PERK, eIF2alpha phosphorylation, ATF4-dependent cascade. *Biochem Biophys Res Comm* **427**, 485-489 (2012).
Mata, M.A. et al. Chemical inhibition of RNA viruses reveals REDD1 as a host defense factor. *Nat Chem Biol* **7**, 712-719 (2011).
138 *P. X. Dinh, A. Das, and A. K. Pattnaik*
Alain, T. et al. Vesicular stomatitis virus oncolysis is potentiated by impairing mTORC1-dependent type I IFN production. *Proc Natl Acad Sci USA* **107**, 1576- 1581 (2010).
Costa-Mattioli, M. & Sonenberg, N. RAPping production of type I interferon in pDCs through mTOR. *Nat Immunol* **9**, 1097-1099 (2008).
Santoro, M.G., Jaffe, B.M. & Esteban, M. Prostaglandin A inhibits the replication of vesicular stomatitis virus: effect on virus glycoprotein. *J Gen Virol* **64** (Pt 12), 2797-2801 (1983).
Pica, F., De Marco, A., De Cesare, F. & Santoro, M.G. Inhibition of vesicular stomatitis virus replication by delta 12-prostaglandin J2 is regulated at two separate levels and is associated with induction of stress protein synthesis. *Antiviral Res* **20**, 193-208 (1993).
D'Agostino P, M., Amenta, J.J. & Reiss, C.S. IFN-beta-induced alteration of VSV protein phosphorylation in neuronal cells. *Viral Immunol* **22**, 353-369 (2009).
Gupta, A.K., Drazba, J.A. & Banerjee, A.K. Specific interaction of heterogeneous nuclear ribonucleoprotein particle U with the leader RNA sequence of vesicular stomatitis virus. *J Virol* **72**, 8532-8540 (1998).
Fujimura, K., Kano, F. & Murata, M. Identification of PCBP2, a facilitator of IRES-mediated translation, as a novel constituent of stress granules and processing bodies. *RNA* **14**, 425-431 (2008).
Zheng, Y. et al. Scaffolding adaptor protein Gab1 is required for TLR3/4- and RIG-I-mediated production of proinflammatory cytokines and type I IFN in macrophages. *J Immunol* **184**, 6447-6456 (2010).
Ezell, S.A. et al. The protein kinase Akt1 regulates the interferon response through phosphorylation of the transcriptional repressor EMSY. *Proc Natl Acad Sci USA* **109**, E613-621 (2012).
Altschuler, L., Wook, J.O., Gurari, D., Chebath, J. & Revel, M. Involvement of receptor-bound protein methyltransferase PRMT1 in antiviral and antiproliferative effects of type I interferons. *J Interferon Cytokine Res* **19**, 189-195 (1999).

Markovits, A., Premecz, G., Bagi, G. & Foldes, I. Oxytocin stimulates translocation of protein kinase C and induces antiviral state in human amnion cells. *Acta Microbiol Hung* **39**, 41-45 (1992).

Borghi, P. et al. Cyclic AMP-mediated inhibition of vesicular stomatitis virus and herpes simplex virus replication in mouse macrophage-like cells. *J Gen Virol* **73** (Pt 11), 2949-2954 (1992).

Lee, N.R., Shin, H.B., Kim, H.I., Choi, M.S. & Inn, K.S. Negative regulation of RIG-I-mediated antiviral signaling by TRK-fused gene (TFG) protein. *Biochem Biophys Res Comm* **437**, 168-172 (2013).

Kim, M.Y. et al. hsp70-dependent antiviral immunity against cytopathic neuronal infection by vesicular stomatitis virus. *J Virol* **87**, 10668-10678 (2013).

Host Functions in VSV Replication 139

Malilas, W. et al. Cancer upregulated gene 2, a novel oncogene, confers resistance to oncolytic vesicular stomatitis virus through STAT1-OASL2 signaling. *Cancer Gene Ther* **20**, 125-132 (2013).

Wongthida, P. et al. Type III IFN interleukin-28 mediates the antitumor efficacy of oncolytic virus VSV in immune-competent mouse models of cancer. *Cancer Res* **70**, 4539-4549 (2010).

Ulker, N. & Samuel, C.E. Mechanism of interferon action: inhibition of vesicular stomatitis virus replication in human amnion U cells by cloned human gamma- interferon. II. Effect on viral macromolecular synthesis. *J Biol Chem* **260**, 4324- 4330 (1985).

Ulker, N. & Samuel, C.E. Mechanism of interferon action: inhibition of vesicular stomatitis virus replication in human amnion U cells by cloned human gamma- interferon. I. Effect on early and late stages of the viral multiplication cycle. *J Biol Chem* **260**, 4319-4323 (1985).

Yang, J., Tugal, D. & Reiss, C.S. The role of the proteasome-ubiquitin pathway in regulation of the IFN-gamma mediated anti-VSV response in neurons. *J Neuroimmunol* **181**, 34-45 (2006).

Lee, A.S., Burdeinick-Kerr, R. & Whelan, S.P. A genome-wide siRNA screen identifies host factors required for vesicular stomatitis virus infection. *J Virol* (2014).

Filone, C.M. et al. Identification of a broad-spectrum inhibitor of viral RNA synthesis: validation of a prototype virus-based approach. *Chem Biol* **20**, 424-433 (2013).