Review

Reviewing host proteins of Rhabdoviridae: Possible leads for lesser studied viruses

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Rhabdoviridae, characterized by bullet-shaped viruses, is known for its diverse host range, which includes plants, arthropods, fishes and humans. Understanding the viral–host interactions of this family can prove beneficial in developing effective therapeutic strategies. The host proteins interacting with animal rhabdoviruses have been reviewed in this report. Several important host proteins commonly interacting with animal rhabdoviruses are being reported, some of which, interestingly, have molecular features, which can serve as potential antiviral targets. This review not only provides the generalized importance of the functions of animal rhabdovirus-associated host proteins for the first time but also compares them among the two most studied viruses, i.e. Rabies virus (RV) and Vesicular Stomatitis virus (VSV). The comparative data can be used for studying emerging viruses such as Chandipura virus (CHPV) and the lesser studied viruses such as Piry virus (PIRYV) and Isfahan virus (ISFV) of the Rhabdoviridae family.

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1. Introduction

The *Rhabdoviridae* family includes approximately 165 viruses with a broad host range. This family consists of six genera: *Vesiculovirus, Lyssavirus, Ephemerovirus, Novirhabdoviruses, Cytorhabdoviruses* and *Nucleorhabdoviruses,* of which *Vesiculovirus* and *Lyssavirus* include viruses that infect mammals, and in many cases humans, whereas the other genera include viruses that infect fishes, invertebrates and plants. The rhabdoviruses are bullet-shaped enveloped viruses with a non-segmented negative-sense single-stranded RNA genome of approximately 11 kb that encodes for five proteins, namely, nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and large subunit (L) of the RNA-dependent RNA polymerase protein (RdRp) (Basak *et al.* 2007).

The association of five viral proteins with host factors is not only important from the pathogenesis point of view but it could also explain the wide host range observed in the family *Rhabdoviridae.* More importantly, the knowledge of virus–host interactions can provide leads for therapeutics. This article thus reviews the host proteins that have possible roles in infection and disease progression of animal rhabdoviruses. On compilation of data for host factors, it was observed that almost all studies had been performed on VSV (genus *Vesiculovirus*) and RV (genus *Lyssavirus*), underlining the fact that, within rhabdoviridae, VSV and RV are the best investigated members and model rhabdovirus

Keywords. host proteins; protein interactions; Rabies virus; Rhabdovirus; Vesicular Stomatitis virus

Abbreviations used: CK, casein kinase; CHPV, Chandipura virus; Cyp A, cyclophilin A; eEF-1, eukaryotic elongation factor; eIF-3 h, eukaryotic initiation factor-3h; GT, guanyltransferase; Hsp70, heat shock protein 70; IRES, internal ribosome entry site; ISFV, Isfahan virus; LC8, Light Chain 8; nAchR, nicotinic acetylcholine receptor; NCAM, neural cell adhesion molecule; NEDD4, neural precursor cell expressed, developmentally down-regulated 4; NPC, nuclear pore complex; PAMP, pathogen-associated molecular pattern; PIRYV, Piry virus; PKR, RNA-activated protein kinase; PRR, pattern recognition receptor; RdRp, RNA-dependent RNA polymerase protein; RIG-I, retinoic-acid-inducible gene-I; RLR, RIG-I-like receptor; RNAP, RNA polymerases; RV, Rabies virus; snRNA, small nuclear RNAs; STAT1, signal transducer and activator of transcription 1; TLR, toll-like receptor; VSV, Vesicular Stomatitis virus; YAP, Yes-kinase-associated protein

pathogens. Host protein interactions have been reviewed for viruses of other families such as Poxviridae (Vaccinia virus) (Zhang *et al.* 2009) and also for plant and insect rhabdoviruses (Ammar *et al.* 2009), but for the first time they are being reviewed for animal viruses of the *Rhabdoviridae* family. Such a compiled list of host proteins for rhabdoviruses will provide an important account of molecular interactions that can be used for understanding other viruses of the family. By this review, we hope to get an insight into the pathogenesis of emerging viruses such as Chandipura virus (CHPV) and the lesser studied viruses such as Piry virus (PIRYV) and Isfahan virus (ISFV) of the *Rhabdoviridae* family through a comparative route.

2. Virus–host protein interactions

2.1 *Interactions of M protein with host proteins*

The rhabdovirus M protein is a multifunctional virion protein that plays a role in gene regulation, cellular pathogenesis and, along with the glycoprotein, enables virion assembly and budding. M protein of VSV inhibits nucleo-cytoplasmic transport by targeting the nucleoporin Nup98 present at the nuclear rim and thus indirectly inhibits host transcription (Kobbe *et al.* 2000). The nucleoporins form the nuclear pore complex (NPC), the largest protein complex in the cell, which is responsible for the exchange of components between the nucleus and cytoplasm. Nucleoporins Nup98 and Nup96 play a role in the bidirectional transport across the NPC. Nup 98 has binding sites for several transport factors. It localizes from within the nucleus to the NPC and back, thus assisting the transport of components between the nucleus and the cytoplasm (Griffis *et al.* 2002). Nup 96 interacts directly with Nup 98 and plays a role in mRNA export as well as in tethering Nup 98 to the nucleus. Interestingly, the expression of Nup98 and Nup96 is upregulated by the interferon γ (Enninga *et al.* 2002), the production of which depends on viral infection of the cell. VSV M protein can also block mRNA transport by disrupting the function of another mRNA export protein, Rae1 (Faria *et al.* 2005), which is also responsible for the nucleo-cytoplasmic transport. The VSV M protein inhibits the mRNA export by a third method involving the inhibition of Ran, which is a GTP-binding protein that is essential for the translocation of RNA and proteins through the nuclear pore complex (Her *et al.* 1997). Transport of nearly all proteins and RNA into and out of the nucleus depends on the asymmetric distribution of the two different forms of Ran across the nuclear envelope. The GTP-bound form of Ran is maintained in the nucleus by a nuclear guanine nucleotide exchange factor, RCC1, while the GDP-bound form of Ran is maintained in the cytoplasm by a cytoplasmic GTPase-activating protein, RanGAP1. M-protein-induced interference with the gradient of Ran-GTP/Ran-GDP across the nuclear envelope would inhibit most nuclear-cytoplasmic transport (Lyles 2000).

M protein selectively inhibits the processing of U1 and U2 small nuclear RNAs (snRNAs; U1,U2 and U3 are the different types), which occurs in the cytoplasm but does not affect the processing of U3 snRNA and mRNA, which occurs in the nucleus, although it does inhibit the transport of the processed mRNA to the cytoplasm (Her *et al.* 1997). The processing of rRNA requires import of newly made ribosomal proteins into the nucleus. Thus, the inhibition of processing of rRNA probably results from an M-protein-induced block in protein import. The only naturally occurring RNAs to escape the inhibitory effects of M protein are tRNAs (Her *et al.* 1997). The relative resistance of tRNA transport appears to be due to a lower requirement for Ran-GTP or due to the existence of a transport pathway that does not depend on Ran (Grimm *et al.* 1997).

The M protein of VSV inhibits host transcription by interfering with the functions of all the three RNA polymerases (RNAP). The exact mechanism by which it interferes with RNAP-I is unknown. But in case of RNAP-II it binds to the initiation factor TFIID (Lyles *et al.* 1996). TFIID is one of the seven initiation factors required by RNAPI-II for transcription, the unavailability of which inhibits transcription by RNAP-II. In case of RNAP-III the M protein binds with TFIIIC and makes it unable for RNAP-III to initiate transcription (Lyles *et al.* 1996).

M protein of RV interacts with eukaryotic initiation factor-3h (eIF-3h) subunit at various levels of translation, thus inhibiting host macromolecular synthesis (Komarova *et al.* 2007). The multisubunit eIF3 complex normally performs the principal role in ribosomal dissociation and anti-association.

Among the major cytopathic effects that result from VSV infection are cell rounding and apoptosis. VSV M protein interacts with tubulin subunits and inhibits its polymerization, thus disrupting the cytoskeleton resulting in cell rounding or even apoptosis (Melki *et al.* 1994). It has also been proposed that the cell-rounding activity of M protein results from cytoskeletal changes necessary for virus assembly (Ye *et al.* 1994).

There exists a highly conserved motif, PPxY [where x is any amino acid (aa)], within the M protein of VSV (PPPY) and RV (PPEY). This PPxY motif interacts strongly and specifically with the WW domain containing cellular proteins such as the Yes-kinase-associated protein (YAP) and the neural precursor cell expressed, developmentally down-regulated 4 (NEDD4) protein and mediate viral budding (Harty *et al.* 1999). NEDD4 is a prototypical protein in a family of E3 ubiquitin ligases.

This membrane-localized ubiquitin ligase normally participates in the endocytosis of proteins from the plasma membrane. Viruses containing the PPxY motif exploit the internalisation function of the NEDD4 protein and achieve viral budding by topologically reversing the process of endocytosis (Ingham *et al.* 2004). The PPxY motif is termed as the late budding domain or the L domain for its function in virus budding and is found to be conserved in the families of *Rhabdoviridae, Retroviridae* and *Filoviridae* (Harty *et al.* 2001).

The M protein of VSV shows striking similarity with the 3 C protease of the Poliovirus (family *Picornaviridae*) with respect to its mode of host transcription inhibition and its molecular targets in the host cell. It is also similar to the influenza virus in regulating host gene expression (Clark *et al.* 1991). The C terminal 180 amino acids of the VSV M protein have been found to be indispensable for its cytopathogenesis, while the N terminal 50 amino acids have been proven to be essential for its function in assembly and budding (Kaptur *et al.* 1991). The inhibition of multiple processes by M protein probably reflects the fact that no single inhibitory mechanism is completely effective. Alternatively, it has been hypothesised that the process that is inhibited depends upon the type of host cell and the duration of infection (Lyles 2000).

2.2 *Interactions of RdRp (L and P proteins) with host proteins*

Purified VSV RdRp (L protein) requires eukaryotic elongation factor (eEF-1) β and γ subunits for its enzymatic activity *in vitro* (Das *et al.* 1998). Interestingly, the purified L protein itself contains a tightly associated eEF-1 α subunit (Das *et al.* 1998). Thus, all three subunits of eEF-1 are associated either directly or indirectly with VSV RdRp. The involvement of multiple host-encoded proteins as subunits by VSV L protein bears striking similarity with the phage Qβ replicase. The associated host proteins in the case of phage Qβ replicase were shown to be essential for the replicase to recognise the template, initiate RNA synthesis and play a fundamental structural role in the replicase function (Blumenthal 1980; Blumenthal and Hill 1980). Similar function has been speculated for VSV L protein (Das *et al.* 1998). The VSV transcriptase is a multiprotein complex consisting of the P protein and cellular proteins, specifically eukaryotic elongation factor (eEF-1) (Das *et al.* 1998), mRNA capping enzyme guanyltransferase (GT) (Gupta *et al.* 2002) and a molecular chaperone heat shock protein 60 (Hsp60) (Qanungo *et al.* 2003), which are tightly associated with the L protein. Although VSV L protein has a unique capping pathway involving GDPs, its association with cellular GT is unanswered. It has been hypothesized by Gupta and co-workers that the L protein itself possesses an active site for capping or the cellular GT bound to it facilitates such reaction by an unknown mechanism (Gupta *et al.* 2002). Similarly, the function of associated Hsp60 is also unknown. Tubulin is another protein that is also required by the L (polymerase) protein of VSV as a positive transcription factor for RNA synthesis (Moyer *et al.* 1986). Moreover, tubulin has been found to functionally replace the acidic domain of the VSV P protein in the process of transcription *in vitro* (Chattopadhyay and Banerjee 1988). The replicase fraction does not contain EF-1α, Hsp60 and GT, which differentiates it from the transcriptase complex, which is clearly associated with those host proteins (Qanungo *et al.* 2003).

P protein of VSV is phosphorylated intracellularly by the ubiquitious cAMP-independent protein kinase casein kinase II (CK II) which remains associated with the ribonucleoprotein particle (RNP) during some steps in replication and during morphogenesis (Gupta *et al.* 1995). The central portion of the P protein (residues 138 to 172) interacts with LC8 (Light Chain 8) dynein (motor protein) and aids in axonal transport of RV along microtubules to the neurons, thus achieving the critical step of long distance transport since the site of RV entry is far from the neuronal cell body (Raux *et al.* 2000). RV P protein also interacts with the coiled-coil and DNA binding domain of signal transducer and activator of transcription 1 (STAT1) through its carboxy domain and inhibits interferon signal transduction pathways (Vidy *et al.* 2005). The RV P protein does not induce downregulation of STAT1 nor does it prevent IFN-induced STAT1 protein-activating tyrosine phosphorylation, but blocks STAT1 nuclear accumulation following IFN-α or IFN-γ treatment by inhibiting the nuclear translocation of phosphorylated STAT1 homodimer as well as STAT1/STAT2 heterodimers (Vidy *et al.* 2005).

2.3 *Interactions of G protein with host proteins*

The molecular chaperones BiP and calnexin present in the endoplasmic reticulum of the host cell bind VSV G protein monomers during folding. BiP binds maximally to early folding intermediates of G protein, whereas calnexin binds a short lag later to more folded molecules. Interaction with calnexin is necessary for efficient folding of G protein and for retention of partially folded forms (Hammond and Helenius 1994).

The nicotinic acetylcholine receptor (nAchR) serves as one of the major host cell receptor for RV (Gastka *et al.* 1996). All RV-susceptible cell lines have the neural cell adhesion molecule (NCAM) on their surface, whereas it is not found on the surface of resistant cell lines (Thoulouze *et al.* 1998). Some groups have also proved the possibility of low affinity nerve growth factor receptor (P75NTR) to serve as the receptor of RV (Tuffereau *et al.* 1998). Phosphatidylserine has been shown to directly bind to VSV and inhibit VSV

Table 1. Different host protein interactors of Vesicular Stomatitis virus and Rabies virus and of both

Protein	Function	Reference
Host proteins interacting with Vesicular Stomatitis virus (VSV)		
Nucleoporin Nup98	Interacts with M protein to inhibit the host cell gene expression	Kobbe *et al.* 2000
Rae1	M protein binds the mRNA export factor Rae1/mrnp41 and inhibits the mRNA nuclear export.	Faria *et al.* 2005
Ran GTP/Ran GDP	M protein interferes with the Ran GTP/Ran GDP gradient across the nuclear envelope and inhibits the transport of RNA and proteins	Lyles 2000
TFIID	M protein binds to TFIID and inhibits transcription initiation by RNA polymerase II	Lyles *et al.* 1996
TFIIIC	M protein binds to TFIIIC and inhibits transcription initiation by RNA polymerase III	Lyles *et al.* 1996
Tubulin	A positive transcription factor for *in vitro* RNA synthesis and cytopathic effects of VSV	Chattopadhyay and Banerjee 1988; Melki *et al.* 1994; Moyer *et al.* 1986
Cyclophilin A	A chaperone protein and one of the cellular factors required in the VSV replication	Bose *et al.* 2003
Eukaryotic elongation factor-1 αβγ (eEF-1)	RNA polymerase of VSV associates with it for its activity	Das *et al.* 1998
Casein Kinase II (CKII)	Phosphorylation of P0 to give activated P1 form of the P protein	Gupta *et al.* 2002
BiP	Essential for VSV G protein folding	Hammond and Helenius 1994
Calnexin	Essential for VSV G protein folding	Hammond and Helenius 1994
Heat shock protein 60 (Hsp60)	Part of transcriptase complex	Qanungo *et al.* 2003
mRNA cap guanylyltransferase (GT)	Part of transcriptase complex	Gupta *et al.* 1995
Annexin A2	Function unknown	Moerdyk-Schauwecker *et al.* 2009
Ubiquitin	Function unknown	Moerdyk-Schauwecker *et al.* 2009
Integrin B1	Function unknown	Moyer *et al.* 1986
Toll -like receptor7(TLR7)	Mediates activation of Interferon α/β production	Kobbe *et al.* 2000
RIG-I-like receptor(RLR)	Mediates activation of Interferon α/β production	Hornung *et al.* 2006
MxA (Mx family of GTPases)	Binds VSV N protein and sequesters viral proteins during type I Interferon response	Staeheli and Pavlovic 1991
Phosphatidylserine	May serve as a binding site for VSV G protein	Staeheli and Pavlovic 1991
Yes-kinase-associated protein (YAP	PPPY domain of M protein found to interact with WW domain of YAP	Harty *et al.* 2001
Neural precursor cell expressed, developmentally downregulated 4 (NEDD4)	PPPY domain of M protein found to interact with WW domain of NEDD4	Harty *et al.* 2001
La	Associates with RNA polymerase III transcripts in their unprocessed form	Wilusz *et al.* 1983
Heat shock protein 70 (Hsp70)	Identified as a major component in virions using immunoblot analyses	Sagara and Kawai 1992
Host proteins interacting with Rabies virus (RV)		
Light chain 8 (LC8) Dynein	Involved in the axonal transport of RV along microtubules through neuron cells	Raux *et al.* 2000
Eukaryotic initiation factor-3 h (eIF-3 h)	Inhibits host Macromolecular synthesis	Komarova *et al.* 2007
Signal transdcuer and activator of transcription 1 (STAT1)	Inhibits Interferon Signal Transduction pathways	Vidy *et al.* 2005
Nicotinic acetylcholine receptor (NAchR)	Acts as a receptor	Gastka *et al.* 1996
Neural cell adhesion molecule (NCAM)	Acts as a receptor in sensory endings and several cell lines	Thoulouze *et al.* 1998

Table 1. (continued)

Protein	Function	Reference
RIG-I-like receptor(RLR)	Mediates activation of Interferon α/β production	Hornung *et al.* 2006
Mx1 (Mx family of GTPases)	Binds RV N protein and sequesters viral proteins during type I Interferon response	Leroy *et al.* 2006
Toll-like receptor 3 (TLR3)	Speculated to play a role in interferon response	Ménager *et al.* 2009
Nerve growth factor receptor (P75NTR)	May serve as the receptor of RV	Tuffereau *et al.* 1998
Yes-kinase-associated protein(YAP)	PPEY domain of M protein found to interact with WW domain of YAP	Harty *et al.* 2001
Neural precursor cell expressed, developmentally downregulated 4 (NEDD4)	PPEY domain of M protein found to interact with WW domain of NEDD4	Harty *et al.* 2001
La	Associates with RNA polymerase III transcripts in their unprocessed form	Kurilla *et al.* 1984
Heat shock protein 70 (Hsp70)	Identified as a major component in virions using immunoblot analyses	Sagara and Kawai 1992
Host proteins interacting with both VSV and RV		
Yes-kinase-associated protein (YAP)	PPxY domain of M protein found to interact with WW domain of YAP	Harty *et al.* 2001
Neural precursor cell expressed, developmentally downregulated 4 (NEDD4)	PPxY domain of M protein found to interact with WW domain of NEDD4	Harty *et al.* 2001
La	Associates with RNA polymerase III transcripts in their unprocessed form	Kurilla *et al.* 1984; Wilusz *et al.* 1983
Heat shock protein 70 (Hsp70)	Identified as a major component in virions using immunoblot analyses	Sagara and Kawai 1992
Mx family of GTPases	Binds to N protein of both VSV and Rabies and sequesters viral proteins during type I Interferon response	Leroy *et al.* 2006; Staeheli and Pavlovic 1991
Toll-like receptor (TLR)	Role in interferon response	Ménager *et al.* 2009; Kobbe *et al.* 2000
RIG-I-like receptor (RLR)	Mediates activation of Interferon α/β production	Hornung *et al.* 2006

attachment and infectivity suggesting that it could function as a binding site for VSV G protein (Schlegel *et al.* 1983). Nevertheless, this remains a debated topic, with some groups claiming that phosphatidylserine is not the receptor for VSV (Coil and Miller 2004).

2.4 *Interaction of N protein with host proteins*

Cyclophilin A (Cyp A), a chaperone protein possessing peptidyl cis-trans prolyl-isomerase activity, is required for VSV replication. Cyp A interacts with the N protein of VSV-NJ and VSV-IND and is incorporated into the released virions of both the serotypes. By virtue of CypA's interaction with VSV N protein, the nucleocapsids are folded into a transcriptionally competent conformation. Thus, the interaction of N with CypA could result in the formation of the correct structure required for optimal transcription efficiency of the VSV-NJ genome (Bose *et al.* 2003).

2.5 *Interactions of the viral RNA with host proteins*

There has been considerable debate over the years about the mechanism of selective translation of viral mRNAs compared to host mRNAs in infected cells. Initially it was just believed that the viral mRNAs compete with the host mRNAs due to their greater abundance. Recently it has been hypothesized that viral mRNAs contain sequences that enhance their translation by binding host factors. These sequences found in rhabdoviruses are analogous to the internal ribosome entry site (IRES) of picornavirus RNAs (Lyles 2000).

La protein is yet another host factor that interacts with the virus. The function of the La host protein binding to the leader RNA of both VSV and RV is not clear; presumably, it regulates the switch between viral RNA transcription and replication in both these viruses apart from inhibiting host macromolecular synthesis in VSV infected cells (Wilusz *et al.* 1983) and stabilising the viral mRNA in RV infected cells (Kurilla *et al.* 1984).

Antiviral response of the host play important role in inhibition of host translation process in VSV infected cells. VSV dsRNA, a replication intermediate, acts as a pattern recognition receptor (PRR) that is recognized by pathogen-associated molecular patterns (PAMPs) of the host (Hardwick and Griffin 1997). The antiviral response thus evoked involves the expression of double-stranded RNA-activated protein kinase (PKR). PKR is primarily an interferon-inducible kinase, but in most cells it serves as a major activator of the host response to dsRNA, even in the absence of interferon. In the presence of dsRNA, PKR phosphorylates the α subunit of the eukaryotic initiation factor 2 (eIF2). Phosphorylation of the α subunit arrests eIF2 in the GDP bound form making it unable to bind to GTP. This prevents the reutilization of eIF2 for translation initiation. eIF4B and the cap binding complex eIF4F are also believed to be inhibited through an unknown mechanism as their addition to extracts from VSV infected cells stimulated translation (Dratewka-Kos *et al.* 1984).

Most of the viral–host interactions initiated by the virus are for its own benefit. These interactions play key role in the virus life cycle, as part of their replication machinery or pathogenesis. Such interactions often prove to be deleterious to the host as they involve deviating host factors from their normal functions. But it so happens that some interactions, most of which are not intentional from the part of the virus and occur due to the over accumulation of viral components within the cell, act as a trigger for the host immune system. Such interactions result in a cascade of pathways leading to the production of antiviral agents. One such viral component is the ssRNA of VSV and RV that acts as a ligand for toll-like receptor-7 (TLR-7) (Jennifer *et al.* 2004) and toll-like receptor-3 (TLR-3) (Ménager *et al.* 2009), respectively. This interaction is responsible for the activation of interferon α/β production during the innate immune response. Another protein, retinoic-acid-inducible gene-I (RIG-I)-like receptor [RLR], is also involved in the activation of interferon α/β production during VSV and RV infection. It has been found to bind to the single-stranded RNA molecules bearing a 5′ triphosphate (Hornung *et al.* 2006).

MxA and Mx1 from the Mx family of GTPases produced during type I interferon response interfere with VSV mRNA synthesis either directly by reducing the activity of the viral RNA polymerase complex or indirectly by destabilising the viral mRNAs (Staeheli and Pavlovic 1991). MxA has also been reported to inhibit the virus replication in case of RV (Leroy *et al.* 2006).

The net effect of viral cytopathogenesis is a result of the balance between virus propagation in the host, the antiviral responses of the host and the viral mechanisms that inhibit that response.

2.6 *Viral–host interactions with unknown functions*

A proteomics approach revealed the presence of five host proteins (tubulin alpha, annexin A2, eEF1α, ubiquitin and integrin 1) associated with VSV virions that were isolated from three different cell types of human, mouse and hamster. The presence of all the five proteins in virions irrespective of the infecting cell type suggests a strong possibility of these proteins being involved in the biology of VSV (Moerdyk-Schauwecker *et al.* 2009). Studies using immunoblot analyses have also detected the heat shock protein 70 (Hsp70) as a minor component of the VSV-NJ and RV virions (Sagara and Kawai 1992).

3. Discussion

After a comprehensive literature search, we identified a spectrum of host proteins that interact with VSV and RV (table 1). The roles of a few of these host cellular proteins have been studied, albeit very recently. Interestingly, on comparison of data, we found that, in spite of having a divergent mode of cytopathogenesis, VSV and RV shared many common interacting host proteins –*La, Hsp70, RLR, TLR, Mx family of GTPases, YAP* and *NEDD4* (table 1).

Table 2. Conserved PPxY motif in M protein

Virus	Accession No.	Protein Sequence	Position
VSV (Indiana)	J02428	KLGIA PPPY EEDTS	24–27
VSV (New Jersey)	M14553	KKMGL PPPY DESCP	24–27
Rabies virus	M31046	DLWLP PPEY VPLKE	35–38
Chandipura virus	AF128868	MDYDS PPSY QDVRR	30–33
Piry virus	D26175	MEWES PPSY NEIKS	33–36
Isfahan virus	AJ810084	MDWDE PPSY SDSRY	28–31

PPxY (where x is any aa) motif is reportedly present in the M protein of key rhabdoviruses. In CHPV, PIRYV and ISFV, the corresponding motif is represented by PPSY (Source: EXPASY, Viral Zone).

The *La* protein binds to the leader RNA of the viruses and is presumably involved in inhibiting host macromolecular synthesis as well as regulating the switch between viral RNA transcription and replication in VSV. In RV, it stabilizes the viral mRNA. Another protein, heat shock protein 70 (Hsp70), was isolated from the virions of both these viruses. However, its function is yet to be identified.

Cytoplasmic receptors such as RLR and membrane-bound receptors such as TLR are involved in the intracellular interferon response of the host against VSV and RV. These receptors are believed to use the RNA of negative-sense single-stranded RNA viruses to initiate a cascade of reactions leading to interferon response. Since all rhabdoviruses are negative-sense single-stranded RNA viruses, these receptors may be involved in the generation of interferon response in hosts against these viruses. The Mx family of GTPases, on the other hand, affects the viral replication by inhibiting the viral mRNA synthesis. This points towards a similar role of this family of proteins in other rhabdoviruses as well.

The WW domains present in NEDD4 and YAP were found to associate with the praline-rich PPxY motif found in the M protein of both VSV and RV. M protein of CHPV, PIRYV and ISFV also contains the sequence PPSY, which conforms to the PPxY motif (table 2). This suggests that there is a high probability that these two proteins may interact with the M protein of CHPV, PIRYV and ISFV as well and may have implications in virus budding and pathogenesis.

It is speculated that antiviral compounds targeting the PPxY motif in rhabdoviruses can be used to inhibit the interaction of host cell proteins and viral proteins required for viral budding (Palese 2003). This demonstrates the importance of the PPSY motif of M protein of CHPV, PIRYV and ISFV in serving as a potential antiviral target.

There are limited studies for finding the host protein interactants of both VSV and RV. In our search, we found that till date around 24 and 13 host proteins interact with VSV and RV, respectively. The list generated was based on specific scientific queries for understanding individual protein interactions by separate research groups. In the genomic context, analysis needs to be done in a global fashion, where a protein interacts with host proteins employing techniques such as yeast-two hybrid, (Guo *et al.* 2001) or tandem affinity purification, where the protein interactions are identified using double-tag affinity purification followed by mass spectroscopy (Krogan *et al.* 2006). There is also a possibility that there may be some proteins from the spectrum of VSV as well as RV host proteins that, although not common between these two viruses, may also be involved in interaction with other lesser studied viruses of the family. Therefore, further studies need to be carried out to identify other host proteins. While more unknown interactions are being identified, the proteins reviewed in this report should be analysed by experimental procedures in context with other viruses of the family.

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