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ORIGINAL PAPER

Reviewing Chandipura: A Vesiculovirus in Human Epidemics

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Abstract Chandipura virus, a member of the rhabdoviridae family and vesiculovirus genera, has recently emerged as human pathogen that is associated with a number of outbreaks in different parts of India. Although, the virus closely resembles with the prototype vesiculovirus, Vesicular Stomatitis Virus, it could be readily distinguished by its ability to infect humans. Studies on Chandipura virus while shed light into distinct stages of viral infection; it may also allow us to identify potential drug targets for antiviral therapy. In this review, we have summarized our current understanding of Chandipura virus life cycle at the molecular detail with particular interest in viral RNA metabolisms, namely transcription, replication and packaging of viral RNA into nucleocapsid structure. Contemporary research on otherwise extensively studied family member Vesicular Stomatitis Virus has also been addressed to present a more comprehensive picture of vesiculovirus life cycle. Finally, we reveal examples of protein economy in Chandipura virus life-cycle whereby each viral protein has evolved complexity to perform multiple tasks.

Keywords Chandipura virus · Transcription · Replication · Encapsidation · Leader RNA · Nucleocapsid protein · Phosphoprotein · Economy · Multi-functional

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Introduction

RNA viruses posses highest mutation rate among all known self-replicating beings (Holland et al. 1982) that offers considerable ''long-term'' evolutionary advantages over its host, organisms with DNA genome repertoire (Elena and Sanjuan 2005). Not surprisingly, out of ~50 new viruses that have been recognized as emergent in the past two decades, most of them are deadly RNA viruses. An outbreak of severe febrile encephalitis in Malaysia with reports of human deaths in 1998–99 was associated with Nipah virus (Chua et al. 2000). Coronavirus associated with Severe Acute Respiratory Syndrome (SARS) posed a threat of epidemic in 2003 with rapid spread across the eastern Asia (Marra 2003). In another recent outbreak, a deadly strain of avian flu virus H5N1 spread across the parts of three different continents from 2004 to 2006 through poultry trading as well as migratory birds (Ducatez et al. 2006; Chen et al. 2005). These occurrences indicated increased susceptibility of the modern day human civilization intimately connected within a global village towards potential viral pandemics.

Chandipura virus (CHPV) was first isolated in 1965 from a patient suffering from febrile illness in a village called Chandipura in India and was shown to have cytopathic effect on cells in the tissue culture (Bhatt and Rodrigues 1967). Later, in 1977 another virus strain was isolated from hedgehog in Ibadan, Nigeria (Clewley et al. 1977). Although, CHPV was known to cause mild symptoms upon human infection and was also isolated from encephalopathy patient in 1980 (Rodrigues et al. 1983), only in 2003 first evidence for its association in human epidemics was obtained when CHPV was identified from patient samples during an outbreak of acute encephalitis with high fatality rate in India (Rao et al. 2004). Subsequently, another outbreak of encephalitis associated with CHPV infection with more than 75% fatality rate was reported in the eastern state of Gujarat in India in 2004 (Chadha et al. 2005). These occurrences indicated possible emergence of Chandipura virus as a deadly human pathogen in Indian Subcontinent.

CHPV was identified as probable causative agent associated with the epidemics based on reliable tools such as serological examination, electron microscopy and other molecular analyses. Although, high mutation rate among RNA viruses could results in evolution of deadly strains, possibilities of CHPV being a passenger or concomitant virus could not be completely ruled out from above mentioned case-studies (Potharaju and Potharaju 2006). Therefore, further investigations are required to obtain evidences that supports the principles forwarded by Koch's postulates to unequivocally establish CHPV as an emerging human pathogen (Nature Reviews Microbiology editorial 2006; Van Ranst 2004). Nonetheless, isolation of CHPV from sand flies earlier in 1967 (Dhanda et al. 1970) and also during the recent epidemic (Rao et al. 2004) lead to suspect sand fly as a potential vector for this viral pathogen.

In this review, we aim to provide a general insight on Chandipura Virus life cycle and discuss our current understanding on molecular events associated with the decoding of its genetic information as well as replication of viral genome. Pathways for encapsidation of progeny genome RNA into a nuclease resistant structure have also been addressed to forward a refined model for nucleocapsid assembly. Understanding gained from the parallel studies in closely related Vesicular Stomatitis Virus (VSV) was discussed to reveal both generalized mechanisms and distinct modes associated with Chandipura virus pathogenesis.

Chandipura Virus: A General Overview

Chandipura virus has been assigned into *vesiculovirus* genus and *rhabdoviridae* family within the virus order *mononegaviridae*. CHPV and other *mononegavirales* are characterized by non-segmented, single stranded RNA genome of anti-message sense (negative sense) (David M. Knipe 2001). Among them, *rhabdoviruses* received major attention due to their wide host range starting from human and other vertebrates, fish, arthopods, members of plant kingdom, and as causative agents for loss in livestocks, agricultural products and in fisheries. Viruses belonging to this family owe its name *rhabdo*, meaning rod-shaped in Greek, to the typical bullet shaped morphology and include human pathogens such as CHPV (Fig. 1a) or rabies. Similarities between CHPV and VSV in genetic makeup, polypeptide composition and life cycle lead to include CHPV within *vesiculovirus* genus (Banerjee 1987a).

Virus Life-cycle

Vesiculovirus life cycle can be divided into discrete steps, namely, adsorption of virus particle, penetration of virus into cell, uncoating and release of core RNP into the cytosol from late endosomal vesicles, transcription of the genome by viral polymerase, translation of viral mRNA, post-translational modifications of viral proteins, replication of viral genome, assembly of progeny particles and finally budding of mature virion. Entire vesiculovirus life cycle within infected cell is cytosolic (Banerjee 1987a). Genome RNA enwrapped with Nuleocapsid protein N acts as a template for sequential transcription starting from 3′end of the genome to synthesize short leader RNA and five monocistronic capped and poly adenylated viral mRNAs. Viral RNA dependent RNA polymerase (RdRp) is composed of Large protein L, the catalytic subunit and phosphorylated form of Phosphoprotein P that acts as a transcriptional activator. Translation of viral mRNAs results in accumulation of viral polypeptides within infected cells and set up stage for the onset of genome replication (Banerjee 1987a; Barr et al. 2002). During replication, the same polymerase switches to replicative mode to copy entire genomic template into an exact polycistronic complement that acts as replication intermediate to produce many more copies of (–) ve sense genomes upon further rounds of replication. Progeny (–) ve sense genomes are also subjected to transcription, referred to as secondary transcription. Notably, virus specific genomic analogues, and not mRNAs, always remain encapsidated by N, while, it is believed that progressive encapsidation of nascent genome RNA during its synthesis is necessary for replication and/or protecting replication product from cellular RNases (Banerjee 1987a;

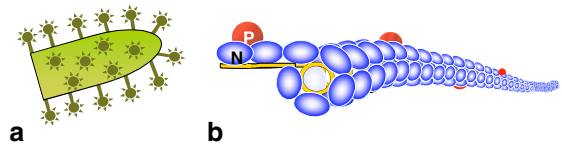

Fig. 1 **(a)** A schematic presentation of bullet shaped Chandipura virus with glycoprotein G protruding out of the viral envelope. (**b**) A proposed view of Chandipura virus genome RNA encapsidated with Nucleocapsid protein. Nucleocapsid protein binds to viral RNA to enclose it in a disc like structure. This disc like structures stacks on each other to generate a helical assembly, as depicted, to form core nucleocapsid. Phosphoprotein P and Large Protein L remain associated with N-RNA

Barr et al. 2002). Below, we will discuss individual components of Chandipura virus and their role in viral life cycle.

Chandipura Virus and its Genome Organization

CHPV is surrounded by lipoprotein envelope, which encloses a helical ribonucleoparticle (RNP) with a non-segmented single strand RNA enwrapped by N. Components of viral RdRp, L and P are also packaged within the mature virion and remained associated with core nucleocapsid particle. Glycoprotein G protrudes externally from the outer membrane while Matrix protein M lies in the inner bilayer of the membrane.

CHPV genome RNA comprises of a 49 nt leader gene (l), followed by five transcriptional units coding for viral polypeptides separated by intragenic spacer regions and a short non-transcribed 46 nt trailer sequence (t) arranged in the order 3′ l-N-P-M-G-L-t 5′. Sequence for (+) leader RNA of CHPV was determined in 1983 (Giorgi et al. 1983), while N and P gene was sequenced in 1987 (Masters and Banerjee 1987). Subsequently, sequences for M and G gene along with parts of L gene was determined (Masters et al. 1989; Taylor et al. 1999). Its only recently, complete sequence for L gene of CHPV was obtained to construct a full-length genome map of this virus (Marriott 2005). Interestingly, comparative sequence analysis proposed CHPV to be evolutionary equidistant from the new world *vesiculoviruses* VSV Indiana (VSVind) and VSV New Jersey (VSVnj) and rather closely related to the Asian *vesiculovirus* Isfahan (Marriott 2005).

Glycoprotein G

Glycoprotein, G is the sole spike protein of CHPV that enables virus absorption, assembly and budding. It also elicits antibody response thus acting as a major antigenic determinant (Neumann et al. 2002). Studies on CHPV-G protein expressed from a cloned DNA revealed presence of a N-terminal cleavable signal peptide, two N-liked glycosylation sites at the N-terminal ectodomain, a membrane anchor domain and a cytosolic domain at the C-terminus (Masters et al. 1989). Previous studies revealed that VSV glycoprotein can exists as trimer (Zagouras and Rose 1993). It may reversibly adopt three distinct conformational states; the native state present on the virus surface and stable above pH 7 (Clague et al. 1990), the activated state that fuses with target membrane (Durrer et al. 1995) and a fusion inactive postfusion state that is stable under low pH condition (Yao et al. 2003). It was proposed that low pH induced conformational change in the G protein within endosome subsequent to viral entry enables membrane fusion to release core particle in two sequential steps into the host cytoplasm (Le Blanc et al. 2005).

Recently, crystal structure of the VSV-G protein ectodomain has been solved in its postfusion state to reveal classic hairpin conformations, large numbers of protonated residues stabilizing low pH state and also a novel fold combining features of class I and class II fusogenic peptides (Roche et al. 2006). In light of the prediction that other rhabdoviral glycoproteins display similar folds (Roche et al. 2006), homology modeling of G protein form Chandipura virus Nagpur strain and sequences derived from the epidemic associated strains may allow us to gain insight into viral pathogenesis. It is interesting to note that almost all of the amino acid substitutions in G protein sequences

of epidemic associated virus strains were observed within the ectodomain, a viral tool for host invasion (Arankalle et al. 2005).

Matrix protein M

The matrix protein, M lies in the inner surface of the virion to tether core nucleocapsid to the membrane. Highly basic N-terminal domain, with eight lysine residues, enables membrane binding (Ogden et al. 1986) and is separated from the rest of the polypeptide by a triple proline sequence (Rose and Gallione 1981). Crystal structure of VSV-M protein revealed contributions from additional domain in membrane association and presence of a flexible link conserved among *mononegavirales* that provide proper alignment to the membrane binding domain (Gaudier et al. 2002). M protein also interacts with and recruits mature nucleocapsid particles to the membrane during viral assembly and budding. Moreover, M protein was shown to inhibit viral transcription presumably by inducing a higher order condensed conformation of the RNP (De et al. 1982).

Research in the last few years, however, recognized Matrix protein as a deadly weapon in rhabdoviral arsenal that perhaps alone may account for the observed cytotoxicity in virus infected cells (Licata and Harty 2003). VSV-M was shown to shut off host transcription by RNA polymerase I and II (Ahmed and Lyles 1998). It was capable of inhibiting nuclear export of host mRNA and snRNA (Petersen et al. 2001) by targeting nucleoporin Nup98 present at the nuclear rim (von Kobbe et al. 2000). M protein mediated inhibition of host gene expression constitute for an example of viral mechanism to suppress cellular interferon response (Enninga et al. 2002). Marriot and co-workers cloned Chandipura virus Matrix protein gene in 1999 to subsequently show its detrimental effect on transcription from cytomegalovirus immediate early promoter in vivo (Taylor et al. 1999). Despite only 28% amino acid sequence identity with VSV, CHPV-M also preserved the ability to inhibit host nucleo-cytoplasmic transport (Petersen et al. 2001). Recently, another full-length clone of CHPV-M was independently constructed by D.J. Chattopadhyay and his co-workers and functional characterizations are underway. Additional works on CHPV-M protein are required to completely understand multiple levels of intervention within the cellular signaling network by human rhabdoviruses.

Ribonucleoprotein (RNP) Particle

Ribonucleoprotein particle is composed of core nucleocapsid and associated viral RdRp subunits. RNP itself is infectious as it contains all the enzymatic activity necessary for RNA synthesis and could produce progeny virus particles when artificially introduced within cell (Thornton et al. 1983).

Nucleocapsid Assembly and Maturation

Core nucleocapsid of Chandipura virus is composed of 11,119 nt long genome RNA (Marriott 2005) tightly enwrapped by ~1,200 molecules of N with requirement of one N molecule for 9 nt (Thomas et al. 1985). While, encapsidated RNA enjoys protection

from cellular RNase, viral RdRp does not recognize genome RNA as template unless presented in an enwrapped conformation (Banerjee 1987a, b; Thomas et al. 1985). Structure of viral RNA inside intact nucleocapsid was examined by Iseni et al. using a combination of chemical probes (Iseni et al. 2000) to reveal that the ribonucleotide bases are in general available for chemical modifications in VSV nucleocapsid, while the phosphate groups present in RNA backbone are not. They also observed that bases present in certain region of the enacpsidated genome RNA are less reactive independent of the associated P or L protein. These observations, along with previous studies (Keene et al. 1981), suggested that N primarily interacts with phosphate backbone to enwrap genome RNA and present sequestered RNA in a specialized conformation.

Ming Luo and his colleagues have recently reported that bacterial co-expression of N and P protein resulted in a complex with 10 molecules of N, 5 molecules of P and a ~90 nt long RNA of bacterial origin. A low salt treatment readily dissociated P to obtain a decamer N complex associated with RNA. Electron micrograph of decamer N revealed a disk-like appearance (Green et al. 2000) with disk size simlar to one turn of helix in viral nucleocapsid particle (Thomas et al. 1985). Single particle electron microscopy revealed that RNA molecule is closely associated with inner surface near the top end of such highly symmetrical disc (Chen et al. 2004). Accordingly, it was proposed that this disc-like particle assembles into a helical structure by stacking on each other to form viral nucleocapsid (Fig. 1b). Finally, crystal structure of these N discs associated with RNA was solved (Green et al. 2006) to reveal that the RNA is tightly bound in a cavity at the interface between two lobes of the N protomer, with nine nucleotides of RNA associated with each N polypeptide. RNA binding cavity within N molecule was considerably positively charged. Residues that are involved in direct association with phosphates of RNA are spread across the N protein. Out of six residues that contact phosphates, four are conserved among different rhabdoviruses, implying a similar mode of RNA binding. Tyr 21, which apparently stacks against nucleotide 1 of the bound RNA, is substituted in CHPV-N by phenylalanine, another aromatic amino acid. An extensive network of interactions between neighboring N molecules was also revealed with each N monomer contact with three other N monomers. A hinge region between two lobes of N protomer was proposed to provide necessary flexibility such that RNP can adopt alternative conformations at different stages of maturation.

Unlike Tobacco Mosaic Virus, vesiculovirus N protein is not completely dissociated from viral RNA during RNA synthesis. How could viral polymerase possibly read sequences during transcription while genome RNA is enwrapped by N? Based on chemical probing experiments it was suggested that bases are available on the surface of the nucleocapsid for polymerase recognition (Iseni et al. 2000). However, crystal structure of the nucleocapsid-like particles revealed that out of 9 nt associated with each N monomer, bases in position 5, 7 and 8 are completely shielded by N thus conceivably prevented from forming RNA duplex during transcription (Green et al. 2006). Therefore, it was postulated that viral RNA is temporarily dissociated from N protein within the active polymerase complex during transcription, a possibility also suggested by Banerjee (1987a, b). Reports of similar structure for rabies virus nucleoprotein-RNA complex (Albertini et al. 2006) and identification of disc-like appearances for CHPV-N (Majumdar et al. 2004) allowed us to built up a conceptual framework towards understanding conserved pathways for rhabdoviral nucleocapsid assembly (discussed later). Nonetheless, nucleocapsids are subjected to further state transitions, presumably

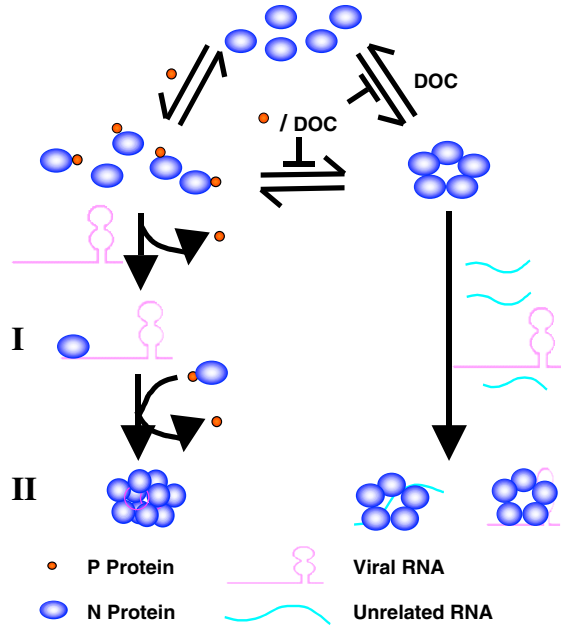

Fig. 2 A Schematic depiction of specific yet processive encapsidation of Chandipura virus genome RNA by Nucleocapsid protein N. The picture describes self-assembly of N protein monomer into oligomer that binds to leader RNA or unrelated RNA. Dissociating detergents such as deoxycholate disrupts those oligomer into monomer units and also prevents further oligomerisation. Phosphoprotein P associates with N to keep N protein in a monomer form. This monomer N is able to recognize specific sequence present on leader chain in the nucleation step (I) and is recruited to the viral RNA with concomitant release of P. Subsequently, additional N molecules associates with RNA bound N monomers in the elongation phase (II). N polymerization mediates a conformational change, thus, generates broad specificity within RNA binding interface of N protein and allows for progressive encapsidation to enclose RNA into a helical conformation. This model posit important role of N polymerization on its RNA binding specificity and encapsidation. A proposed stem-loop structural element within leader sequence has also been depicted

involving M protein, from an undulating ribbon to tightly coiled helix before packaging within mature virus (Thomas et al. 1985).

Chandipura Virus N protein and Encapsidation

Chandipura virus N gene was sequenced almost two decades ago to reveal ~50% sequence homology to N protein of VSVind or VSVnj serotypes (Masters and Banerjee 1987). However, it is only recently a full-length clone of N, its recombinant expression, biochemical and biophysical characterizations has been reported (Majumder et al. 2001). CHPV-N gene codes for a 422 amino acid polypeptide with no reports for any significant post-translational modifications. Comparison of rhabdovirus and paramyxovirus N proteins revealed a region with significant identity at the center of the polypeptide (Masters and Banerjee 1987) that has been implicated for N–N association (Banerjee 1987a). Recent crystallographic data on VSV-N protein indicated that this central region forms constituents of both N- and C-terminal lobe and thus probably instrumental in maintaining overall architecture of the protein (Green and Luo 2006).

The major biological property of N is to bind with nascent leader RNA to initiate encapsidation of replication product concurrent to synthesis (Blumberg et al. 1983, Wertz 1983; Patton et al. 1984; Banerjee 1987a). However, aggregation prone nature of N poses a major obstacle towards biochemical characterizations. Encapsidation competent N synthesized in a cell-free system was not sufficient for extensive structure-function analysis (Patton et al. 1983). Native viral protein obtained after dissociating from RNA (Blumberg et al. 1984) or recombinant protein obtained by over-expressing within eukaryotic cellular milieu (Sprague et al. 1983) forms aggregates. Bacterially over-expressed protein, recovered by denaturation/ refolding, required high salt to prevent precipitation, therefore, compromising the scope for wide biochemical applications (Das and Banerjee 1993). However, its was observed that Phosphoprotein P, through, its C-terminus interacts (Takacs et al. 1993) and keeps N in a soluble form (Howard and Wertz 1989; Green et al. 2000; Gupta and Banerjee 1997). Accordingly, N-P complexes of various molar ratios was observed within infected cells (Masters and Banerjee 1988b; La Ferla and Peluso 1989). Gupta et al. successfully co-expressed N and P protein from two independent transcriptional units within bacteria to obtain soluble N–P complex (Gupta and Banerjee 1997). Their expression condition resulted in formation of large set of aggregates, which was prevented by co-expression of N and P from a single polycistronic unit that apparently maintained precise molar ratio of N and P protein to enable nucleocapsid-like particles formation (Green et al. 2000).

However, Basak et al. were able to express a soluble form of CHPV-N protein in bacteria circumventing the need for co-expressed P (Majumder et al. 2001). Transmission electron microscopy of homogenously purified CHPV-N removed from detergent revealed similar disc-like appearances (Majumdar et al. 2004) as obtained for VSV N–P complex. Subsequent studies revealed a role for osmolytes in preventing further aggregation of this oligomer N (Majumder et al. 2001). P was identified as a N specific chaperone that prevented N aggregation in vitro and in vivo independent of its phosphorylation status (Majumdar et al. 2004). One important difference between N preparations described for CHPV and those reported by Green et al. was the absence of detectable amount of bacterial RNA in CHPV-N (Majumdar et al. 2004; Bhattacharya et al. 2006). Rather, purified CHPV-N was shown to interact with RNA of viral or non-viral origin in vitro (Majumdar et al. 2004; Bhattacharya et al. 2006; Majumder et al. 2001), substantiating the conclusion that N preparation was not saturated for RNA binding. Considering a high degree of sequence homology, these apparent contradictions were striking and may arise from high salt purification protocol used to obtain CHPV-N, which could potentially dissociate bound RNA (Bhattacharya et al. 2006; Majumder et al. 2001). However, it seems possible that N rapidly polymerizes as soon as it is synthesized thus were unable to enclose RNA into the disc like structures, when expressed alone in bacteria (Bhattacharya et al. 2006; Majumdar et al. 2004). Whereas, co-expressed P prevented such self-assembly and allows for defined polymerization of N protein on RNA chain. Based on their study in VSV, Green et al. (2000) predicted a transient yet indispensable role for P in appropriate assembly of N into a nucleocapsid-like particles, whereas, absence of P would result in an aggregated N that is incapable of RNA binding. Therefore, studies on CHPV suggest existence of additional possible mechanisms for rhabdoviral N protein assembly in a P independent manner (Majumdar et al. 2004).

Although, N protein displays broad sequence specificity that is consistent to the observed mode of RNA binding in crystal structure, proper initiation of the

encapsidation requires specific recognition of the sequence elements present at the genome termini (Blumberg et al. 1981, 1983; Pattnaik et al. 1995). How does N maintain a dual status, i.e. its ability to recognize specific sequence on nascent RNA (nucleation) and still progressively encapsidate RNA of diverse sequences (elongation)? While, VSV-N crystal structure provided insight into the molecular interactions present within mature nucleocapsid, recent studies on CHPV-N described a dynamic model for N-mediated nulcleocapsid assembly (Bhattacharya et al. 2006). In their experiment, Bhattacharya et al. used a dissociating detergent deoxycholate to disrupt oligomeric disc structure of bacterially expressed N protein to obtain monomer N. Furthermore, they removed the detergent from N preparation in presence of Phosphoprotein to reveal that P prevents re-assembly of N into oligomer and maintain it in a monomer form, in vitro. Subsequently, they compared RNA binding specificities of monomer N and its oligomeric counterpart. These course of studies strikingly revealed that monomer N could recognize specific sequence present within the first 21 nucleotides of (+) leader RNA whereas oligomer N shows a compromised RNA binding specificity. Finally, viral minigenome length RNA sequestered N from its P bound monomer form to initiate appropriate encapsidation into a nuclease resistant structures.

Based on this analysis and new insights gained from structural studies, we propose a two-step model for encapsidation that requires monomer N as a building block (Fig. 2). In the nucleation step, monomer N that poses an intrinsic RNA binding specificity recognizes viral sequences on nascent leader RNA to initiate nucleocapsid assembly. Subsequent N–N association during elongation phase results in subtle conformational change to allow for compromised binding specificity so that newly polymerized N on RNA molecule can bind to heterogenous sequences. While, a fast reversible interaction between N and P protein ensures supply of additional N monomers, N–N as well as N-RNA association, traps N from N–P complex within encapsidation complex during elongation. In this proposal, P modulates oligomeric status of N protein to allow for appropriate encapsidation of viral RNA.

This mechanistic model was able to provide explanation for many of the published reports. Previous studies with VSV-defective interfering (DI) particle revealed that the 5′terminal 36 nucleotides and 3′terminal 51 nucleotides of the genome are sufficient for replication, encapsidation and budding (Pattnaik et al. 1995). In vitro assembly assay with synthetic nucleocapsid identified the first 19 nt from the 5′end of the (+) sense RNA as necessary component for encapsidation (Moyer et al. 1991). Moreover, a heterologous sequence when fused downstream to the first 19 nt sequence from viral RNA, supported encapsidation, thus confirming a compromised specificity in the ensuing encapsidation process (Moyer et al. 1991). Also, a large network of N–N interactions was shown to stabilize nucleocapsid assembly, therefore, consistent to the need for association between N proteins in enduring encapsidation. N protein expressed in bacteria or in mammalian cell in absence of P assembles into aggregate (Das and Banerjee 1993; Majumdar et al. 2004). This oligomeric N was shown to form RNase resistant complexes with leader RNA (Blumberg et al. 1983), but was unable to encapsidate longer VSV RNA in vitro (Moyer et al. 1991). Oligomeric N of CHPV once formed was only able to loosely associate with RNA instead of forming true encapsidation complex (Bhattacharya et al. 2006). Finally, complex formation with P was proposed to suppress non-specific RNA binding ability of N, therefore, channel N protein towards viral specific sequences within infected cells (Masters and Banerjee 1988a). Our current model, consistent to this observation, proposes that N protein maintained in monomer form through its association with P would exhibit such RNA binding specificity.

However, disc-like structures obtained by co-expressing VSV N and P protein was non-specifically bound to RNA (Green et al. 2000, 2006). These results were in apparent contrast to a proposed function of P to prevent non-specific RNA binding (Masters and Banerjee 1988a) and also to the observed RNA binding specificity of CHPV N-P complex (Bhattacharya et al. 2006). VSV expression system allowed N to form 2:1 complex with P and therefore it appears that stoichiometry of N–P complex plays a critical role in RNA binding specificity. Such suboptimal level of P would result in a trimeric assembly with two N molecules and one P protein, while, N–N association within those complexes may allow for non-specific RNA binding. However, N–P complex with one monomer from each protein provides RNA recognition specificity of N. It is interesting to note that a 1:1 molar complex between N and P protein was proposed to be most efficient in supporting viral replication (Peluso 1988; La Ferla and Peluso 1989).

Nonetheless, above studies established a requirement for stoichiometric amount of P, independent of its role in transcription, to prevent both oligomerisation and aggregation of N and to ensure supply of soluble encapsidation competent N. However, based on current proposal, in would be necessary to elucidate monomer N protein structure to understand its RNA binding specificity in Chandipura virus system. Proposed conformation alterations due to N–N association also need to be investigated. As N protein lacks any well-characterized RNA binding motifs; most striking aspect of this proposal is an intrinsic RNA binding specificity that is hardwired within N molecule. If true, this may allow for development of small molecule inhibitors of nucleation as potential antiviral agents. Further mutagenic analysis on N protein to dissect oligomerisation, P binding and RNA binding will shed light on Chandipura virus nucleocapsid assembly pathways. Nonetheless, encapsdiation process exemplifies coordinated operation of multiple viral components to perform single function, viz genome packaging.

Leader RNA and Trailer Sequence

Leader gene positioned at the extreme 3′end of the genome is one of the first to be transcribed and codes for 49 nt-untranslated, uncapped and non-polyadenylated leader RNA of (+) polarity. VSV leader RNA was recovered from virus-infected cells in a nucleocapsid-like structure (Blumberg and Kolakofsky 1981) and was postulated to be an abortive replication product and not synthesized during transcription (Barr et al. 2002, Whelan and Wertz 2002). However, as discussed later, post-synthesis encapsdiation of leader transcript appears to be possible within virus-infected cells. In this regard, it would be important to investigate temporal profile for synthesis of leader RNA within CHPV infected cells to ensure it to be an immediate early viral transcript.

Precise physiological function of l-RNA has remained unclear. Although, it was proposed to localize into the nucleus (Kurilla et al. 1982) and interferes with host DNA-dependent RNA synthesis (McGowan et al. 1982). VSV leader RNA was shown to interact with components of hnRNP U in the nucleus which may explain its inhibitory function on host mRNA synthesis (Gupta et al. 1998). VSV l-RNA was also shown to binds with La, a protein that modulate RNA polymerase III transcription (Kurilla and Keene 1983). Studies on Rinderpest virus, a paramyxovirus, not only identified similar interaction between leader RNA and La protein but also revealed a possible role for La in the cytoplasm in modulating viral RNA synthesis by its ability to bind nascent l-RNA (Raha et al. 2004b).

Most important function of leader sequence, however, is to provide specific nucleation signal for initiation of N-mediated encapsidation of genomic analogues. Critical sequence elements on leader chain for N binding was identified to reside at the genome termini (Pattnaik et al. 1995) and apparently first 21 nucleotide of l-RNA was sufficient for nucleocapsid assembly (Li and Pattnaik 1999). In vitro studies with CHPV-N revealed that first half of the leader RNA (1–20) was sufficient for specific encapsidation complex formation (Bhattacharya et al. 2006). Comparison of the sequences revealed that only two nucleotides, 8th and 19th residue from 5′end of the anti-genome RNA, within the first half of the leader gene differ among VSVind, VSVnj or CHPV. Whereas second half of the leader gene showed considerable sequence diversity (Marriott 2005). Consistently, the second half of the l-RNA of CHPV was shown dispensable for specific encapsidation in vitro (Bhattacharya et al. 2006). However, recent studies revealed potential for presence of a structural element within second half of the CHPV leader RNA that may regulate viral replication (Basak et al. 2003). Nonetheless, trailer sequence present at the 5′end of the viral genome, specially extreme 20 nt, shows extensive sequence complementarities with leader region thus strongly supports proposed copy back mechanism in generating DI particles (Nichol and Holland 1987).

Promoter and Terminator Elements

Efforts by several groups led to identify cis-acting signals embedded within vesiculovirus genome that modulate transcription or mRNA modifications. In vitro reconstituted synthetic nucleocapsid analogues indicated that first 15 nt at the 3′end of the genome plays an essential role in modulating polymerase entry to the template (Smallwood and Moyer 1993). However, G. W. Wertz and coworkers employed reverse genetics to report necessity of two distinct elements at 19–29 and 34–46 from the 3′end of the genome, that regulate VSV transcription activity from sub-genomic template (Whelan and Wertz 1999, Walpita and Flick 2005). In a separate study, Li et al. also proposed a role for sequences from 25 nt to 47 nt in modulating viral transcription beside first half of the leader gene (Li and Pattnaik 1999).

Presence of core promoter element associated with each transcription unit was further investigated by mutagenic analysis of bipartite minigenome replicon to identify 3′-UYG-5′ tri-nucleotide at the beginning of each five protein coding genes as necessary element for transcription initiation and capping of the mRNA (Stillman and Whitt 1999). In this regard, vesiculovirus uses promoter elements that are downstream of initiation site and resembles with downstream promoter elements found within mammalian transcriptional repertoire (Kadonaga 2002). Leader gene present at the extreme 3′end of the genome of CHPV and other vesiculoviruses, however, lacks this conserved tri-nucleotide and poses 3′-UGC-5′ sequence. Strikingly, sequence analysis among different *mononegavirales* revealed a more general consensus 3′-UNC-NNNUUNN-5′ at the beginning of transcriptional units (Stillman and Whitt 1997), which significantly differ in the +3 residue from a conserved VSV sequence proposed by mutagenesis analysis. Therefore, distinct promoter sequence requirement of vesiculovirus RdRp could be envisioned whereby optimal transcription from the protein coding ORF may require a G in +3 site to synthesize capped RNA.

Nevertheless, similar mutagenesis study was employed to propose intergenic non-transcribed dinucleotide 3′G/CA5′ as an essential element for efficient transcription termination (Stillman and Whitt 1997). A conserved tetranucleotide sequence (3′-AUAC-5′) followed by a U7 tract present at the end of each gene was proposed to ensure proper termination and poly-adenylation of mRNA (Barr et al. 1997). Except for G/L junction, the gene junctions are highly conserved between CHPV and VSVind serotype indicating that a general mechanism is employed by vesiculoviruses to regulate gene-expression. While, for VSVind, G/L junction still has a dinucleotide in the intergenic space, VSVnj or CHPV contains 20 and 21 nucleotide in between G and L transcription units, respectively (Marriott 2005). However, direct investigations to identify cis-acting signals required for CHPV gene-expression has been impeded due to lack of reverse genetics system. Recently, a minigenome-based replicon of Chandipura virus was constructed and efforts are currently underway to reveal sequence codes for transcriptional initiation and termination (A. Majumdar and D. Chattopadhyay 2006, Unpublished data).

It is worth mentioning that research on eukaryotic transcription has highlighted critical function of distant enhancer elements, regulations *via* conformational alterations of chromatin and finally important role for chromatin modifying enzymes. While, minigenome based studies are, otherwise, informative does not allow studying viral gene expression within its proper full-length nucleocapsid context. Therefore, parallel experimental approaches must be undertaken to investigate possible conserved mechanisms underlying gene-expression in mammal and mammalian RNA viruses.

Transcription and Viral RNA Dependant RNA Polymerase

Vesiculovirus transcription is characterized by actinomycin D resistant synthesis of leader RNA and viral mRNAs. Order of transcription of VSV genes was determined by in vitro transcriptional mapping analysis using UV-radiation. Such studies revealed that VSV mRNAs are synthesized in an obligatory sequential manner after polymerase entry at single 3′end of the genome termini i.e. at the beginning of leader gene (Ball and White 1976, Abraham and Banerjee 1976; Testa et al. 1980). Determination of relative molar ratios of different viral mRNAs within infected cells revealed that their abundance decreased with increasing distance from the 3′ promoter in an order N>P>M>G>L, thus implying a mechanism that also ensures polar transcription (Villarreal et al. 1976, Iverson and Rose 1981). More recently, an engineered version of VSV was utilized where an additional transcriptional unit (I) was inserted at N–P, P–M, M–G or G–L gene junction. Quantitation of I mRNA relative to the 3′ proximal VSV N mRNA expressed within cells infected with recombinant virus, consistently, revealed a gradual decrease in I mRNA synthesis with increase in distance from 3′ termini of the genome (Wertz et al. 2002).

To explain polar and sequential mRNA synthesis, Emerson et al. postulated stop-start model for transcription (Fig. 3) that remains most widely accepted, till date (Emerson 1982). In this model, subsequent to single site entry at the beginning of leader gene, viral polymerase sequentially transcribes the genome with progressive attenuation at each gene boundary to result in decreasing amount of transcripts for genes that are distant from the entry site. Each termination event may lead to the polymerase to fall off from the template or may allow for re-initiation at the downstream promoter. Therefore, transcription from downstream genes is dependent on termination of

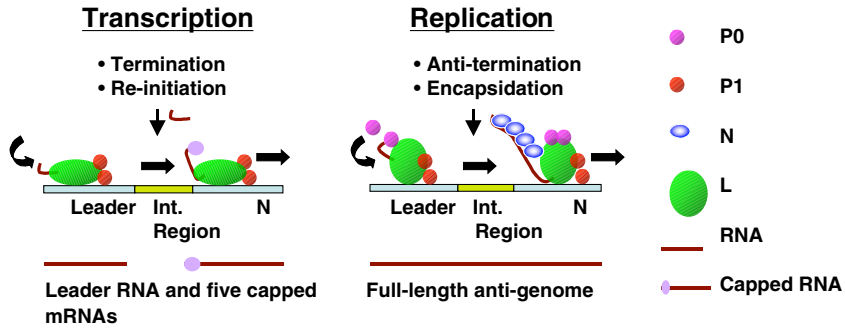

Fig. 3 RNA synthesis events of Chandipura Virus. Viral polymerase composed of L and phosphorylated form of P protein transcribes the genome RNA with progressive attenuation at each intergenic region (Int Region) to synthesize leader RNA and five capped and poly adenylated mRNA. RdRp was proposed to remain associated with N-RNA while transcribing the genomic template and reinitiate synthesis of downstream genes after termination. However, during replication mode, the same polymerase read-through the termination signals present at the intergenic region to eventually copy the entire genome. N protein is recruited on nascent genome RNA to protect it form cellular RNase action as it is synthesized. Unphosphorylated form of Phosphoprotein recruited to leader RNA has been proposed to modulate polymerase activity during replication to bring about anti-termination

upstream genes and reinitiation. This proposal explained observed polarity during transcription, while, anti-termination by polymerase at the gene boundaries results in synthesis of replication product (Banerjee a, b,). Consistent to single site entry model, VSV polymerase was able to predominantly produce ppAC dinucleotide representing 5′ dinucleotide of the leader RNA in an incomplete in vitro transcription reaction that was supplemented with only ATP and CTP and not ppAACA tetranucleotide that represents beginning of a mRNA, (Emerson 1982).

An alternative model for multiple entry site was also proposed that describes multiple independent initiations at each gene promoter. Experimental support for this model was gathered when an internal polymerase entry site was described to explain VSV-DI particle synthesis (Schubert et al. 1979). Furthermore, a mutant version of polymerase was shown to be able to mediate internal entry at the beginning of N gene in vitro (Chuang and Perrault 1997). While, this model lacks supports of experiments done on wild type virus, it was also unable to explain experimentally observed polar transcription in vesiculoviruses. However, a modified version of this model has recently been proposed to suggest two separate RNA synthesis initiation sites, at the beginning of N gene or leader gene, during transcription or replication, respectively (Whelan and Wertz 2002; Barr et al. 2002). Merit of such proposal will be further discussed in later section in light of Chandipura virus research.

Large Protein L

Viral transcriptase is composed of L and P protein. L retains the catalytic activity of RNA polymerization, capping and polyadenylation. Vesiculovirus L protein shares a conserved structural module similar to that of a right hand, with palm, thumb and fingers domain, whereas, the palm domain structure is particularly conserved in almost all RNA-dependent RNA polymerases (Ahlquist 2002). Not surprisingly, comparison of

deduced amino acid sequence of L protein of CHPV with that of different rhabdoviruses exhibited a high degree of homology along the length of the protein. Four conserved motifs present in VSV (Poch et al. 1989) are also present within CHPV-L in a central block that is thought to mediate RNA polymerization (Marriott 2005).

A unique feature of VSV mRNA synthesis is involvement of L protein in 5'end modification of the nascent mRNA that includes methylation and capping. However, unlike cellular guanylyltransferase, L protein incorporates GDP rather than GMP in the capped structure as Gp(alpha)p(beta)-p(alpha)A. The 5′end modification events were proposed to be successive to transcription initiation, whereby, nascent mRNA termini maintains contact with transcribing polymerase until modified (Stillman and Whitt 1999). Notably, leader RNA lacks 5′ cap structure. This may have resulted from the lack of a consensus trinucleotide sequence at the 5′end of leader RNA that is otherwise present in the other mRNA species; or may reflect requirement for a minimum transcript length for capping. Addition of poly(A) tail to the viral mRNA is also attributed to the L protein, where, polymerase slippage during transcription termination at U7 tract is believed to add A residues at the 3′end of mRNA (Barr et al. 1997). A protein kinase activity was also found to be associated with the purified VSV-L that has been termed as L associated kinase, LAK. It is still not clear, if the protein kinase activity is intrinsic to the L polypeptide or reflects a co-purified cellular kinase (Banerjee 1987a, b). Nevertheless, L protein remains associated with translation elongation factor, EF1; while, EF1–$\beta\gamma$ complex was proposed to constitute LAK activity, EF1–α may participate in the capping by virtue of GTP/GDP binding activity (Das et al. 1998). Therefore, it seems likely that L protein in conjunction with Phosphoprotein P and other cellular components, synthesize viral mRNA within infected cells and offers for mechanisms that could be potential drug targets, such as its unique capping or RNA dependent RNA polymerase activity. Nevertheless, a recombinant expression system to study structure and function of CHPV-L is yet not available and is required for additional analyses.

Phosphoprotein P in Transcriptional Regulation

Successful in vitro reconstitution of Chandipura virus transcription with purified components by Chattopadhyay et al. (1997) was an important step towards developing mechanistic insight into transcription. To this end, RNP particle obtained from detergent disrupted virus was treated with high salt buffer to dissociate N-RNA from L and P. Subsequently, N-RNA collected on the glycerol cushion or released L and P protein collected from the top of the gradient was further purified following rigorous biochemical protocol. When, purified L protein was incubated with N-RNA in a reaction mixture that allows for in vitro transcription, it was unable to synthesize viral mRNA. However, addition of viral P protein along with L, and not P alone, allowed for viral mRNA synthesis. These studies confirmed a role of Phosphoprotein as an activator of viral transcription (Chattopadhyay et al. 1997) and this proposed function was consistent to the observations made in VSV (Banerjee 1987b).

Chattopadhyay and Chattopadhyay (1994) cloned Phosphoprotein gene of CHPV to express in bacteria and used purified recombinant protein to understand importance of post-translational modifications of P. P, a 32.5 kDa protein when analyzed on SDS-PAGE shows anomalous migration with apparent molecular weight of 50 kDa. Studies with recombinant P protein critically showed that negatively charged N-terminal

domain was responsible for such anomalous migration. However, when viral P was substituted with its bacterially expressed counterpart, it failed to support transcription from N-RNA template, implying requirement for additional modifications for P protein function (Chattopadhyay et al. 1997). P of both VSVind and VSVnj serotypes was extensively phosphorylated within infected cells at multiple sites (Hsu and Kingsbury 1982; Banerjee 1987a; Clinton et al. 1979). Accordingly, a role for host derived kinases was proposed in modifying P at specific residues (Barik and Banerjee 1992a; Clinton et al. 1982). Casein Kinase II (CKII) was shown to be responsible for phosphorylating P of both VSV serotypes (Banerjee 1987b; Barik and Banerjee 1992a). Studies with recombinant CHPV-P revealed CKII, indeed similarly phosphorylates P in vitro (Chattopadhyay and Chattopadhyay 1994), although, CHPV-P exhibited less than 20% homology with P protein from other vesiculoviruses (Masters and Banerjee 1987). Further investigations identified serine-62 at the N-terminal acidic domain of CHPV P protein was specifically modified by CKII (Chattopadhyay et al. 1997). Only CKII phosphorylated form of recombinant P could effectively substitute P protein purified from virus to support transcription from nucleocapsid template in vitro (Chattopadhyay et al. 1997). A mutated version of P protein with alanine substituted for serine^{62}, when tested in vivo was unable to activate transcription and rather inhibited viral mRNA synthesis in a trans-dominant manner (Basak et al. 2003). Therefore, CKII mediated phosphorylation appeared to be indispensable for P to act as a transcriptional activator. The C-terminal domain of P was proposed to associate with N-RNA template during transcription, thus tethers polymerase with its template and provide processivity during transcription (Gao and Lenard 1995).

VSV-P protein existed within virus infected cells in two distinct phosphorylated states, namely P1 and P2, while unphosphorylated form of P was designated as P0. It was proposed that CKII mediated phosphorylation at the N-terminal region of P0 generates P1 form, which undergoes further round of modification by L-associated kinase at its C-terminal domain to form P2. This sequential phosphorylation event was proposed to modulate transcriptional activation property of VSV-P (Barik and Banerjee 1992a, b; Banerjee 1987b; Chattopadhyay and Banerjee 1987). Mutational analysis identified serine^{62} in CHPV-P as single phophorylation site in vivo to suggest existence of only P0 and P1 forms (Basak et al. 2003). However, a high turn over rate of phosphates incorporated by LAK could potentially result in a failure to identify such C-terminal phosphorylations of P in vivo. Nevertheless, detergent disrupted Chandipura virus incubated in an in vitro transcription reaction produced leader RNA and viral mRNAs, thus, confirming packaging of an active form of polymerase within mature virion (Basak et al. 2003).

Interestingly, studies on transcription complexes of respiratory syncitial virus, a paramyxovirus, indicated that P protein phosphorylation by CKII is required for promoter clearance and transcription elongation (Dupuy et al. 1999). These observation, therefore, suggest a post-initiation function for P-protein phosphorylation in transcription and in parallel with the proposed role for C-terminal domain phosphorylation of eukaryotic RNA polymerase II. Analysis in Chandipura virus, although, is consistent to a role for P1 in transcription initiation, additional function of P protein phosphorylation in elongation remains to be tested.

How does phosphorylation of P protein regulate its function as transcription activator? Phosphorylated P forms dimer (Chattopadhyay et al. 1997) and N-terminal 46 amino acids were proposed to be responsible for phosphorylation-mediated dimerisation (Raha et al. 2000). Accordingly, a critical role for phosphorylation

mediated dimerisation was proposed that regulate P protein function in transcription *via* efficient polymerase interaction (Gao and Lenard 1995). However, large zone gel-filtration technique was employed to reveal gradual appearance of dimer and tetramer species within unphosphorylated P protein pool in a concentration dependent manner. This observation was further substantiated by measuring sedimentation coefficient of those different oligomer species using analytical ultracentrifugation technique and measuring hydrated diameter by dynamic light scattering (Basak et al. 2004). Furthermore, Ding et al. (2006) has recently reported crystal structure for dimerisation domain of VSVind P protein, first of its kind for any vesiculovirus P. Intriguingly, such analysis indicated that a central domain, spanning 107–177 residues, mediate dimer assembly of the P protein in its unphosphorylated form. This central domain is composed of a α-helix surrounded by two β-hairpins. While, α-helix forms dimer, two β-hairpins from each P protein engages into domain swapping to form four stranded β-sheet and provides stabilizing force. In light of Chandipura research, it seems possible that N-terminal domain of P may provide additional stabilizing interactions by exposing coiled-coil motif upon phosphorylation (Raha et al. 1999, 2000), although, such domain is probably not required for dimerisation per se. Whereas, central domain assembles P into a dimer even in its unphosphorylated form. Therefore, dimerisation alone was not sufficient to explain transcription activation property of phosphorylated P.

Biophysical studies with CHPV-P protein revealed that phosphorylation at serine^{62} induced a major structural change within N-terminal domain of P protein to expose cystein^{57} on the protein surface (Raha et al. 1999). Phosphorylation also resulted in tryptophan residues to burry inside protein core, while, maintaining overall flexibility of the N-terminus. Accordingly, such conformation changes within the N-terminal domain of P were proposed to facilitate precise polymerase contact by P1 to result in optimal transcription (Raha et al. 1999). Lack of such N-terminal phosphorylation in P may also induce an altered conformation in interacting L protein, thus forming replicase complex, as discussed later (Basak et al. 2003; Gupta et al. 2003).

Replication and its Regulation

Chandipura virus replicative cycle is characterized by read-through of the gene boundaries by viral polymerase to synthesize an exact complement of (–) sense genome RNA. The molecular mechanism that allowed for a switch in polymerase function from transcription to replication, in other words, to ignore termination signals at the gene boundaries, has remained obscure. Studies in recent years on both CHPV and VSV led to a different proposal to explain *vesiculovirus* transcription-replication switch.

Two Separate Promoters for Transcription and Replication

According to this proposal, initiation of viral transcription predominantly occurs at the beginning of N gene, while, polymerase initiates replication at the beginning of leader gene. Both the processes utilize same nucleocapsid template, whereas, modifications of the viral polymerase allow for binding at separate sites during replication (Whelan and Wertz 2002; Barr et al. 2002). Indeed, a complex from VSV-infected cells composed of L, P and N protein was isolated that showed replication activity, in vitro (Gupta et al. 2003). Phophorylation of P was shown to be dispensable for the activity of this proposed tripartite replicase complex, thus, separating it from the classical L-P1 transcriptase

complex. Accordingly, it was postulated that the integration of de novo synthesized N protein into a replicase complex resulted in differential promoter recognition by polymerase to initiate replication phase.

Although, such model could not satisfactorily explain generation of leader RNA during viral RNA synthesis (Blumberg and Kolakofsky 1981). In this proposal, abortive replication attempts by polymerase resulted in accumulation of leader RNA within cells. However, in vitro transcription with Chandipura virus nucleocapsid resulted in synthesis of leader RNA (Basak et al. 2003). Even as replication byproduct, presence of discrete leader RNA species within virus-infected cells implies a strong attenuation signal for polymerase at the leader-N boundary, that needs to be suppressed for productive replication. Also this model was proposed based on studies on an engineered version of VSV that can substantially differ from wild type virus in nucleocapsid conformation, thus, may allow for observed internal entry of the polymerase.

Involvement of N protein and an auxiliary function for P

Viral replication was shown to critically depend on *de novo* synthesis of N protein (Patton et al. 1984) and accordingly a role for N in genome replication was proposed. N mediated encapsidation of nascent leader RNA suppress termination signal at the leader-N gene boundaries to eventually bring about the replicative phase (Blumberg et al. 1981). Accordingly, selective nucleation signal for N binding was identified within first 20 nt of (+) leader RNA for both VSV and CHPV system, as discussed. However, accumulation of critical amount of replication competent N is controlled by P protein (Bhattacharya et al. 2006). Essentially, P maintains N in a monomer form that engages in specific nucleation on the leader chain to form nucleocapsid.

How ensuing encapsidation of nascent RNA would influence termination or anti-termination decision of transcribing polymerase has remained debatable. One possible explanation was provided in studies with Influenza virus, where a direct interaction between RNA bound N proteins and polymerase was proposed (Biswas et al. 1998). In VSV, encapsidation was proposed to oppose polymerase pausing at the gene-boundaries to bring about anti-termination. However, clear biochemical evidence for such proposal is still missing (Banerjee 1987a). Chandipura virus, when allowed to multiply within cells with a preformed pool of N–P1 complex, showed negligible enhancement in replication, implying existence for additional mechanism in catalyzing transcription-replication switch (unpublished observation). Also, a stoichiometric amount of N is required to encapsidate viral RNA to protect it from RNase action and stabilize the single stranded genome within nucleocapsid core. Therefore, it could be questioned if N is actually required for the onset of genome replication (catalyzing replication) or its role is confined in protecting replication product from degradation (stoichiometric role). It seems possible that a major role for N in providing protection to progeny genome RNA may have resulted in an over-estimation of its function in the onset of replication.

Role for Unphosphorylated P protein

Recently, research in both CHPV and VSV indicated a rather surprising function for P in its unphosphorylated form in viral replication. A minigenome-based assay using VSV DI-RNA revealed that N-terminal phosphorylations of P protein, although, necessary for transcription, were dispensable for viral replication in vivo (Pattnaik et al. 1997). On

the other hand, C-terminal basic residues of VSV P protein was shown to be required for transcription and not for replication (Das et al. 1997). A tripartite complex composed of transcriptionally inactive mutant of P, and wild type L and N protein efficiently synthesized 42 S replication product in vitro (Gupta et al. 2003). These studies predicted possible engagement of distinct biochemical forms of P in viral transcription or replication processes. Chandipura virus, when allowed to multiply in presence of a pre-existing pool of phosphorylation defective mutant of P, revealed a two-log increase in the viral yield as compared to that of phosphorylated P protein. Presence of such phosphorylation defective P within the cellular milieu, resulted in an temporal alteration with early onset for genome replication and also mediated quantitative effect with an increase in viral genome synthesis (Basak et al. 2003). These studies were not only consistent to the similar observations in VSV but also conducted in a manner that allowed understanding the role of P protein phosphorylation in transcription and replication in the context of wild type virus. Moreover, detergent disrupted Chandipura virus, when incubated with a phosphorylation defective mutant of P in an in vitro transcription reaction, synthesized products that corresponds to read-through RNA of leader-N junction. These experiments critically showed that unphosphorylated form of P alone could catalyze anti-termination of leader-N junction in vitro.

However, Basak et al. subsequently presented another intriguing observation that connected a role for P0 in viral replication with events on nascent leader RNA chain. In their study, CHPV-P protein was shown to specifically bind with leader RNA in its unphosphorylated form, whereas, CKII mediated phosphorylation abrogated its RNA binding ability (Basak et al. 2004; Basak 2003). Leader RNA-P0 interaction was distinct from N-mediated encapsidation of viral RNA. Moreover, oligomeric status of P0 was shown to partly alter its RNA binding pocket thus fine-tunes P0 assembly on leader chain (Basak et al. 2004). Accordingly, a new model was proposed to explain Chandipura virus genome replication (Basak et al. 2003). In this proposal, recruitment to nascent leader chain allows P0 to interact with transcribing polymerase in the vicinity and alters polymerase conformation in a manner that enables read-through at the gene-boundaries (Fig. 3). Consistently, structural differences between activation domains of P0 and P1 was reported in CHPV (Raha et al. 1999). Theoretical analysis of prokaryotic transcription also revealed that a transcriptional activator may acts as a repressor by mediating differential contact with polymerase (Roy et al. 1998). Therefore, this model predicts that such differential contact may enable anti-termination by mammalian virus RNA polymerase during transcription. Nonetheless, progressive encapsidation of nascent RNA by N maintains processivity of replicase complex to allow for synthesis of ~11.1 kb (+)ve sense genome. Progeny (+)ve sense genome, however, lacks transcription termination elements, thus, exclusively acts as template for replication to synthesize more copies of (–)ve sense genome RNA. M2–1 phosphoprotein of human respiratory syncytial virus, a paramyxovirus, was shown to bind leader RNA whereby binding affinity was reduced upon phosphorylation (Cuesta et al. 2000). Similar phosphoregulation of RNA binding of P protein was also reported for Rinderpest virus, another paramyxovirus, to postulate a conserved function of leader RNA-P0 interaction in mononegavirus life-cycle (Raha et al. 2004a). Notably, the proposed mechanism for Chandipura virus to read-through its gene-boundaries during replication bears remarkable similarity to the N mediated anti-termination strategy employed by bacteriophage λ (Rees et al. 1996; Oppenheim et al. 2005). It also constitutes an

example of protein economy, whereby; a single protein in two different forms has been used in two distinct steps of viral RNA synthesis.

Chandipura virus P protein posses a consensus CKII site enclosing serine-62 (-**Ser**-Glu-Glu-Asp-), as discussed earlier. CKII, being a ubiquitous kinase raises a question of possible existence of unphosphorylated P protein within virus infected cells. However, Basak et al. has recently postulated a phosphatase activity that is induced upon Chandipura virus infection within BHK-21 cells and capable of dephosphorylating P protein (Basak 2003). Induction of this phosphatase activity remarkably coincides with the onset of genome replication. Curiously, recent studies identified a conserved Glu64Asp mutation within P protein in all epidemic isolates (Arankalle et al. 2005). In light of proposed function of unphosphorylated P protein in boosting up viral replication, functional consequences of such mutations in viral life-cycle need to be tested.

Concluding Remarks

In this review, we have attempted to provide details of Chandipura virus life cycle. Clearly, our understanding of the virus partly relies on contemporary studies on VSV, and remarkable similarities between CHPV and VSV provide basis for some of the generalized conclusions. While, proposed mechanism for CHPV replication that involves RNA binding by Phosphoprotein needs to be tested in VSV, existence of distinct mechanisms in different serotypes of vesiculoviruses could not be completely ruled out. Reverse genetic tools need to be employed along with biochemical experiments for further characterizing CHPV pathogenesis at molecular details. While retaining general academic interest for mechanism of RNA synthesis in rhabdoviruses, future Chandipura research should also focus on development of potential anti-viral therapeutic interventions that allows for specific inhibition of viral growth without any pleotropic effect on cellular metabolism. Available reagents and a large body of information on N-P, N-leader RNA or P0-leader RNA interactions should be utilized to screen chemical library to identify compounds that inhibits such interactions in vitro thus detrimental to viral multiplication in vivo.

Interestingly, we noticed that almost each protein of Chandipura virus executes multiple different tasks within viral life cycle, therefore, constitutes for an example of protein complexity and economy. Catalytic activity for RNA dependent RNA polymerization, 5′ end capping of mRNA and poly-adenylation activity is contained within L gene. Matrix protein M, beside its role in viral assembly, is also used for interfering with host cellular metabolism. Nucleocapsid protein N has multiple targets whereby it interacts with both P protein and viral RNA to form precise nucleocapsid template. Phosphoprotein P in its distinct phosphorylated state can act as either transcriptional activator or an anti-terminator. We propose that fragility of single stranded RNA molecule and replicative advantage of compact genome due to shorter replication cycle imparts constrain on Chandipura virus genome length. Accordingly, Chandipura virus has evolved an economic way to use single viral protein in multiple different related functions rather encoding separate genes with specialized missions. This proposed mode for evolution of proteins encoded within RNA genome predicts utilization of distinct structural motifs and novel functional mechanisms by rhabdoviruses.

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References

Abraham G, Banerjee AK (1976) Sequential transcription of the genes of vesicular stomatitis virus. Proc Natl Acad Sci USA 73:1504–1508

Ahlquist P (2002) RNA-dependent RNA polymerases, viruses, and RNA silencing. Science 296:1270–1273

Ahmed M, Lyles DS (1998) Effect of vesicular stomatitis virus matrix protein on transcription directed by host RNA polymerases I, II, and III. J Virol 72:8413–8419

Albertini AA, Wernimont AK, Muziol T, Ravelli RB, Clapier CR, Schoehn G, Weissenhorn W, Ruigrok RW (2006) Crystal structure of the rabies virus Nucleoprotein-RNA complex. Science 313:360–363

Arankalle VA, Prabhakar SS, Madhukar WA, Hanumaih, Dattatraya PS, Chandra MA (2005) G, N, and P gene-based analysis of Chandipura viruses, India. Emerg Infect Dis 11:123–126

Ball LA, White CN (1976) Order of transcription of genes of vesicular stomatitis virus. Proc Natl Acad Sci USA 73:442–446

Banerjee AK (1987a) Transcription and replication of rhabdoviruses. Microbiol Rev 51:66–87

Banerjee AK (1987b) The transcription complex of vesicular stomatitis virus. Cell 48:363–364

Barik S, Banerjee AK (1992a) 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

Barik S, Banerjee AK (1992b) Sequential phosphorylation of the phosphoprotein of vesicular stomatitis virus by cellular and viral protein kinases is essential for transcription activation. J Virol 66:1109–1118

Barr JN, Whelan SP, Wertz GW (1997) cis-Acting signals involved in termination of vesicular stomatitis virus mRNA synthesis include the conserved AUAC and the U7 signal for polyadenylation. J Virol 71:8718–8725

Barr JN, Whelan SP, Wertz GW (2002) Transcriptional control of the RNA-dependent RNA polymerase of vesicular stomatitis virus. Biochim Biophys Acta 1577:337–353

Basak S (2003) Studies on RNA-Protein interactions of Chandipura virus. Calcutta University, Kolkata

Basak S, Polley S, Basu M, Chattopadhyay D, Roy S (2004) Monomer and dimer of Chandipura virus unphosphorylated P-protein binds leader RNA differently: implications for viral RNA synthesis. J Mol Biol 339:1089–1101

Basak S, Raha T, Chattopadhyay D, Majumder A, Shaila MS, Chattopadhyay DJ (2003) Leader RNA binding ability of Chandipura virus P protein is regulated by its phosphorylation status: a possible role in genome transcription-replication switch. Virology 307:372–385

Bhatt PN, Rodrigues FM (1967) Chandipura: a new Arbovirus isolated in India from patients with febrile illness. Indian J Med Res 55:1295–1305

Bhattacharya R, Basak S, Chattopadhyay DJ (2006) Initiation of encapsidation as evidenced by deoxycholate-treated Nucleocapsid protein in the Chandipura virus life cycle. Virology 349:197–211

Biswas SK, Boutz PL, Nayak DP (1998) Influenza virus nucleoprotein interacts with influenza virus polymerase proteins. J Virol 72:5493–5501

Blumberg BM, Giorgi C, Kolakofsky D (1983) N protein of vesicular stomatitis virus selectively encapsidates leader RNA in vitro. Cell 32:559–567

Blumberg BM, Giorgi C, Rose K, Kolakofsky D (1984) Preparation and analysis of the nucleocapsid proteins of vesicular stomatitis virus and sendai virus, and analysis of the sendai virus leader-NP gene region. J Gen Virol 65(Pt 4):769–779

Blumberg BM, Kolakofsky D (1981) Intracellular vesicular stomatitis virus leader RNAs are found in nucleocapsid structures. J Virol 40:568–576

Blumberg BM, Leppert M, Kolakofsky D (1981) Interaction of VSV leader RNA and nucleocapsid protein may control VSV genome replication. Cell 23:837–845

Chadha MS, Arankalle VA, Jadi RS, Joshi MV, Thakare JP, Mahadev PV, Mishra AC (2005) An outbreak of Chandipura virus encephalitis in the eastern districts of Gujarat state, India. Am J Trop Med Hyg 73:566–570

Chattopadhyay D, Banerjee AK (1987) Phosphorylation within a specific domain of the phosphoprotein of vesicular stomatitis virus regulates transcription in vitro. Cell 49:407–414

Chattopadhyay D, Chattopadhyay D (1994) Cloning of the chandipura virus phosphoprotein encoding gene and its expression in Escherichia coli. Cell Mol Biol Res 40:693–698

Chattopadhyay D, Raha T, Chattopadhyay D (1997) Single serine phosphorylation within the acidic domain of Chandipura virus P protein regulates the transcription in vitro. Virology 239:11–19

Chen H, Smith GJ, Zhang SY, Qin K, Wang J, Li KS, Webster RG, Peiris JS, Guan Y (2005) Avian flu: H5N1 virus outbreak in migratory waterfowl. Nature 436:191–192

Chen Z, Green TJ, Luo M, Li H (2004) Visualizing the RNA molecule in the bacterially expressed vesicular stomatitis virus nucleoprotein-RNA complex. Structure 12:227–235

Chua KB, Bellini WJ, Rota PA, Harcourt BH, Tamin A, Lam SK, Ksiazek TG, Rollin PE, Zaki SR, Shieh W et al (2000) Nipah virus: a recently emergent deadly paramyxovirus. Science 288:1432–1435

Chuang JL, Perrault J (1997) Initiation of vesicular stomatitis virus mutant polR1 transcription internally at the N gene in vitro. J Virol 71:1466–1475

Clague MJ, Schoch C, Zech L, Blumenthal R (1990) Gating kinetics of pH-activated membrane fusion of vesicular stomatitis virus with cells: stopped-flow measurements by dequenching of octadecylrhodamine fluorescence. Biochemistry 29:1303–1308

Clewley JP, Bishop DH, Kang CY, Coffin J, Schnitzlein WM, Reichmann ME, Shope RE (1977) Oligonucleotide fingerprints of RNA species obtained from rhabdoviruses belonging to the vesicular stomatitis virus subgroup. J Virol 23:152–166

Clinton GM, Burge BW, Huang AS (1979) Phosphoproteins of vesicular stomatitis virus: identity and interconversion of phosphorylated forms. Virology 99:84–94

Clinton GM, Guerina NG, Guo HY, Huang AS (1982) Host-dependent phosphorylation and kinase activity associated with vesicular stomatitis virus. J Biol Chem 257:3313–3319

Cuesta I, Geng X, Asenjo A, Villanueva N (2000) Structural phosphoprotein M2-1 of the human respiratory syncytial virus is an RNA binding protein. J Virol 74:9858–9867

Das T, Banerjee AK (1993) Expression of the vesicular stomatitis virus nucleocapsid protein gene in Escherichia coli: analysis of its biological activity in vitro. Virology 193:340–347

Das T, Mathur M, Gupta AK, Janssen GM, Banerjee AK (1998) RNA polymerase of vesicular stomatitis virus specifically associates with translation elongation factor-1 alphabetagamma for its activity. Proc Natl Acad Sci USA 95:1449–1454

Das T, Pattnaik AK, Takacs AM, Li T, Hwang LN, Banerjee AK (1997) Basic amino acid residues at the carboxy-terminal eleven amino acid region of the phosphoprotein (P) are required for transcription but not for replication of vesicular stomatitis virus genome RNA. Virology 238:103–114

David M. Knipe BR, Howley PM, Straus SE, Fields BN, Griffin DE, Martin MA, Lamb RA (ed) (2001) Fields virology, 4th edn. (Lippincott Williams & Wilkins)

De BP, Thornton GB, Luk D, Banerjee AK (1982) Purified matrix protein of vesicular stomatitis virus blocks viral transcription in vitro. Proc Natl Acad Sci USA 79:7137–7141

Dhanda V, Rodrigues FM, Ghosh SN (1970) Isolation of Chandipura virus from sandflies in Aurangabad. Indian J Med Res 58:179–180

Ding H, Green TJ, Lu S, Luo M (2006) Crystal structure of the oligomerization domain of the Phosphoprotein of vesicular stomatitis virus. J Virol 80:2808–2814

Ducatez MF, Olinger CM, Owoade AA, De Landtsheer S, Ammerlaan W, Niesters HG, Osterhaus AD, Fouchier RA, Muller CP (2006) Avian flu: multiple introductions of H5N1 in Nigeria. Nature 442:37

Dupuy LC, Dobson S, Bitko V, Barik S (1999) Casein kinase 2-mediated phosphorylation of respiratory syncytial virus phosphoprotein P is essential for the transcription elongation activity of the viral polymerase; phosphorylation by casein kinase 1 occurs mainly at Ser(215) and is without effect. J Virol 73:8384–8392

Durrer P, Gaudin Y, Ruigrok RW, Graf R, Brunner J (1995) Photolabeling identifies a putative fusion domain in the envelope glycoprotein of rabies and vesicular stomatitis viruses. J Biol Chem 270:17575–17581

Elena SF, Sanjuan R (2005) RNA viruses as complex adaptive systems. Biosystems 81:31–41

Emerson SU (1982) Reconstitution studies detect a single polymerase entry site on the vesicular stomatitis virus genome. Cell 31:635–642

Enninga J, Levy DE, Blobel G, Fontoura BM (2002) Role of nucleoporin induction in releasing an mRNA nuclear export block. Science 295:1523–1525

Gao Y, Lenard J (1995) Cooperative binding of multimeric phosphoprotein (P) of vesicular stomatitis virus to polymerase (L) and template: pathways of assembly. J Virol 69:7718–7723
Gaudier M, Gaudin Y, Knossow M (2002) Crystal structure of vesicular stomatitis virus matrix protein. Embo J 21:2886–2892
Giorgi C, Blumberg B, Kolakofsky D (1983) Sequence determination of the (+) leader RNA regions of the vesicular stomatitis virus Chandipura, Cocal, and Piry serotype genomes. J Virol 46:125–130
Green TJ, Luo M (2006) Resolution improvement of X-ray diffraction data of crystals of a vesicular stomatitis virus nucleocapsid protein oligomer complexed with RNA. Acta Crystallogr D Biol Crystallogr 62:498–504
Green TJ, Macpherson S, Qiu S, Lebowitz J, Wertz GW, Luo M (2000) Study of the assembly of vesicular stomatitis virus N protein: role of the P protein. J Virol 74:9515–9524
Green TJ, Zhang X, Wertz GW, Luo M (2006) Structure of the Vesicular Stomatitis Virus Nucleoprotein-RNA Complex. Science 313:357–360
Gupta AK, Banerjee AK (1997) Expression and purification of vesicular stomatitis virus N-P complex from Escherichia coli: role in genome RNA transcription and replication in vitro. J Virol 71:4264–4271
Gupta AK, Drazba JA, Banerjee AK (1998) Specific interaction of heterogeneous nuclear ribonucleoprotein particle U with the leader RNA sequence of vesicular stomatitis virus. J Virol 72:8532–8540
Gupta AK, Shaji D, Banerjee AK (2003) Identification of a novel tripartite complex involved in replication of vesicular stomatitis virus genome RNA. J Virol 77:732–738
Holland J, Spindler K, Horodyski F, Grabau E, Nichol S, VandePol S (1982) Rapid evolution of RNA genomes. Science 215:1577–1585
Howard M, Wertz G (1989) Vesicular stomatitis virus RNA replication: a role for the NS protein. J Gen Virol 70(Pt 10):2683–2694
Hsu CH, Kingsbury DW (1982) NS phosphoprotein of vesicular stomatitis virus: subspecies separated by electrophoresis and isoelectric focusing. J Virol 42:342–345
Iseni F, Baudin F, Blondel D, Ruigrok RW (2000) Structure of the RNA inside the vesicular stomatitis virus nucleocapsid. Rna 6:270–281
Iverson LE, Rose JK (1981) Localized attenuation and discontinuous synthesis during vesicular stomatitis virus transcription. Cell 23:477–484
Kadonaga JT (2002) The DPE, a core promoter element for transcription by RNA polymerase II. Exp Mol Med 34:259–264
Keene JD, Thornton BJ, Emerson SU (1981) Sequence-specific contacts between the RNA polymerase of vesicular stomatitis virus and the leader RNA gene. Proc Natl Acad Sci USA 78:6191–6195
Kurilla MG, Keene JD (1983) The leader RNA of vesicular stomatitis virus is bound by a cellular protein reactive with anti-La lupus antibodies. Cell 34:837–845
Kurilla MG, Piwnica-Worms H, Keene JD (1982) Rapid and transient localization of the leader RNA of vesicular stomatitis virus in the nuclei of infected cells. Proc Natl Acad Sci USA 79:5240–5244
La Ferla FM, Peluso RW (1989) The 1:1 N-NS protein complex of vesicular stomatitis virus is essential for efficient genome replication. J Virol 63:3852–3857
Le Blanc I, Luyet PP, Pons V, Ferguson C, Emans N, Petiot A, Mayran N, Demaurex N, Faure J, Sadoul R et al (2005) Endosome-to-cytosol transport of viral nucleocapsids. Nat Cell Biol 7:653–664
Li T, Pattnaik AK (1999) Overlapping signals for transcription and replication at the 3' terminus of the vesicular stomatitis virus genome. J Virol 73:444–452
Licata JM, Harty RN (2003) Rhabdoviruses and apoptosis. Int Rev Immunol 22:451–476
Majumdar A, Bhattacharya R, Basak S, Shaila MS, Chattopadhyay D, Roy S (2004) P-protein of Chandipura virus is an N-protein-specific chaperone that acts at the nucleation stage. Biochemistry 43:2863–2870
Majumder A, Basak S, Raha T, Chowdhury SP, Chattopadhyay D, Roy S (2001) Effect of osmolytes and chaperone-like action of P-protein on folding of nucleocapsid protein of Chandipura virus. J Biol Chem 276:30948–30955
Marra EA (2003) The Genome sequence of the SARS-associated coronavirus. Science 300:1399–1404
Marriott AC (2005) Complete genome sequences of Chandipura and Isfahan vesiculoviruses. Arch Virol 150:671–680
Masters PS, Banerjee AK (1987) Sequences of Chandipura virus N and NS genes: evidence for high mutability of the NS gene within vesiculoviruses. Virology 157:298–306
Masters PS, Banerjee AK (1988a) Complex formation with vesicular stomatitis virus phosphoprotein NS prevents binding of nucleocapsid protein N to nonspecific RNA. J Virol 62:2658–2564
Masters PS, Banerjee AK (1988b) Resolution of multiple complexes of phosphoprotein NS with nucleocapsid protein N of vesicular stomatitis virus. J Virol 62:2651–2657

Masters PS, Bhella RS, Butcher M, Patel B, Ghosh HP, Banerjee AK (1989) Structure and expression of the glycoprotein gene of Chandipura virus. Virology 171:285–290
McGowan JJ, Emerson SU, Wagner RR (1982) The plus-strand leader RNA of VSV inhibits DNA-dependent transcription of adenovirus and SV40 genes in a soluble whole-cell extract. Cell 28:325–333
Moyer SA, Smallwood-Kentro S, Haddad A, Prevec L (1991) Assembly and transcription of synthetic vesicular stomatitis virus nucleocapsids. J Virol 65:2170–2178
Nature Reviews Microbiology editorial (2006) Cause and effect. Nat Rev Microbiol 4:414
Neumann G, Whitt MA, Kawaoka Y (2002) A decade after the generation of a negative-sense RNA virus from cloned cDNA - what have we learned? J Gen Virol 83:2635–2662
Nichol ST, Holland JJ (1987) Genome RNA terminus conservation and diversity among vesiculoviruses. J Virol 61:200–205
Ogden JR, Pal R, Wagner RR (1986) Mapping regions of the matrix protein of vesicular stomatitis virus which bind to ribonucleocapsids, liposomes, and monoclonal antibodies. J Virol 58:860–868
Oppenheim AB, Kobiler O, Stavans J, Court DL, Adhya S (2005) Switches in bacteriophage lambda development. Annu Rev Genet 39:409–429
Pattnaik AK, Ball LA, LeGrone A, Wertz GW (1995) The termini of VSV DI particle RNAs are sufficient to signal RNA encapsidation, replication, and budding to generate infectious particles. Virology 206:760–764
Pattnaik AK, Hwang L, Li T, Englund N, Mathur M, Das T, Banerjee AK (1997) 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
Patton JT, Davis NL, Wertz GW (1983) Cell-free synthesis and assembly of vesicular stomatitis virus nucleocapsids. J Virol 45:155–164
Patton JT, Davis NL, Wertz GW (1984) N protein alone satisfies the requirement for protein synthesis during RNA replication of vesicular stomatitis virus. J Virol 49:303–309
Peluso RW (1988) Kinetic, quantitative, and functional analysis of multiple forms of the vesicular stomatitis virus nucleocapsid protein in infected cells. J Virol 62:2799–2807
Petersen JM, Her LS, Dahlberg JE (2001) Multiple vesiculoviral matrix proteins inhibit both nuclear export and import. Proc Natl Acad Sci USA 98:8590–8595
Poch O, Sauvaget I, Delarue M, Tordo N (1989) Identification of four conserved motifs among the RNA-dependent polymerase encoding elements. Embo J 8:3867–3874
Potharaju NR, Potharaju AK (2006) Is Chandipura virus an emerging human pathogen? Arch Dis Child 91:279–280
Raha T, Chattopadhyay D, Roy S (1999) A phosphorylation-induced major structural change in the N-terminal domain of the P protein of Chandipura virus. Biochemistry 38:2110–2116
Raha T, Kaushik R, Shaila MS (2004a) Phosphoprotein P of Rinderpest virus binds to plus sense leader RNA: regulation by phosphorylation. Virus Res 104:191–200
Raha T, Pudi R, Das S, Shaila MS (2004b) Leader RNA of Rinderpest virus binds specifically with cellular La protein: a possible role in virus replication. Virus Res 104:101–109
Raha T, Samal E, Majumdar A, Basak S, Chattopadhyay D, Chattopadhyay DJ (2000) N-terminal region of P protein of Chandipura virus is responsible for phosphorylation-mediated homodimerization. Protein Eng 13:437–444
Rao BL, Basu A, Wairagkar NS, Gore MM, Arankalle VA, Thakare JP, Jadi RS, Rao KA, Mishra AC (2004) A large outbreak of acute encephalitis with high fatality rate in children in Andhra Pradesh, India, in 2003, associated with Chandipura virus. Lancet 364:869–874
Rees WA, Weitzel SE, Yager TD, Das A, von Hippel PH (1996) Bacteriophage lambda N protein alone can induce transcription antitermination in vitro. Proc Natl Acad Sci USA 93:342–346
Roche S, Bressanelli S, Rey FA, Gaudin Y (2006) Crystal structure of the low-pH form of the vesicular stomatitis virus glycoprotein G. Science 313:187–191
Rodrigues JJ, Singh PB, Dave DS, Prasan R, Ayachit V, Shaikh BH, Pavri KM (1983) Isolation of Chandipura virus from the blood in acute encephalopathy syndrome. Indian J Med Res 77:303–307
Rose JK, Gallione CJ (1981) Nucleotide sequences of the mRNA's encoding the vesicular stomatitis virus G and M proteins determined from cDNA clones containing the complete coding regions. J Virol 39:519–528
Roy S, Garges S, Adhya S (1998) Activation and repression of transcription by differential contact: two sides of a coin. J Biol Chem 273:14059–14062
Schubert M, Keene JD, Lazzarini RA (1979) A specific internal RNA polymerase recognition site of VSV RNA is involved in the generation of DI particles. Cell 18:749–757

Smallwood S, Moyer SA (1993) Promoter analysis of the vesicular stomatitis virus RNA polymerase. Virology 192:254–263

Sprague J, Condra JH, Arnheiter H, Lazzarini RA (1983) Expression of a recombinant DNA gene coding for the vesicular stomatitis virus nucleocapsid protein. J Virol 45:773–781

Stillman EA, Whitt MA (1997) Mutational analyses of the intergenic dinucleotide and the transcriptional start sequence of vesicular stomatitis virus (VSV) define sequences required for efficient termination and initiation of VSV transcripts. J Virol 71:2127–2137

Stillman EA, Whitt MA (1999) Transcript initiation and 5'-end modifications are separable events during vesicular stomatitis virus transcription. J Virol 73:7199–7209

Takacs AM, Das T, Banerjee AK (1993) Mapping of interacting domains between the nucleocapsid protein and the phosphoprotein of vesicular stomatitis virus by using a two-hybrid system. Proc Natl Acad Sci USA 90:10375–10379

Taylor A, Easton AJ, Marriott AC (1999) Matrix protein of Chandipura virus inhibits transcription from an RNA polymerase II promoter. Virus Genes 19:223–228

Testa D, Chanda PK, Banerjee AK (1980) In vitro synthesis of the full-length complement of the negative-strand genome RNA of vesicular stomatitis virus. Proc Natl Acad Sci USA 77:294–298

Thomas D, Newcomb WW, Brown JC, Wall JS, Hainfeld JF, Trus BL, Steven AC (1985) Mass and molecular composition of vesicular stomatitis virus: a scanning transmission electron microscopy analysis. J Virol 54:598–607

Thornton GB, Kopchick JJ, Stacey DW, Banerjee AK (1983) Microinjection of vesicular stomatitis virus ribonucleoprotein into animal cells yields infectious virus. Biochem Biophys Res Commun 116:1160–1167

Van Ranst M (2004). Chandipura virus: an emerging human pathogen? Lancet 364:821–822

Villarreal LP, Breindl M, Holland JJ (1976). Determination of molar ratios of vesicular stomatitis virus induced RNA species in BHK21 cells. Biochemistry 15:1663–1667

von Kobbe C, van Deursen JM, Rodrigues JP, Sitterlin D, Bachi A, Wu X, Wilm M, Carmo-Fonseca M, Izaurralde E (2000). Vesicular stomatitis virus matrix protein inhibits host cell gene expression by targeting the nucleoporin Nup98. Mol Cell 6:1243–1252

Walpita P, Flick R (2005). Reverse genetics of negative-stranded RNA viruses: a global perspective. FEMS Microbiol Lett 244:9–18

Wertz GW (1983). Replication of vesicular stomatitis virus defective interfering particle RNA in vitro: transition from synthesis of defective interfering leader RNA to synthesis of full-length defective interfering RNA. J Virol 46:513–522

Wertz GW, Moudy R, Ball LA (2002). Adding genes to the RNA genome of vesicular stomatitis virus: positional effects on stability of expression. J Virol 76:7642–7650

Whelan SP, Wertz GW (1999). Regulation of RNA synthesis by the genomic termini of vesicular stomatitis virus: identification of distinct sequences essential for transcription but not replication. J Virol 73:297–306

Whelan SP, Wertz GW (2002). Transcription and replication initiate at separate sites on the vesicular stomatitis virus genome. Proc Natl Acad Sci USA 99:9178–9183

Yao Y, Ghosh K, Epand RF, Epand RM, Ghosh HP (2003) Membrane fusion activity of vesicular stomatitis virus glycoprotein G is induced by low pH but not by heat or denaturant. Virology 310:319–332

Zagouras P, Rose JK (1993) Dynamic equilibrium between vesicular stomatitis virus glycoprotein monomers and trimers in the Golgi and at the cell surface. J Virol 67:7533–7538