Casein kinase II is the P protein phosphorylating cellular kinase associated with the ribonucleoprotein complex of purified vesicular stomatitis virus

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Protein kinase activities associated with a highly purified transcriptionally active ribonucleoprotein complex from the virions of vesicular stomatitis virus (VSV) were isolated and characterized. Based upon several biochemical and immunological criteria, the protein kinase activity, which phosphorylated the bacterially expressed unphosphorylated (P₀) protein, was shown to be cellular casein kinase II (CKII). These studies included inhibition of the protein kinase by specific inhibitors, phosphorylation of mutant phosphoproteins (P), immunoprecipitation by CKII antibody and Western blot analyses, and finally its ability to activate P₀ to synthesize RNA in a transcription–reconstitution reaction. The P protein is phosphorylated intracellularly by cellular CKII. The present study demonstrates that VSV specifically packages CKII which remains strongly associated with the ribonucleoprotein complex during morphogenesis.

Introduction

It has been well documented that many enveloped viruses package cellular macromolecules during morphogenesis of the mature virion. Protein kinases are specifically associated with several such viruses, including vesicular stomatitis virus (VSV) (Clinton et al., 1982; Harmon et al., 1983; Imblum & Wagner, 1974; Moyer & Summers, 1974; Sinacore & Lucas-Lenard, 1982; Strand & August, 1971). In the case of VSV, detergent-disrupted purified virions contain protein kinase activities that phosphorylate predominantly the structural proteins P and M in vitro. The P protein, which is phosphorylated intracellularly by a protein kinase, is a transcription factor for the RNA polymerase L; M, the matrix protein, is located underneath the lipid bilayer (Banerjee, 1987). Recently, based on several properties including differential utilization of ATP and GTP as phosphate donors, salt sensitivity, etc., Beckes & Perrault (1991) and Beckes et al. (1989) characterized three different protein kinase activities associated with the virion; one activity was specific for M protein phosphorylation and the other two for P protein phosphorylation. The bulk of the M protein phosphorylating activity in the purified virion can be removed by low salt (0-4 M-NaCl) buffer treatment of the virus followed by sedimentation of the ribonucleoprotein (RNP) complex by centrifugation (Beckes & Perrault, 1991). The highly purified transcriptionally active RNP complex thus obtained is devoid of glycoprotein (G) and M proteins, and retains significant protein kinase activity which preferentially phosphorylates the RNP-associated P protein in vitro. At least two protein kinase activities seem to be associated with the RNP; one is manifested by a host enzyme and the other possibly by the L (polymerase) protein, which has been shown to possess a P protein phosphorylating activity (Sánchez et al., 1985; Hammond et al., 1992). However, when the RNP is further extracted with high salt buffer to remove the associated L and P proteins, the protein kinase activity present in the high salt soluble fraction appears to be predominantly of cellular origin since its activity does not co-elute with either L or P protein (Harmon et al., 1983; Massey et al., 1989). The protein kinase activity present in this fraction is able to phosphorylate both P protein and casein. Massey et al. (1989) reported that possibly two cellular protein kinase activities are present in this high salt fraction in view of the slightly altered elution profile of the activities that phosphorylate these two substrates.

The precise identity of the RNP-associated protein kinase(s) of VSV and its role in transcription has yet to

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be determined. However, the important role of P protein phosphorylation in transcription has been well documented (Clinton et al., 1978; Hsu et al., 1982; Kingsford & Emerson, 1980; Masters & Banerjee, 1986; Sinacore & Lucas-Lenard, 1982; Testa et al., 1980; Watanabe et al., 1974; Witt & Summers, 1980). A recent series of observations indicate that the P protein of VSV is phosphorylated intracellularly by the ubiquitous cAMP-independent protein kinase, casein kinase II (CKII) (Barik & Banerjee, 1991, 1992). This has been shown by purifying the protein kinase activity from cell extracts, which specifically phosphorylate P (the unphosphorylated form of P protein purified from E. coli expressing P protein; Barik & Banerjee, 1992), as a substrate. By means of various biochemical and immunological studies, the protein kinase was shown to be indistinguishable from CKII (Barik & Banerjee, 1992b). The CKII-mediated phosphorylation of P was shown to be essential for the biological activity of the P protein in in vitro transcription–reconstruction assays (Barik & Banerjee, 1991, 1992; Takacs et al., 1992). A cascade phosphorylation pathway is proposed where a stepwise phosphorylation by CKII and L protein-associated kinase is required for transcriptional activation of the P protein (Barik & Banerjee, 1992a). A similar result was obtained by Beckes & Perrault (1992) using purified RNP containing associated protein kinases. The precise phosphorylation sites have been subsequently identified within the acidic domain of the P protein; the two serine residues reside within the typical casein kinase II consensus motif (Takacs et al., 1992). The L-kinase-mediated serine phosphorylation sites have been shown previously to be within the L-protein binding domain (domain II) of the P protein (Chattopadhyay & Banerjee, 1987).

The involvement of cellular CKII in phosphorylation and activation of VSV P protein prompted us to study the nature of the protein kinase(s) present in the purified RNP of the virion of VSV. In this report we demonstrate that VSV specifically packages CKII which remains strongly associated with the purified RNP.

Methods

Cell cultures and virus. Monolayers of baby hamster kidney cells (BHK-21) were grown in Eagle's minimal essential medium (Gibco) containing 5% fetal calf serum. The cells were infected with VSV (Serotype Indiana IND) Mudd Summers strain, at an m.o.i. of 0.05, and the virus was purified as described previously (Barik & Banerjee, 1991).

Cloning, expression and purification of wild-type and mutant P proteins. Plasmids containing mutant P genes were constructed by the revised megaprimer polymerase chain reaction (PCR) method as described previously (Barik & Galinski, 1991; Takacs et al., 1992).

For the expression of the P protein in bacteria, the wild-type and all P mutant genes were subcloned into the vector pET-3a. The primers used were 5′ ATTCGCATATTGATAATCTCACAAGATT 3′ (upstream) and 5′ ATTCGGGATCTTCACAGAGATATTTGAC 3′ (downstream), where the upstream and the downstream primers contain NdeI (CATATG) and BamHI (GGATCC) sites, respectively, along with a hexanucleotide clamp sequence at the upstream of each restriction site. The PCR-amplified products were then digested with NdeI and BamHI and subsequently ligated into NdeI-BamHI cut pET-3a. The positive clone in each case was identified by restriction analysis and the appropriate mutation was characterized by DNA sequencing. For expression, the positive recombinant plasmids containing various mutant P genes were then introduced into E. coli BL21/DE3. Recombinant P proteins were purified by the method described previously (Barik & Banerjee, 1991).

The histidine-tagged P protein was made by PCR amplification of the wild-type P gene with oligonucleotide primers, with the downstream primer containing sequences corresponding to seven histidine residues just before the stop codon. The histidine-tagged P protein was then purified by nickel affinity column chromatography.

Isolation and purification of the RNP-associated protein kinase (RNP–PK). Purified VSV IND was used as the source to isolate cellular protein kinase (if any) associated with the RNP. Purified virus (10 mg) was first treated with a low salt buffer (containing 40 mM-Tris-HCl pH 8.0, 400 mM-NaCl, 5% glycerol, 1 mM-DTT and 2% Triton X-100) by incubation on ice for 90 min with gentle rocking. The low-salt-treated virus was then centrifuged through 30% (v/v) glycerol cushion at 4 °C in order to purify the viral RNP. After centrifugation at 45000 rpm in an SW60 rotor for 2 h, the pellet containing the RNP was collected from the top of 100% glycerol cushion and suspended in Tris–EDTA buffer. The purified RNP in Tris–EDTA buffer was then treated as described above but with a buffer containing 1 M-NaCl and lacking Triton X-100 in order to isolate L, P, and cellular kinase(s). The high-salt-treated RNP was then centrifuged through 30% glycerol as described above. The soluble supernatant (at a protein concentration of 35 μg/ml) collected from the top of the 30% glycerol was dialysed against buffer A (50 mM-Tris-HCl pH 7.5, 0.1 mM-EDTA, 5% glycerol and 1 mM-DTT) containing 100 mM-NaCl and was loaded onto a 2.5 ml phosphocellulose-cation column pre-equilibrated with the same buffer A. After loading, the column was washed with 12 ml of buffer A containing 100 mM-NaCl and subjected to a 15 ml gradient of 0.1–1.2 M-NaCl in the same buffer. Each fraction (250 μl) was then assayed for P phosphorylating activity using bacterially expressed VSV P protein as the substrate. The protein concentration in the active pooled fraction was 0.2–0.3 μg/ml.

Protein kinase assay. A 20 μl kinase reaction mixture containing 0.3–0.5 μg of either wild-type or mutant P protein, 5 μCi [γ-32P]ATP, 50 μg ATP, 50 mM-Tris–HCl pH 8.0, 100 mM-NaCl, 5 mM-MgCl2, 2 mM-DTT, and the indicated amount of kinase was incubated at 30 °C for 30 min. The reaction was stopped by adding sample loading buffer and electrophoresed on a 10% polyacrylamide gel containing SDS (Laemmli, 1970). The gel was then subjected to autoradiography.

Isolation and purification of L protein. The isolation and purification of L protein was performed essentially as described previously (De & Banerjee, 1984) with minor modifications. The low salt and high salt fractionation of virus was done in the same way as that for RNP–PK. The high salt fraction containing L and cellular kinase was dialysed against phosphocellulose buffer (20 mM-Tris–HCl pH 7.4, 10 mM glycerol and 1 mM-DTT) and loaded onto a 20 ml phosphocellulose column. Bound L protein was eluted with a 0–1 M-NaCl salt gradient (12 ml) in phosphocellulose buffer. Fractions in which the L protein was completely free of P protein (as identified by silver staining) and devoid of CKII activity (as checked by phosphorylation of bacterially expressed VSV P protein as substrate) were pooled. If necessary, the L protein was further rechromatographed on a second phospho-
cellulose column to remove any remaining contaminant viral P protein and cellular kinase.

Reconstitution of VSV transcription in vitro. VSV transcription in vitro was performed essentially as described previously (De & Banerjee, 1984) except that bacterially expressed recombinant P protein was used instead of viral P protein. To examine the effect of RNP-PK on VSV transcription in vitro, bacterially expressed histidine-tagged P protein was first phosphorylated with RNP-PK. The phosphorylated P protein (P<sub>r</sub>) was then purified by affinity chromatography through a nickel column (Novagen). N-RNA template was heated at 55 °C for 30 s in order to destroy any residual cellular kinase activity. VSV transcription was then reconstituted with phosphorylated P protein (P<sub>r</sub>), kinase-free L protein and kinase-free N-RNA template as described previously (Barik & Banerjee, 1992a).

Immunoprecipitation of kinase activity. An anti-CKII antibody raised against the peptide (SH123 (CVVKILKPVKKKKIKREIKILE) was used to precipitate CKII from BHK cell extract or RNP-PK preparation (Je et al., 1991). The L-peptide antibody raised against amino acids 5–19 numbered from the NH<sub>2</sub> terminus of the L protein (DFETDEDFNEDDY) (Schubert et al., 1985) was used as a negative control. The immunoprecipitate recovered after A–Sepharose treatment as the secondary antibody was washed repeatedly with a buffer containing 50 mM-HEPES pH 7.4, 250 mM-NaCl and 0.1% NP40. The immunoprecipitate was then used as the source of kinase activity (assayed in terms of P<sub>0</sub> phosphorylation).

Western blot analyses. A total of 10 mg of purified virus was used to obtain the high salt (1 M-NaCl) soluble supernatant fraction as described above. The soluble fraction (~150 µl) containing RNP–PK was concentrated to 150 µl by using a Centricon concentrator. The concentrated fraction was then analysed in a 10 % SDS-polyacrylamide gel and the resolved proteins were transferred to nitrocellulose membrane by electroblotting. The blot was blocked overnight with blocking buffer containing 10% nonfat milk and 2% fetal bovine serum in PBS. After blocking, the membrane was treated with CKII antibody in the same buffer for 3 h at room temperature. Subsequent probing with secondary antibody was carried out according to the manufacturer’s (Bio-Rad) protocol. Finally, the blot was developed with enhanced chemiluminescence reagent (Pharmacia) and exposed to X-ray film.

The L protein antibody and CKII antibody were kindly provided by Drs Manfred Schubert (NIH, Bethesda, MD) and Dan Marshak (Cold Spring Harbor Laboratories, NY), respectively. Recombinant CKII was purchased from Boehringer Mannheim. The specific activity of the enzyme is 1 unit/mg protein, where 1 unit is the enzyme activity which catalyses the transfer of 1 µmol phosphate from ATP to the synthetic substrate RRDDDDDDD at 37 °C in 1 min under the manufacturer’s assay conditions.

Results

Purification of RNP–PK

The cellular protein kinase(s) packaged within purified virions of VSV was extracted by disruption with the non-ionic detergent Triton X-100 and 0.4 M-NaCl and extensively washed as detailed in the flowsheet in Fig. 1(a). This procedure essentially removes both G and M proteins from the RNP and the bulk of the membrane-associated protein kinases. The remaining transcriptionally active RNP complex retains significant protein kinase activity (Beckes & Perrault, 1991). The RNP was further treated with buffer containing 1.0 M-NaCl. The high-salt supernatant containing the RNP–PK and the L and P proteins was dialysed against buffer containing 0.1 M-NaCl and the protein kinase activity was fractionated on a phosphocellulose column using a linear salt gradient from 0.1–1.2 M-NaCl. By this chromatographic technique most of the P protein is excluded from the column; the L protein is strongly bound and can be eluted at 1 M-NaCl (De & Banerjee, 1984; data not shown). As shown in Fig. 1(b), the protein kinase activity as judged by the phosphorylation of bacterially expressed unphosphorylated (P<sub>0</sub>) protein was eluted as a single peak at 0.6 M-NaCl. Each fraction was also assayed for phosphorylation of casein. Both P protein and casein phosphorylating activities were eluted coincidentally (data not shown). These results indicate that a P<sub>0</sub> phosphorylating activity is present in the fraction.

Characterization of RNP–PK

Effect of inhibitors and studies on phosphorylation of mutant P proteins. To study whether multiple protein kinases that phosphorylate P<sub>0</sub> were eluted in a single peak of activity we used inhibitors known to inhibit at least two ubiquitous cellular protein kinases, i.e. cellular CKII and cellular protein kinase C (PKC) activities. As shown in Fig. 2, heparin, a specific inhibitor of CKII (Hathway et al., 1980), completely inhibited the protein kinase activity whereas staurosporine, a specific inhibitor of PKC (Tamaoki et al., 1986) failed to inhibit the activity. Moreover, the basic protein protamine-stimulated protein kinase activity in vitro, indicating that CKII is present in the RNP–PK preparation (Ahmed et al., 1986). To further confirm that CKII is present in the RNP–PK we compared the abilities of RNP–PK and purified CKII to phosphorylate several mutant P proteins. We have previously shown that in VSV serotype New Jersey (NJ) the CKII-phosphorylated serine residues are at positions 59 and 61, which are located within the typical CKII consensus motif in the acidic domain I of the P protein (Takacs et al., 1992). Purified CKII failed to phosphorylate these sites when these residues were altered to alanine. These two serine residues of the P protein of VSV (NJ) correspond to S<sup>24</sup> and S<sup>32</sup> of VSV (IN), which are also located within the CKII consensus motif (Fig. 3a; unpublished observation). The mutant protein P2 contains unaltered serine residues at positions 58 and 62, whereas in P3 and P5 these residues have been altered to alanine. The P protein mutants P2, P3 and P5 along with P<sub>0</sub> were used as substrates for phosphorylation by purified CKII and the RNP–PK. As shown in Fig. 3(b), like CKII the RNP–PK failed to phosphorylate P3 and P5, whereas P<sub>0</sub> and P2 were efficiently phosphorylated by both enzymes. A faint band appearing in both P3
and P5 is a contaminant phosphorylated P protein present in the RNP–PK preparation (Fig. 3b, '−' lane). An additional band migrating faster than P1 (Fig. 3b, lane P2; also see Fig. 2, last lane) is generated by the degradation of P protein during purification. These results strongly indicate that the protein kinase in RNP–PK shares the same motif within the P protein, similar to purified CKII.

**Immunoprecipitation of CKII activity from RNP–PK by CKII-specific antibody.** To further confirm that RNP–PK indeed contains CKII, we used a CKII antibody to isolate CKII from the RNP–PK preparation. The BHK cell extract and RNP–PK preparations were incubated with CKII antibody followed by precipitation with Protein A–Sepharose (see Methods). The washed precipitates were tested for CKII activity using P₀ as substrate. As shown in Fig. 4, the fraction that was immunoprecipitated by CKII antibody from both the BHK cell extract and the RNP–PK preparation effectively phosphorylated P₀. In control experiments, corresponding fractions obtained by anti-L protein antibody did not show any P₀ phosphorylating activity. These results further confirm that CKII activity is present in the RNP–PK preparation.

**Western blot analyses of RNP–PK.** In order to confirm convincingly that CKII is indeed present in the RNP–PK, we isolated from 10 mg of purified virus the high-salt-soluble supernatant fraction after washing RNP with 1 M-NaCl (Fig. 1) and concentrated it to 150 μl. Two portions of the fraction were assayed for the presence of CKII by western blot analysis using CKII antibody. As shown in Fig. 5, CKII subunits α and α' were clearly detectable when compared with authentic CKII used as the control. Bacterially expressed N and P proteins (used as negative controls) did not cross-react with CKII antibody (data not shown). From the intensity of the α
and α' subunit bands in RNP–PK, as measured by densitometric analyses and compared with the corresponding bands obtained with known concentration the amount of CKII associated with the RNP was calculated to be approximately 50 ng/mg of virus. Thus, the western blot analysis clearly demonstrates that CKII is selectively packaged within the virion and remains associated with the transcribing RNP.

**Transcriptional activation of P o by RNP–PK**

Finally, to confirm that RNP–PK, like CKII, can also activate P o, transcription–reconstitution experiments were performed using kinase-free L and N–RNA template along with RNP–PK-phosphorylated P protein. However, to study the RNP–PK-mediated activation of P o, it was important to remove any contaminating phosphorylated viral P protein present in the RNP–PK preparation which may complicate the interpretation of the data. To achieve this, we first purified bacterially expressed P o which contained six histidine residues at the C terminus of the protein (see Methods) and subsequently phosphorylated this protein with RNP–PK using unlabelled ATP. The phosphorylated P o was then purified by nickel affinity column chromatography. RNP–PK containing any contaminant viral P protein will presumably not bind to the column due to lack of the histidine tag. The phosphorylated P o protein thus obtained was used in the transcription–reconstitution reaction. As shown in Fig. 6, the P o which was phosphorylated to P1 by RNP–PK effectively stimulated transcription. Moreover, addition of heparin had no effect on RNA synthesis indicating that the P o was phosphorylated to P1 by the RNP–PK. These results strongly support the suggestion that RNP–PK, like CKII, is also capable of converting inactive P o to biologically active P1 which, in turn, supports transcription.
Fig. 4. Immunoprecipitation of CKII activity and phosphorylation of P protein. BHK cell extract or the RNP-PK preparation were immunoprecipitated either by CKII antibody or by L-peptide antibody along with Protein A-Sepharose and used for protein kinase activity using P₀ as the substrate, as detailed in Methods.

Fig. 5. Western blot analyses. A high-salt-soluble supernatant was prepared from 10 mg of purified virus, as described in Methods. The indicated sample volumes of concentrated fraction (designated RNP-PK) were electrophoresed on a 10% SDS-polyacrylamide gel, electroblotted onto nitrocellulose membrane, and probed with rabbit IgG raised against synthetic human CKII α peptide. CKII protein was detected by enhanced chemiluminescence using a peroxidase-linked goat anti-rabbit secondary antibody. Pure CKII (200 ng) was used as a positive control. The positions of protein size markers are indicated on the left.

Fig. 6. Role of RNP-PK in VSV transcription. In vitro VSV transcription was reconstituted using kinase-free N–RNA template (heat inactivated) and L protein along with histidine-tagged P1. The P1 was made by the phosphorylation of P₀ with RNP-PK and purified by nickel affinity column chromatography, as described in Methods. The corresponding VSV transcripts are indicated by G, N, P and M.

Discussion

Recent work from our laboratory indicates that cellular CKII plays an important role in the life-cycle of VSV (Barik & Banerjee, 1992b). This ubiquitous cellular protein kinase phosphorylates unphosphorylated P₀, rendering it biologically active. The phosphorylated P then interacts with the RNA polymerase L and forms a transcriptionally active complex which appears to be further phosphorylated by the L-associated protein kinase during transcription in vitro and presumably in vivo. These findings prompted us to take a closer look at the nature of the protein kinases in the virion, especially to characterize the RNP-associated protein kinase in virions which in vitro efficiently phosphorylated the associated P protein. In the present study, we extracted
the detergent-disrupted purified virion with low salt treatment to ensure that all of the membrane-associated protein kinases were removed from the RNP. This fraction contained M protein phosphorylating activity (Beckes et al., 1989). The precise identity of the M protein kinase remains unknown. The protein kinase activity associated with the purified RNP (RNP–PK) containing both L and P proteins was purified by chromatography on a phosphocellulose column. Most of the P protein did not bind to phosphocellulose but the L protein bound tightly (De & Banerjee, 1984). The RNP–PK was eluted as a single peak at a salt concentration of 0.6 M (Fig. 1b). This fraction contained no detectable protein band other than a trace quantity of the P protein (data not shown). Using several criteria, such as the effect of inhibitors, the degree of phosphorylation of mutant P proteins, immunoprecipitation and Western blot analyses, the RNP–PK was found to contain the CKII activity. Moreover RNP–PK, like CKII, fully activated P0 to the transcriptionally active form P1. Thus, it appears that several protein kinase activities are present in the purified virion of VSV; the bulk of the cellular protein kinases can be removed from the transcribing RNP (Harmon et al., 1983; Massey et al., 1989, 1990), whereas viral L-protein associated kinase and the CKII activity remain tightly associated with the RNP. Since P protein is exclusively phosphorylated intracellularly by CKII, it is conceivable that it remains associated with the RNP during morphogenesis. A strong association of CKII with RNP in the purified virion clearly shows that CKII has high affinity for the RNP.

Similar to our results, Beckes & Perrault (1991) showed that two protein kinase activities are discernible using purified RNP as the enzyme source. Although not characterized, one activity appeared to strongly resemble CKII activity, based on several biochemical properties. However, its inability to phosphorylate a synthetic CKII peptide raised doubt about its precise identity. Our highly concentrated RNP–PK preparation was able to phosphorylate synthetic CKII peptide (data not shown) and clearly showed the presence of CKII by Western blot analysis (Fig. 5). The amount of CKII present in the virion is indeed low, approximately 50 ng/mg of virus. However, its specific activity is similar to or higher than the commercially available CKII, 1 unit/mg of protein. It seems that the small amount of RNP used in the previous studies may have been the reason for not detecting CKII by phosphorylation of the CKII peptide. Additionally, the source of the CKII antibody may also be an important factor in determining whether or not CKII is detected by Western blot analyses. It should be noted that although conventional substrates like casein and phosvitin are phosphorylated reasonably well by CKII, the level of phosphorylation of P0 appears to be extremely high and efficient demonstrating that it is by far the best substrate for CKII. Thus, it is reasonable to conclude that CKII and the L-associated protein kinase may be the two principal protein kinases associated with the transcribing RNP. A recent report suggested that some PKC may also be present in the RNP of VSV since staurosporine (10 μM) inhibited P protein phosphorylation by 50% in vitro (Rigaut et al., 1993). It is not clear whether the high concentration of staurosporine used in the study would have inhibited CKII non-specifically, since nanomolar concentrations are needed to inhibit PKC activity (Tamaoki et al., 1986). It is important to note that in our studies the CKII activity was monitored by its phosphorylation of P0 and casein (data not shown). Thus, other protein kinases which do not phosphorylate P0 or casein may be present in RNP–PK preparations which were not assayed under our reaction conditions.

Since cellular CKII phosphorylates P protein during the life-cycle of the virus, it is possible that it becomes associated with the RNP during some step in replication and remains associated during morphogenesis. The CKII remains tightly associated with the RNP even in the presence of 400 mM-NaCl, indicating that it has a high affinity for any or all of the polypeptide constituents of the RNP. However, the precise function, if any, of the CKII packaged in the virion is presently not clear. A large fraction of the P protein associated with the RNP is already phosphorylated in vivo during virus maturation (Harmon et al., 1983; Sinacore & Lucas-Lenard, 1982). However, the RNP-associated CKII is still able to phosphorylate the associated P protein in vitro, suggesting that RNP must package a certain amount of unphosphorylated P protein which is converted into the phosphorylated form in vitro. Precise quantification of the amount of unphosphorylated and phosphorylated forms of P protein within the RNP remains unknown and should be quantified to address this issue. The involvement of CKII in the life-cycle of VSV presents an opportunity for the use of specific agents to inhibit its activity with potential applications for developing antiviral drug(s) to effectively combat replication of the virus.

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References


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