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β-Catenin Associates With Human Parainfluenza Virus Type 3 Ribonucleoprotein Complex and Activates Transcription of Viral Genome RNA In Vitro

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Several studies have indicated that human parainfluenza virus type 3 (HPIV-3) requires polymeric actin for transcription of its genome RNA in vitro and in vivo. In the current study, we have identified β-catenin, an actin-bound protein, as one of the transcriptional activators for HPIV-3 genome RNA. β-Catenin was packaged within the purified HPIV-3 virions and was associated with the HPIV-3 ribonucleoproteins (RNP) from infected cells. Moreover, purified β-catenin interacted with bacterially expressed HPIV-3 nucleocapsid protein (N) and phosphoprotein (P) fused to glutathione *S*-transferase (GST). Double-labeled immunofluorescent confocal microscopic analysis revealed colocalization of β-catenin with HPIV-3 RNP at cell periphery in infected cells. The HPIV-3 RNP-associated β-catenin functioned as a transactivator of HPIV-3 genome, because purified β-catenin stimulated transcription of viral RNP in an in vitro transcription assay. These results demonstrate that β-catenin, a multifunctional protein that is involved in cell–cell adhesion and embryogenesis, acts as one of the transcriptional activators of HPIV-3 genome RNA.

Key words: β-Catenin; Human parainfluenza virus type 3; In vitro transcription; Actin

THE human parainfluenza virus type 3 (HPIV-3), a paramyxovirus belonging to the nonsegmented negative-strand RNA virus family, infects the lung epithelial cells in children and causes severe respiratory tract illnesses (12). The HPIV-3 genome, consisting of a linear negative sense single-stranded RNA, is packaged within a helical nucleocapsid and undergoes transcription and replication in the cytoplasm of infected cells (19). Three virus-encoded proteins, the nucleocapsid protein, N (68 kDa), the phosphoprotein, P (90 kDa), and the RNA-dependent RNA polymerase, L (251 kDa), are tightly associated with the RNA genome to form the transcribing ribonucleoprotein (RNP) complex (1,2,19). Several studies have shown that cellular actin in the polymerized form is required for the RNP complex to transcribe the HPIV-3 genome RNA in vitro (14–16). A direct involvement of actin in transcription was further supported by in vivo studies with HPIV-3-infected cells, where viral RNP was shown to colocalize with the actin microfilament and disruption of actin filament by actin depolymerizing drug, cytochalasin D, resulted in the inhibition of viral RNA synthesis (21). In vitro transcriptional studies further demonstrated that transcription activation activity of cellular actin was lost when purified extensively by column chromatography, suggesting the involvement of putative actin binding protein(s) in its activity. Thus, it was postulated that actin microfilament may serve as a template for interaction of viral RNP complex with actin while bound to cellular factor(s), acting as transcriptional activator(s). In this report, we have identified β-

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catenin, a 94-kDa cytosolic actin-bound protein involved in cell–cell adhesion and embryogenesis, functioning as one of the transcriptional activators of HPIV-3 genome RNA.

MATERIALS AND METHODS

Materials

Polyclonal and monoclonal antibodies against β-catenin were purchased from Sigma Chemicals (St. Louis, MO). HPIV-3 RNP antibody was raised in rabbits as described previously (21). Monoclonal and polyclonal actin antibodies and goat anti-mouse and anti-rabbit horseradish peroxidase IgG were purchased from Boehringer Mannheim (Indianapolis, IN). Texas Red conjugated anti-rabbit and fluorescein isothiocyanate (FITC) conjugated anti-mouse antibodies were purchased from Chemicon (Temecula, CA). Nickel-agarose beads and glutathione-sepharose were purchased from Novagen (Madison, WI). Protein A-sepharose was purchased from Amersham Pharmacia (Sweden). The recombinant hexa-histidine (his)-tagged β-catenin was a gift from Dr. Pierre D. McCrea (University of Texas M. D. Anderson Cancer Center, Houston, TX).

Cells and Viruses

HPIV-3 (HA-1, NIH 47885) was propagated in CV-1 cells as described previously (5–7,21). Human lung epithelial A549 cells were used for infection experiments, as described previously (5–7,20).

Isolation of Viral RNP Complexes

The RNP complex was isolated from purified HPIV-3 virions essentially as described previously (15). The vesicular stomatitis virus (VSV) RNP was isolated from purified viruses as described earlier (8). Intracellular RNP from HPIV-3 infected cells was isolated as described by Tongeguzzo and Ghosh (29).

In Vitro Binding of His-Tagged β-Catenin With GST-N and GST-P

Recombinant full-length β-catenin was purified from baculovirus-infected Hi-5 cells (Pharmingen, San Diego, CA) infected with AcMNPV phage expressing hexa-his-tagged β-catenin as described previously (28). The purified β-catenin obtained following elution from nickel-agarose affinity column was subjected to 7.5% SDS-PAGE and Coomassie blue staining to confirm its purity.

The HPIV-3 N and P proteins were expressed as a glutathione *S*-transferase (GST) fusion protein in *Escherichia coli* BL-21 along with the control GST plasmid. The GST and GST fusion protein(s) expressing bacterial extract were added to the glutathione-sepharose beads precleared with control bacterial extract to remove nonspecific proteins. Following incubation of the extract with the beads for 12 h at 4°C, the beads were washed extensively and the bound proteins were released from the beads by boiling in the presence of SDS buffer. The released proteins were subjected to SDS-PAGE analysis and Coomassie staining to check the purity of the glutathione bead-bound GST, GST-N and GST-P proteins. In each case, the amounts of GST and GST fusion protein(s) bound to the glutathione beads were similar and the proteins were homogeneous.

To study in vitro binding of purified β-catenin with GST-N and GST-P proteins, equal amount of purified his-tagged β-catenin was added to the washed GST, GST-N and GST-P protein-bound glutathione beads. Following incubation at 4°C for 12 h, the beads were washed extensively and the bound proteins were released in the presence of SDS sample buffer. The released proteins were subjected to 7.5% SDS-PAGE and Western blot analysis with β-catenin antibody. A portion of the glutathione-bound proteins was also subjected to Western blot analysis with GST and HPIV-3 RNP antibodies to monitor the expression of GST, GST-N, and GST-P during the in vitro binding studies.

Double Labeled Immunofluorescence Confocal Microscopy

A549 cells grown on coverslips were infected with HPIV-3 at 1 pfu/cell. At 36 h postinfection, the cells were washed with phosphate-buffered saline followed by fixation with 3.6% paraformaldehyde and permeabilization with 1% Triton X-100. The fixed cells were treated with a mixture of rabbit anti-RNP and mouse monoclonal anti-β-catenin antibodies followed by incubation with Texas Red conjugated anti-rabbit and FITC conjugated anti-mouse secondary antibodies. The coverslips were finally washed, mounted, and examined using a Leica CLSM confocal laser-scanning microscope as described previously (7,21).

In Vitro Transcription

Fifty microliters of in vitro transcription reactions (17) was prepared in the presence of 100 mM HEPES-KOH (pH 8.0), 100 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, 1 mM each of ATP, GTP, and UTP, 10 μM CTP, 15 μCi of [α-^{32}P]CTP, 25 units of RNase inhibitor, and 2 μg of purified HPIV-3 RNP. The reaction was carried out in the presence or absence of immunoprecipitated β-catenin and/or actin pellet (P)

(actin or β-catenin from uninfected A549 cell lysate bound to washed and precleared protein A-sepharose beads) and β-catenin immunodepleted supernatant (S) (uninfected A549 cell lysate not bound to the β-catenin immunopellet), along with control rabbit serum immunoprecipitated pellet and immunosupernatant. In another set of transcription reaction, actin and β-catenin immunopellets were added together to the reaction mixture. For these experiments, uninfected A549 cell lysates (100 μg protein/reaction) were immunoprecipitated with the antibodies in the presence of washed protein A-sepharose beads. The immunopellets comprising the proteins bound to the beads were extensively washed prior to using them for in vitro transcription assay. The immunodepleted β-catenin supernatant obtained following incubation of the cell lysate with β-catenin antibody bound beads was further immunoprecipitated with β-catenin antibody to remove residual β-catenin protein from the supernatant. The doubly immunoprecipitated supernatant was used for the in vitro transcription assay.

In addition, the in vitro transcription assay reaction was carried out in the absence or presence of two different concentrations (1 and 0.5 μg) of purified recombinant his-tagged β-catenin. As a control, reaction was also performed in the presence of purified RNP alone. The reaction mixture was incubated at 30°C for 3 h, and the in vitro synthesized ^{32}P-labeled RNA products were purified by phenol extraction and ethanol precipitation and analyzed by 5% polyacrylamide-urea electrophoresis, followed by autoradiography.

RESULTS

Association of β-Catenin With HPIV-3 RNP

Earlier studies have demonstrated that optimal transcription of HPIV-3 RNP, associated with the actin microfilament, required not only actin but additional actin-bound host protein(s) (15). Based on this observation, it was speculated (21) that actin-interacting cellular transcriptional activators may be required for actin to fully function as the transcriptional activator of HPIV-3 genome. β-Catenin, an actin-bound protein, became our logical choice as one of the actin-bound transcriptional activators because it constitutes one of the abundant proteins that are bound to actin filament in the cytoplasm and it posses transactivation function (4,13). Thus, to initially investigate whether β-catenin indeed interacts with the viral RNP, HPIV-3 RNP isolated from infected human lung epithelial A549 cells and purified HPIV-3 virions were subjected to Western blot analysis with β-catenin antibody. We utilized human lung epithelial A549 cells for these studies, because HPIV-3 is a human lung tropic virus that primarily infects human lung epithelial cells during productive infection. As shown in Figure 1, β-catenin antibody reacted with the endogenous β-catenin present in the A549 cell lysate (lane 1), as well as with the β-catenin that is associated with the HPIV-3 RNP isolated from the HPIV-3 virion particles (lane 2) and RNP isolated from infected A549 cells (lane 3). The specificity of the interaction of β-catenin with HPIV-3 RNP was borne out by the observation that β-catenin was not detected in the RNP isolated from either VSV (a HPIV-3-related virus belonging to the nonsegmented negative-strand RNA virus family) infected cells or purified virions (data not shown). These results indicated that HPIV-3 RNP interacts with β-catenin in infected cells and remains associated with it during viral assembly and budding.

In Vitro Interaction of β-Catenin With HPIV-3 N and P Proteins

To further study the authenticity of β-catenin–RNP interaction, we tested the in vitro association of purified his-tagged β-catenin with bacterially expressed GST-N and GST-P proteins. We chose N and P proteins for these studies because these proteins are the major constituents of the viral RNP complex (12,19). SDS-PAGE analysis and Coomassie blue staining of purified his-tagged β-catenin, eluted from the nickel-agarose beads, revealed that β-catenin was purified to near homogeneity with two detectable bands of 94 and 72 kDa (Fig. 2A). The native molecular weight of β-catenin is 94 kDa, whereas the 72-kDa band appears to be a degraded portion of β-catenin, because the β-catenin antibody reacted in Western blot analysis with both the 94- and the 72-kDa bands (Fig. 2B,

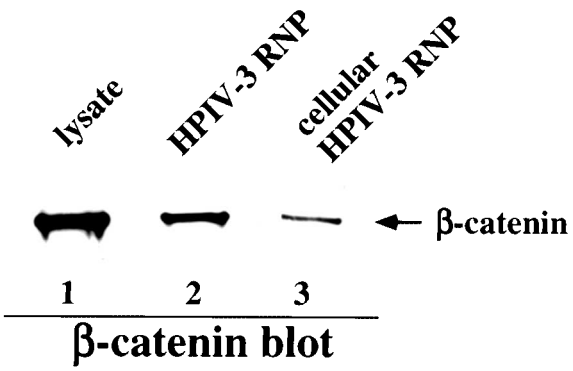

Figure 1. Association of β-catenin with HPIV-3 RNP. A549 cell lysate (lane 1), RNP isolated from purified HPIV-3 virions (15 μg) (lane 2), and RNP isolated from HPIV-3-infected A549 cells (10 μg) (lane 3) were subjected to 7.5% SDS-PAGE and Western blot analysis with β-catenin antibody.

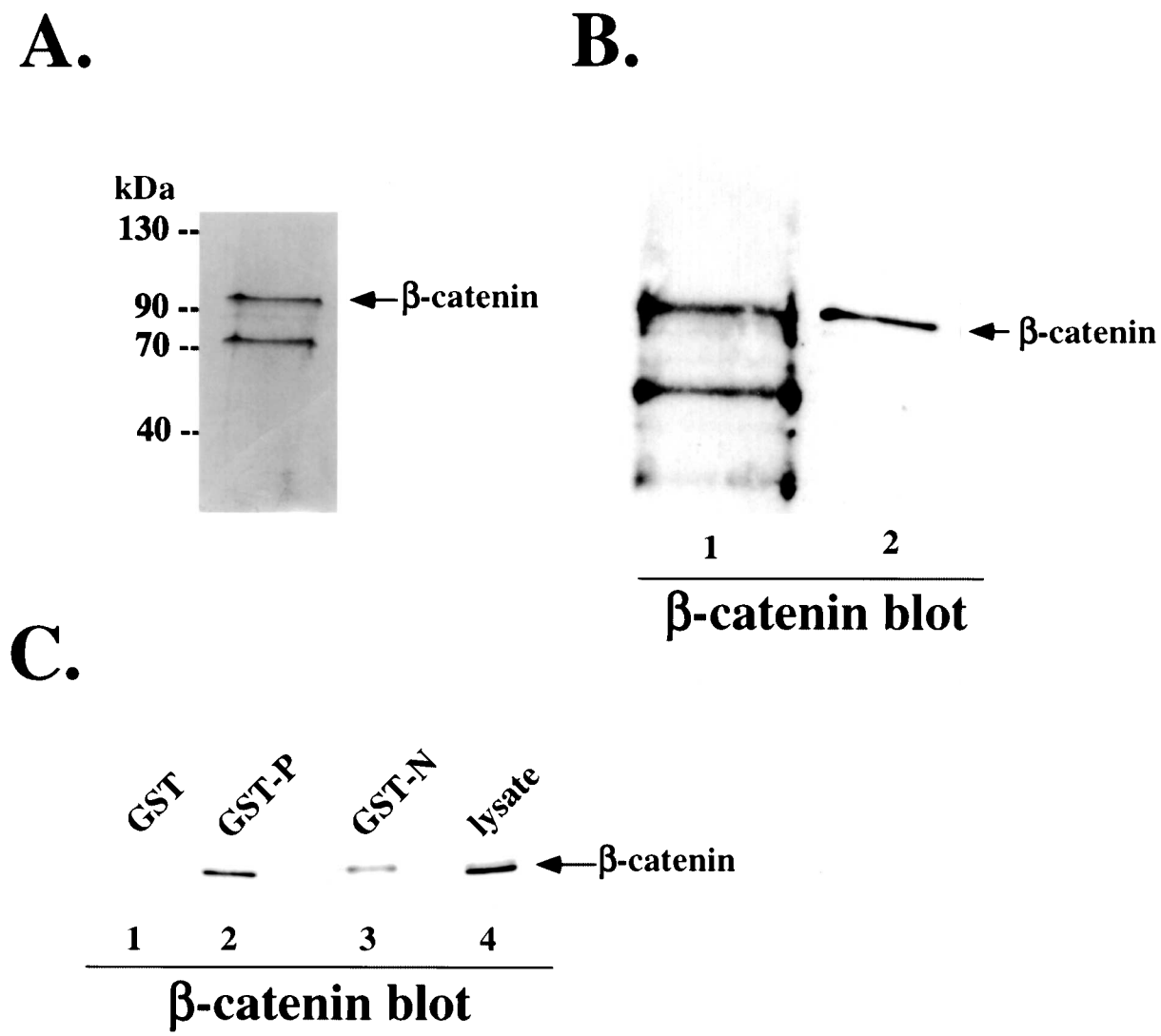

Figure 2. Iin vitro binding of his-tagged β-catenin with GST-N and GST-P. (A) Purified his-tagged β-catenin (2 μg) was subjected to 7.5% SDS-PAGE and Coomassie blue staining. (B) Purified his-tagged β-catenin (0.2 μg) (lane 1) and A549 cell lysate (12 μg) (lane 2) were subjected to 7.5% SDS-PAGE and Western blot analysis with β-catenin antibody. (C) GST alone (lane 1), GST-P (lane 2), and GST-N (lane 3) (8 μg each) bound to the glutathione beads were incubated with purified his-tagged β-catenin (0.5 μg). The in vitro binding of β-catenin with GST and GST fusion proteins were visualized following Western blot analysis of glutathione-sepharose-bound proteins with β-catenin antibody. Cell lysate from A549 cells (lane 4) was used as a control.

lane 1). The endogenous 94-kDa β-catenin from A549 cell lysate (lane 2) was also recognized by the same antibody. For the in vitro binding assay (Fig. 2C), the bacterially expressed GST-N, GST-P, and GST alone (control) were bound to glutathione beads and incubated with equal amounts of purified his-tagged β-catenin. The amounts of GST and GST fusion proteins bound to the glutathione beads were not only homogeneous (based on Coomassie blue staining of glutathione-bound proteins), but equal amounts of GST, GST-N, and GST-P proteins were present in each cases as deduced by Western blot analysis with HPIV-3 RNP (5) and GST antibodies (data not shown). Following incubation, the beads were washed extensively and the bound proteins were subjected to SDS-PAGE and Western blot analysis with β-catenin antibody. Consistent with its direct physical interaction with RNP, β-catenin interacted with both GST-N (lane 3) and GST-P (lane 2), but not with control GST (lane 1). However, the amount of β-catenin associated with GST-N was significantly less compared with that of GST-P. The interaction of β-catenin with HPIV-3 N and P proteins was specific, because we observed a discernable difference in the amount of β-catenin binding to GST-N and GST-P, notwithstanding, equivalent amounts of protein were present in

both cases. These results thus demonstrated that β-catenin interacts directly with HPIV-3 N and P, perhaps with greater affinity with P protein of the viral RNP complex.

Colocalization of β-Catenin With HPIV-3 RNP in Infected Cells

To investigate the physiological and functional significance, if any, of the interaction of β-catenin with HPIV-3 RNP, we carried out double-labeled immunofluorescence confocal microscopic analysis (Fig. 3) of HPIV-3-infected A549 cells. For these studies, uninfected and HPIV-3-infected A549 cells were labeled with monoclonal anti-β-catenin and polyclonal anti-RNP antibodies, followed by incubation with Texas Red conjugated anti-rabbit (to visualize RNP) and FITC conjugated anti-mouse (to visualize β-catenin) secondary antibodies. Figure 3A and B displays the same set of double-labeled uninfected cells incubated with anti-β-catenin (A) and anti-RNP (B) antibodies. Likewise, Figure 3C and D shows the same set of double-labeled infected cells incubated with anti-β-catenin (C) and anti-RNP (D) antibodies. As shown in the figure, viral RNP colocalized with β-catenin at the cell periphery (compare C and D). HPIV-3 RNP from infected cells (Fig. 3D) was predominantly cytoplasmic and a portion of it colocalized with the β-catenin at the cell periphery (Fig. 3C), near the cell–cell borders, where β-catenin is localized. In uninfected cells, RNP was not detected (Fig. 3B), and in these cells β-catenin staining revealed its usual localization at the cell periphery, just beneath the cell membrane (Fig. 3A). Merging of the red (RNP label) and green (β-catenin label) colors revealed colocalization of RNP and β-catenin at the cell periphery as visualized by the merged yellow color (Fig. 3E). Although β-catenin interacts and colocalizes with viral RNP, we noticed no change in β-catenin protein levels by Western blot analysis of HPIV-3-infected A549 cells (data not shown). Interestingly, even in HPIV-3-infected giant multinucleated cells (Fig. 3C, D, E), resulting from HPIV-3 glycoprotein-mediated cell fusion and syncytia formation, β-catenin was located at the cell periphery (Fig. 3C, E), suggesting that alteration in cellular morphology induced by HPIV-3 infection has no effect on the intracellular localization of β-catenin.

In Vitro Transcriptional Activation of HPIV-3 Genome RNA by Immunoprecipitated β-Catenin From A549 Cells

Because actin plays an important role in transactivating HPIV-3 genome, next we addressed whether β-catenin, an actin-bound HPIV-3 RNP-interacting protein, is able to stimulate viral transcription. To this end, an in vitro HPIV-3 transcription assay was performed in the presence of β-catenin.

To ascertain the function of viral RNP-bound β-catenin, immunoprecipitated β-catenin from uninfected A549 cell lysate (Fig. 4) was added to an in vitro HPIV-3 transcription assay. The assay constitutes RNP (containing viral N, P, and L proteins bound to viral genomic RNA) purified from the virus, which synthesizes a significant amount of mRNA in the transcription reaction, only when cell lysate or other viral transcription stimulatory factors like actin is added to the reaction (14,15).

As expected, addition of actin immunopellet (P) stimulated RNA synthesis significantly (lane 2) compared with the control purified RNP alone (lane 1). However, addition of β-catenin immunopellet (P) together with actin immunopellet (lane 3) further stimulated mRNA synthesis (compare lanes 2 and 3). Moreover, addition of β-catenin immunopellet (P) alone in the absence of actin pellet also stimulated transcription (lane 5) compared with the pellet obtained following immunoprecipitation of uninfected cell lysate with control rabbit serum (lane 4). The basal levels of transcription observed with control RNP alone (lane 1) (in the absence of any exogenously added factors) is due to the presence of residual actin that remains bound to the RNP even after extensive purification.

From this series of experiments, it appears that the transcriptional stimulation observed following addition of actin and β-catenin immunopellet together (Fig. 4, lane 3) was significantly higher (three- to fourfold) compared with the addition of β-catenin or actin pellet alone (compare lanes 2 and 5 with lane 3). Coupled with the fact that β-catenin remains bound with actin in the cytosol, the pellets obtained by immunoprecipitation with individual antibodies contain both actin and β-catenin in different amounts and they stimulated transcription accordingly. It is important to note that β-catenin-depleted immunosupernatant (S) also stimulated transcription (lane 7), albeit at a lower efficiency compared with the control rabbit serum-depleted immunosupernatant (S) (lane 6). Because β-catenin supernatant (lane 7) contains essentially free actin and the control supernatant (lane 6) contains both actin and β-catenin, the decreased stimulation by the β-catenin-depleted supernatant strongly suggests that β-catenin functions as one of the transcriptional coactivators for HPIV-3 genome. Moreover, the higher transcriptional stimulation achieved by the addition of actin and β-catenin immunopellet together (lane 3) compared with their addition alone (lane 2 and lane 5), suggests that β-catenin-bound actin may have higher transcriptional

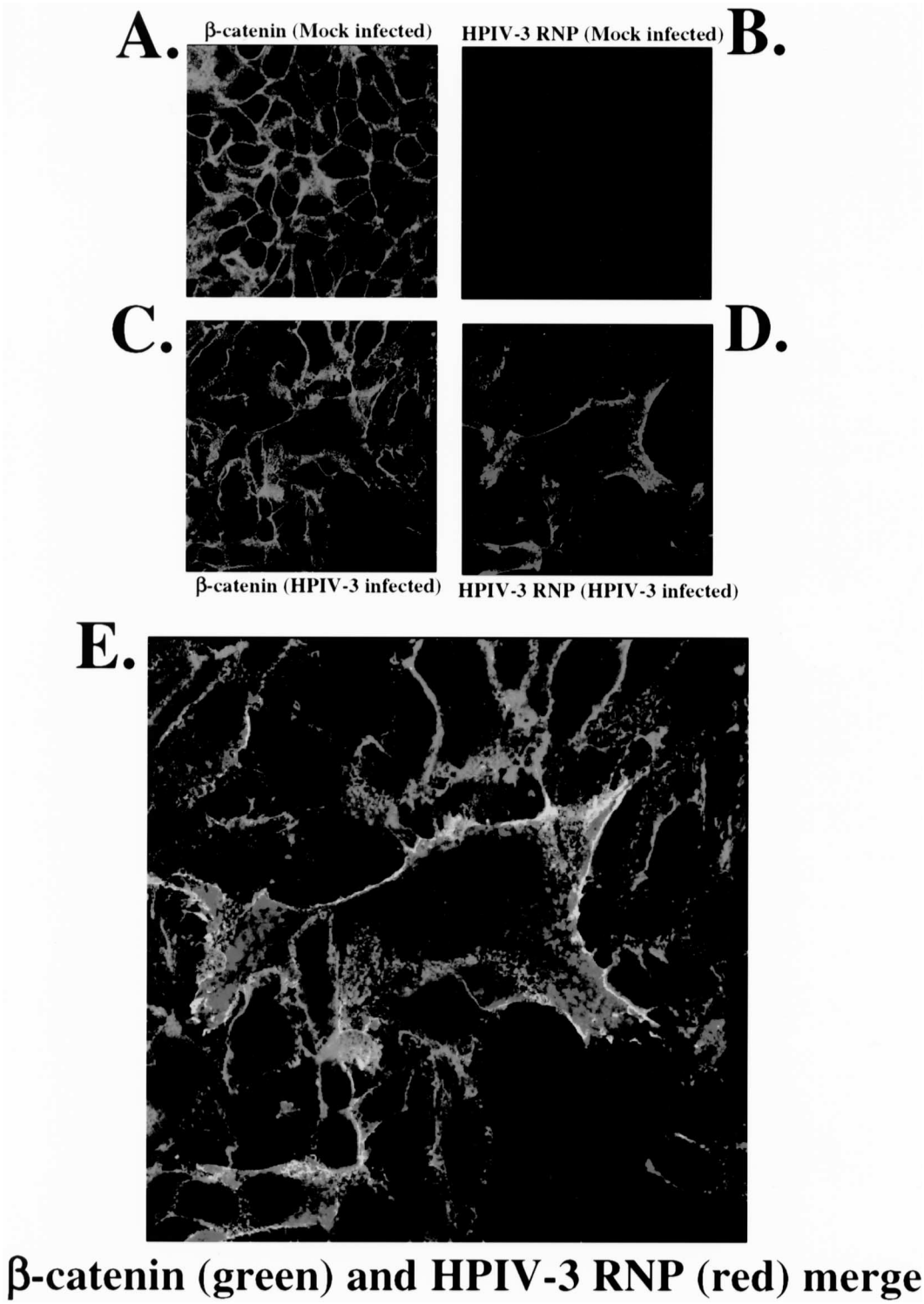

Figure 3. Colocalization of β-catenin with HPIV-3 RNP in A549 cells. Double-labeled immunofluorescence confocal microscopy analysis was performed with mock-infected (A and B) and HPIV-3-infected (C and D) A549 cells double labeled with anti-rabbit HPIV-3 RNP (B and D) and anti-mouse β-catenin (A and C) antibodies. Mock Infected (A, B) and infected (C, D) represents same set of cells double labeled with RNP and β-catenin antibodies. RNP was visualized by staining mock-infected (B) and HPIV-3-infected (D) cells with Texas Red conjugated anti-rabbit antibody, while β-catenin was visualized following incubation of mock-infected (A) and HPIV-3-infected (C) cells with FITC conjugated anti-mouse antibody. (E) Merged image of RNP (red) and β-catenin (green) showing colocalization (yellow) of β-catenin and HPIV-3 RNP at the cell periphery.

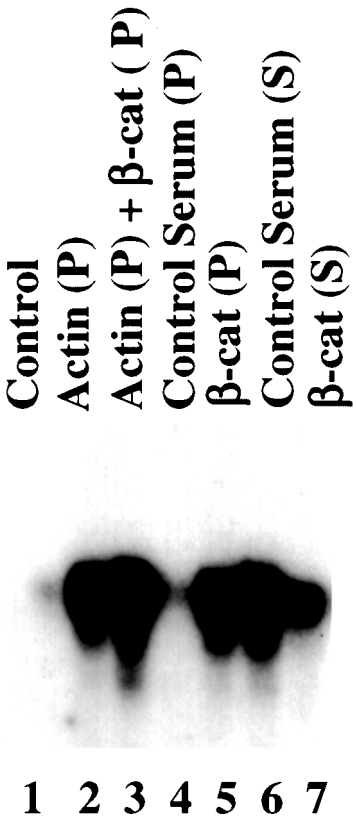

Figure 4. In vitro transcriptional activation of HPIV-3 genome by immunoprecipitated β-catenin (β-cat). In vitro transcription was carried out as described in Materials and Methods in a transcription reaction mixture containing purified HPIV-3 RNP. Washed immunoprotein A-sepharose bead pellets (P) obtained following immunoprecipitation of uninfected A549 cell lysate with either actin (lane 2) or β-catenin (lane 5) antibodies were added to the transcription reaction either separately (lane 2 and lane 5) or together (lane 3). Similarly, immunopellets obtained following immunoprecipitation with control rabbit serum (lane 4) and the corresponding immunodepleted supernatant (S) obtained from control rabbit serum (lane 6) or β-catenin (devoid of β-catenin) (lane 7) immunoprecipitates were added to the transcription reaction mixture and analyzed as described in the Materials and Methods.

stimulatory activity compared with the β-catenin free actin and they may act cooperatively to optimally stimulate the HPIV-3 genome RNA transcription.

In Vitro Transcriptional Activation of HPIV-3 Genome RNA by Purified β-Catenin

Because β-catenin immunopellets may arguably contain additional β-catenin-associated protein(s), the possibility remains that these β-catenin-associated protein(s) may stimulate HPIV-3 RNP (Fig. 5). To demonstrate a direct involvement of β-catenin in transactivating viral RNP, in vitro transcription assay was performed in the presence of purified his-tagged

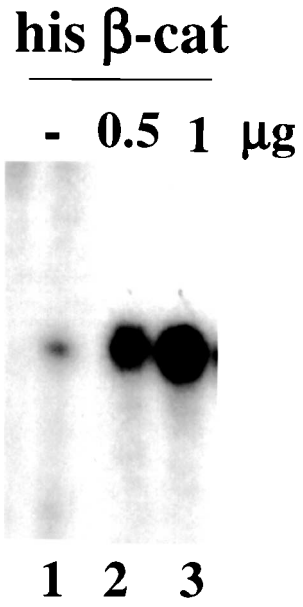

Figure 5. In vitro transcriptional activation of HPIV-3 genome by purified β-catenin (β-cat). In vitro transcription reaction was carried out in the absence (lane 1) or presence of 0.5 μg (lane 2) and 1 μg (lane 3) of purified his-tagged β-catenin. The transcription products were analyzed as described in Materials and Methods.

β-catenin (Fig. 5). Addition of 0.5 μg (lane 2) and 1 μg (lane 3) of purified β-catenin stimulated in vitro transcription in a dose-dependent manner, compared with the control RNP alone (lane 1). These results indicated that β-catenin alone, in the absence of its associated proteins, activates transcription of HPIV-3 RNP that is bound to residual actin. It is important to note that purified β-catenin preparation was devoid of actin and β-catenin binding proteins α/γ-catenins (3), as deduced by Western blot analysis with their corresponding antibodies (data not shown). Thus, the transactivating function observed with purified β-catenin seems to be solely due to β-catenin in the absence of any associated cellular factors.

DISCUSSION

In this article we have demonstrated a role for β-catenin in stimulating HPIV-3 transcription by virtue of its interaction in vivo (Fig. 1) and in vitro (Fig. 2) with HPIV-3 RNP. Furthermore, β-catenin colocalized with peripheral HPIV-3 RNP near the cell–cell contact (Fig. 3). Finally, the RNP-bound β-catenin acted as a transactivator for HPIV-3 genome in vitro (Figs. 4 and 5). Taken together, it seems actin-associated β-catenin optimizes viral gene expression by interacting with HPIV-3 RNP.

Our focus on β-catenin as a candidate activator of HPIV-3 genome transcription stemmed from earlier studies demonstrating an important role of actin microfilament in HPIV-3 transcription (15,16,21). These studies demonstrated: a) HPIV-3 RNP colocalizes with actin microfilament in infected cells and disruption of microfilament results in inhibition of HPIV-3 transcription (21), and b) purified actin stimulated viral genome transcription in an in vitro assay (14–16). During the course of these studies, it was observed that following extensive purification of actin, several purified actin preparations lost their transactivating potential, thus suggesting that actin-bound cellular factor(s) may confer an essential function for optimal activation of HPIV-3 transcription. In an effort to identify these factor(s), we decided to test whether several well-known actin-bound proteins are capable of activating HPIV-3 genome transcription. These studies yielded β-catenin (4,13), an actin-bound cytosolic protein, as one of the transcriptional activators of HPIV-3 genome.

Besides being an important actin binding protein, β-catenin is a multifunctional cytoplasmic protein involved in cell–cell adhesion at adherens junctions in normal cells. Interestingly, β-catenin is also a central component of the wingless signal transduction cascade, which is operative during embryogenesis. Activation of wingless pathway leads to the translocation of cytosolic β-catenin into the nucleus. In the nucleus, β-catenin interacts with transcriptional factors of the T-cell factor (TCF) family to transcriptionaly activate the expression of target genes that are involved in embryo development (23). In addition, nuclear translocated β-catenin also acts as a transactivator of genes involved in tumorogenesis of normal tissues (4). Although nuclear translocated β-catenin interacts with TCF transcription factors to express target genes by DNA-dependent RNA polymerase II, no such function has been attributed to cytosolic β-catenin from normal cells. In that context, our present work suggests that the transactivating function of cytosolic β-catenin may be utilized by cytoplasmic viruses to augment the efficiency of their gene expression. Thus, cytosolic β-catenin binds to the viral polymerase subunit, the P protein along with the nucleocapsid protein N, to stimulate the transcription of HPIV-3 gene by RNA-dependent RNA polymerase (L protein).

The mechanism(s) by which β-catenin transactivates HPIV-3 transcription remains unclear. In that context, it is important to note that although β-catenin colocalizes with viral RNP at the cell periphery (Fig. 3), the major bulk of RNP is nonperipheral. Moreover, in vitro transcription results (Fig. 4, lane 7) indicated that β-catenin free immunosupernatant (S), containing free actin, also supports transcription, albeit to a lesser extent compared with β-catenin immunopellet (P) (Fig. 4, lane 5). Based on these results, we postulate that nonperipheral RNP associated with β-catenin free actin also undergoes transcription. In that case, it is plausible that additional unidentified actin-bound and/or unbound host factor(s) supports viral transcription.

Besides HPIV-3, cytoskeletal proteins are known to play an important role in the transcription of several other nonsegmented negative-strand RNA viral genome. For example while tubulin is utilized by measles virus (24), Sendai virus (25), and VSV (25) for transcription, actin is necessary for respiratory syncytial virus (RSV) transcription (9). Apart from these viruses, a role of actin in human immunodeficiency virus-1 reverse transcriptase activity has been demonstrated (22). The cytoskeletal requirement for the transcription of these viruses is partly or wholly dependent on the availability of cytoskeletal-bound specific host factors that stimulate or sometime inhibit transcription. The two best examples are HPIV-3 and RSV, both belonging to the same family (Paramyxoviridae) of viruses. Recent studies implicated the requirement of profilin, an actin modulatory protein, for RSV transcription (10). Moreover, RSV interacted with a small GTPase, Rho A, to induce actin reorganization required for efficient transcription of RSV (26,27). Similar function of Rho A during HPIV-3 infection was proposed recently (27). In contrast to the requirement of host factors for efficient replication, GAPDH, a cellular glycolytic enzyme that is known to interact with actin, inhibited HPIV-3 transcription (11,18). Future studies would certainly provide insight into the role of cytoskeletal proteins as well as the mechanism of β-catenin-mediated transcriptional activation of HPIV-3 genome.

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