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Recovery of Paramyxovirus Simian Virus 5 with a V Protein Lacking the Conserved Cysteine-rich Domain: The Multifunctional V Protein Blocks both Interferon-β Induction and Interferon Signaling

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The V protein of the Paramyxovirus simian virus 5 (SV5) is a multifunctional protein containing an N-terminal 164 residue domain that is shared with the P protein and a distinct C-terminal domain that is cysteine-rich and which is highly conserved among Paramyxoviruses. We report the recovery from Vero cells [interferon (IFN) nonproducing cells] of a recombinant SV5 (rSV5) that lacks the V protein C-terminal specific domain (rSV5VΔC). In Vero cells rSV5VΔC forms large plaques and grows at a rate and titer similar to those of rSV5. In BHK or CV-1 cells rSV5VΔC forms small plaques and grows poorly. However, even when grown in Vero cells rSV5VΔC reverts to pseudo-wild-type virus in four to five passages, indicating the importance of the V protein for successful replication of SV5. Whereas rSV5 grows in many cell types with minimal cytopathic effect (CPE), rSV5VΔC causes extensive CPE in the same cell types. To overcome the antiviral state induced by IFN, many viruses have evolved mechanisms to counteract the effects of IFN by blocking the production of IFN and abrogating IFN signaling. Whereas rSV5 blocks IFN signaling by mediating the degradation of STAT1, rSV5VΔC does not cause the degradation of STAT1 and IFN signaling occurs through formation of the ISGF3 transcription complex. Furthermore, we find that rSV5 infection of cells prevents production of IFN-β. The transcription factor IRF-3 which is required for transcription of the IFN-β gene is not translocated from the cytoplasm to the nucleus in rSV5-infected cells. In contrast, in rSV5VΔC-infected cells IRF-3 is localized predominantly in the nucleus and IFN-β is produced. By using ectopic expression of IRF-3, it was shown that after dsRNA treatment and expression of the V protein IRF-3 remained in the cytoplasm, whereas after dsRNA treatment and expression of the P protein (which lacks the C-terminal cysteine-rich domain) IRF-3 was localized predominantly in the nucleus. Thus, SV5 blocks two distinct pathways of the innate immune response, both of which require the presence of the C-terminal specific cysteine-rich domain of the multifunctional SV5 V protein.

INTRODUCTION

The *Paramyxoviridae* family of nonsegmented negative-sense RNA viruses contains many important established and emerging human and animal pathogens, including Sendai virus, human parainfluenza viruses I–IV, mumps virus, Newcastle disease virus, measles virus, rinderpest virus, and the members of the recently classified *Henipavirus* genus, the newly identified Nipah and Hendra viruses. Members of this family are enveloped viruses with a nonsegmented, single-stranded, negative-sense RNA genome of ~15–16,000 nucleotides. Within the *Paramyxoviridae* simian parainfluenza virus 5 (SV5) is a prototype of the *Rubulavirus* genus. The SV5 genome contains 15,246 nucleotides encoding eight proteins from seven genes (reviewed in Lamb and Kolakofsky, 2001). SV5, similar to all Paramyxoviruses, consists of a core of genomic RNA encapsidated by nucleocapsid (NP) protein to which is bound an RNA polymerase complex composed of large (L) protein and phosphoprotein (P). This core is surrounded by a lipid envelope that is coated with matrix (M) protein on its inner surface and penetrated by spike glycoproteins that function for attachment (hemagglutinin-neuraminidase) (HN) and fusion (F). Minor structural components of SV5 include the SH integral membrane protein (He *et al.,* 1998; Hiebert *et al.,* 1985, 1988), which plays a role in blocking apoptosis in infected cells (He *et al.,* 2001) and the V protein (discussed below).

The V and P proteins (Paterson *et al.,* 1984; Peluso *et al.,* 1977) are both transcribed from the V/P gene by a process formally termed pseudotemplated addition of nucleotides and popularly dubbed "RNA editing" (Paterson *et al.,* 1989; Thomas *et al.,* 1988; Vidal *et al.,* 1990). The SV5 V mRNA is a faithful transcript of the V/P gene,

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whereas the P mRNA contains two nontemplated G residues which are added cotranscriptionally by the viral RNA polymerase, presumably by a stuttering mechanism (Thomas *et al.,* 1988; Vidal *et al.,* 1990; reviewed in Lamb and Kolakofsky, 2001). The consequence of the addition of two G residues to create the P mRNA is that the translational reading frame is changed relative to the V protein after the editing site. SV5 V and P proteins share an N-terminal domain of 164 amino acids but have unique C-terminal domains (Thomas *et al.,* 1988). The process of pseudotemplated transcription at a specific site in the V/P gene occurs for almost all members of the subfamily *Paramyxovirinae* (reviewed in Lamb and Kolakofsky, 2001). However, whereas for the Rubulaviruses the V mRNA is transcribed directly from the genome RNA, for the Respiroviruses and the Morbilliviruses the P mRNA is transcribed directly from the genome RNA and the V mRNA contains the additional pseudotemplated G nucleotide(s) (reviewed in Jacques and Kolakofsky, 1991).

The sequence of the unique C-terminal domain of the V proteins is highly conserved among the paramyxoviruses. This domain, containing seven cysteine residues, is analogous to but different from classic zinc finger and RING finger domains (Thomas *et al.,* 1988; reviewed in Borden, 2000; Klug and Rhodes, 1987; Klug and Schwabe, 1995). Nonetheless, the measles virus, SV5, Newcastle disease virus, and Sendai virus V proteins bind zinc ions (Huang *et al.,* 2000; Liston and Briedis, 1994; Paterson *et al.,* 1995; Steward *et al.,* 1995). The SV5 V protein is incorporated into virions (~350 molecules per virion) and is found associated with the nucleocapsid (Paterson *et al.,* 1995). For Sendai virus and measles virus, however, the V protein does not appear to be incorporated into virions. Recombinant Sendai virus, measles virus, and rinderpest virus unable to synthesize the V protein [V(−) viruses] have been recovered from cloned DNA, indicating the V protein is not essential for these paramyxoviruses for replication in tissue culture (Baron and Barrett, 2000; Delenda *et al.,* 1997; Kato *et al.,* 1997a; Schneider *et al.,* 1997), although the V proteins appear to be important for pathogenicity of these viruses in animals (Baron and Barrett, 2000; Kato *et al.,* 1997a,b; Tober *et al.,* 1998). Sendai virus deficient in V protein shows increased antigenome synthesis and transcription (Kato *et al.,* 1997a) and expression of the V protein decreased replication in a defective interfering (DI) RNA replication assay, indicating that the V protein may negatively regulate viral replication (Curran *et al.,* 1991). Rinderpest virus unable to synthesis V protein shows an increase in synthesis of genome and antigenome RNAs. For the Rubulaviruses, whose V proteins are of a mass considerably less than those of the *Respirovirus* or *Morbillivirus* genera, a recombinant virus lacking the V protein has not been reported.

Although a role for the SV5 V protein in regulating RNA replication has not been shown, the SV5 V protein is known to have many functions. It has been shown to interact with soluble viral NP (Randall and Bermingham, 1996) and the shared N-terminal domain of V and P binds single-stranded RNA through a basic region (Lin *et al.,* 1997). The SV5 V protein interacts with a cellular protein (DDB1), the 127-kDa subunit of the damage-specific DNA-binding protein (DDB) and this interaction requires the presence of the V protein-specific C-terminal domain (Lin *et al.,* 1998a). Expression of the SV5 V protein, via its C-terminal domain, also acts to slow the cell cycle (Lin and Lamb, 2000). V protein binding to DDB1 and the effect of V protein on the cell cycle may be related as DDB has also been found to interact with CUL-4A (Shiyanov *et al.,* 1999), a member of the cullin family of E3 ubiquitin ligases. Although the function of CUL-4A is not known, the related CUL-1 and CUL-3 proteins are involved in the ubiquitin-mediated degradation of cyclin E (reviewed in Winston *et al.,* 1999) and cyclin D and $p21^{CIP}$ (Yu *et al.,* 1998). Interestingly, the changes in the cell cycle caused by expression of V protein can be partially reversed by coexpression of DDB1 (Lin and Lamb, 2000). Last, the SV5 V protein interferes with establishment of the antiviral state induced by interferons, as discussed below.

Interferons (IFNs) are the primary innate antiviral cytokines produced in virus-infected cells (reviewed in Biron and Sen, 2001; Darnell, 1997; Stark *et al.,* 1998). Induction of IFN-β is not fully understood but it is thought to involve signaling by dsRNA formed in virus-infected cells leading to the formation of a large transcription complex which contains among many factors NF-κB, members of the interferon regulatory factor (IRF) family, including IRF-1, IRF-3, and IRF-7 ATF-2 homodimers or ATF-2/c-*Jun* heterodimers (reviewed in Taniguchi *et al.,* 2001; see also accompanying article, Poole *et al.,* 2002). Both type I (IFN-α/β) and type II (IFN-γ) IFNs can induce an antiviral state. IFN signaling is initiated when IFN binds to its receptor on the cell surface, which leads to phosphorylation of cytoplasmic STAT proteins to produce DNA-binding complexes that become translocated into the nucleus. IFN-α/β responses are regulated primarily through the activated transcription complex, ISGF3, that is composed of a heterodimer of STAT1 and STAT2 in association with a DNA-binding subunit p48 (IRF-9). ISGF3 binds to a DNA element, ISRE, found in the promoter of IFN-α/β-inducible genes. IFN-γ responses are mediated via a transcription complex consisting of STAT1 homodimers. The STAT1 homodimer binds to a DNA element, GAS, found in the promoter of IFN-γ inducible genes.

To overcome the antiviral state induced by interferon, many viruses have evolved mechanisms to counteract the effects of IFN by blocking the production of IFN and abrogating IFN signaling (reviewed in Biron and Sen, 2001; Goodbourn *et al.,* 2000). For the paramyxovirus Sendai virus a block in IFN signaling occurs (Didcock *et al.,* 1999a; Young *et al.,* 2000) through the viral C protein leading, directly or indirectly, to inactivation of STAT1

(Garcin *et al.,* 1999; Takeuchi *et al.,* 2001). The Rubulaviruses, SV5, mumps virus, and hPIV2 all interfere with IFN-induced antiviral responses by targeting STAT1 (SV5 and mumps virus) or STAT2 (hPIV2) proteins for proteasomal degradation (Didcock *et al.,* 1999b; Kubota *et al.,* 2001; Parisien *et al.,* 2001, 2002a,b). For SV5, mumps virus, and hPIV2, the degradation of STAT1 or STAT2 is mediated by the V protein (Andrejeva *et al.,* 2002b; Didcock *et al.,* 1999b; Kubota *et al.,* 2001; Parisien *et al.,* 2001). Hence the multifunctional V protein has yet another role in the paramyxovirus lifecycle. For SV5-induced STAT1 degradation and hPIV2-induced STAT2 degradation, the targeting complex minimally requires expression of V, STAT1, and STAT2 (Parisien *et al.,* 2002b). The ability of SV5 to induce degradation of STAT1 and interfere with IFN signaling is species specific and this finding may in part explain species tropism. Degradation of STAT1 occurs in SV5-infected primate cells but is greatly reduced in SV5-infected murine cells (Didcock *et al.,* 1999a). However, ectopic expression of human STAT2 in mouse cells overcomes the species-specific block and SV5 virus infection now antagonizes the mouse IFN signaling pathway by causing degradation of mouse STAT1 (Parisien *et al.,* 2002a). It is known that sequence differences in the 164 residue N-terminal shared V/P domain of SV5 have an important effect on IFN signaling and the degradation of STAT1 (Chatziandreou *et al.,* 2002; Young *et al.,* 2001). However, the V protein-specific C-terminal domain is also involved in targeting STAT1 for degradation: for mumps virus and hPIV2, expression of the V protein containing a deletion of the C-terminal specific domain greatly decreases the affect of V on STAT1 signaling (Kubota *et al.,* 2001; Nishio *et al.,* 2001). Although the mechanism by which the SV5 V protein mediates the proteasomal degradation of STAT 1 is not known, it seems possible it could involve the association of V protein with DDB1 and the E3 ubiquitin ligase protein cullin 4A.

Here we report recovery of a recombinant SV5 (rSV5) that lacks the V protein C-terminal specific domain (VΔC) and we describe properties of the rSV5VΔC virus including its inability to mediate STAT1 degradation and concomitantly its inability to defeat STAT 1 signaling. Furthermore, we also show that whereas wt SV5 infection prevents production of IFN-β, rSV5VΔC infection permits production of IFN-β. We find that the transcription factor IRF-3 is not transported from the cytoplasm to the nucleus in wt SV5-infected cells but in rSV5VΔC-infected cells IRF-3 is localized predominantly to the nucleus. It was found that the V protein, but not the P protein, directly or indirectly causes IRF-3 to remain cytoplasmically localized after dsRNA stimulation. Thus, SV5 blocks two distinct pathways of the innate immune response, both of which require the presence of the C-terminal-specific domain of the multifunctional SV5 V protein.

RESULTS

Recovery of rSV5 containing a deletion of the C-terminal domain of the V protein

To study the role of the V protein in the SV5 lifecycle, many attempts were made to generate a recombinant virus that would express only the P mRNA and not the V mRNA (due to mutation of the RNA editing site—for experimental approach used, see Delenda *et al.,* 1997). Even though the genome length was maintained as a multiple of six nucleotides (Calain and Roux, 1993), all attempts over 3 years to recover a virus that could not express the V protein failed, even when the V protein was expressed *in trans* from an expression plasmid during the initial transfection. It has been shown previously that the V protein C-terminal unique domain is involved in several functions of the V protein (cell-cycle regulation, DDB1 interactions, STAT1 degradation). Thus we attempted to recover a virus containing a V protein in which the C-terminal unique domain was deleted. Two translational stop codons were introduced into the V reading frame, one after residue 169 and one at the equivalent of V residue 172. Neither mutation changed the amino acid sequence of the P protein. We used a modification (Waning *et al.,* 2002) of the SV5 reverse genetics system (He *et al.,* 1997) that improved the ease of recovery of rSV5 from cloned DNA. Rather than infecting cells with vaccinia virus-expressing bacteriophage T7 RNA polymerase, the cell-line BSR T7/5 (Buchholz *et al.,* 1999), which stably expresses T7 RNA polymerase, was used. The wt and mutant infectious SV5 cDNA plasmids were cotransfected together with expression plasmids encoding the viral proteins NP, P, and L into BSR T7/5 cells such that SV5 (+)-strand antigenome RNA and mRNAs for NP, P, and L were transcribed. The cDNA genome construct encoding VΔC gave rise to recombinant virus (rSV5VΔC) after passage from the BSR T7/5 cells onto Vero cells in the first attempt. Vero cells were selected for amplification of the virus stock because Vero cells do not produce interferon and we considered it likely that an altered V protein may have altered properties with respect to its effect on IFN signaling. We were unable to demonstrate that defects observed with rSV5VΔC could be complemented by V protein expressed in transfection/infection experiments due to extensive cytopathic effects (see Fig. 5).

Confirmation of the engineered mutations in the rSV5VΔC stocks was obtained by nucleotide sequencing of RT-PCR products derived from vRNA (Fig. 1). All of the recovered viruses contained a tyrosine codon at V residue 166 due to the deliberate introduction of a restriction endonuclease site in the V/P gene to facilitate the production of the mutant. However, after four to five passages in Vero cells and plaque purification, rSV5VΔC virus was found to revert to a pseudo-wild-type with both stop codons being altered. The natural residues in the P protein G170 and I172 were unchanged but new residues

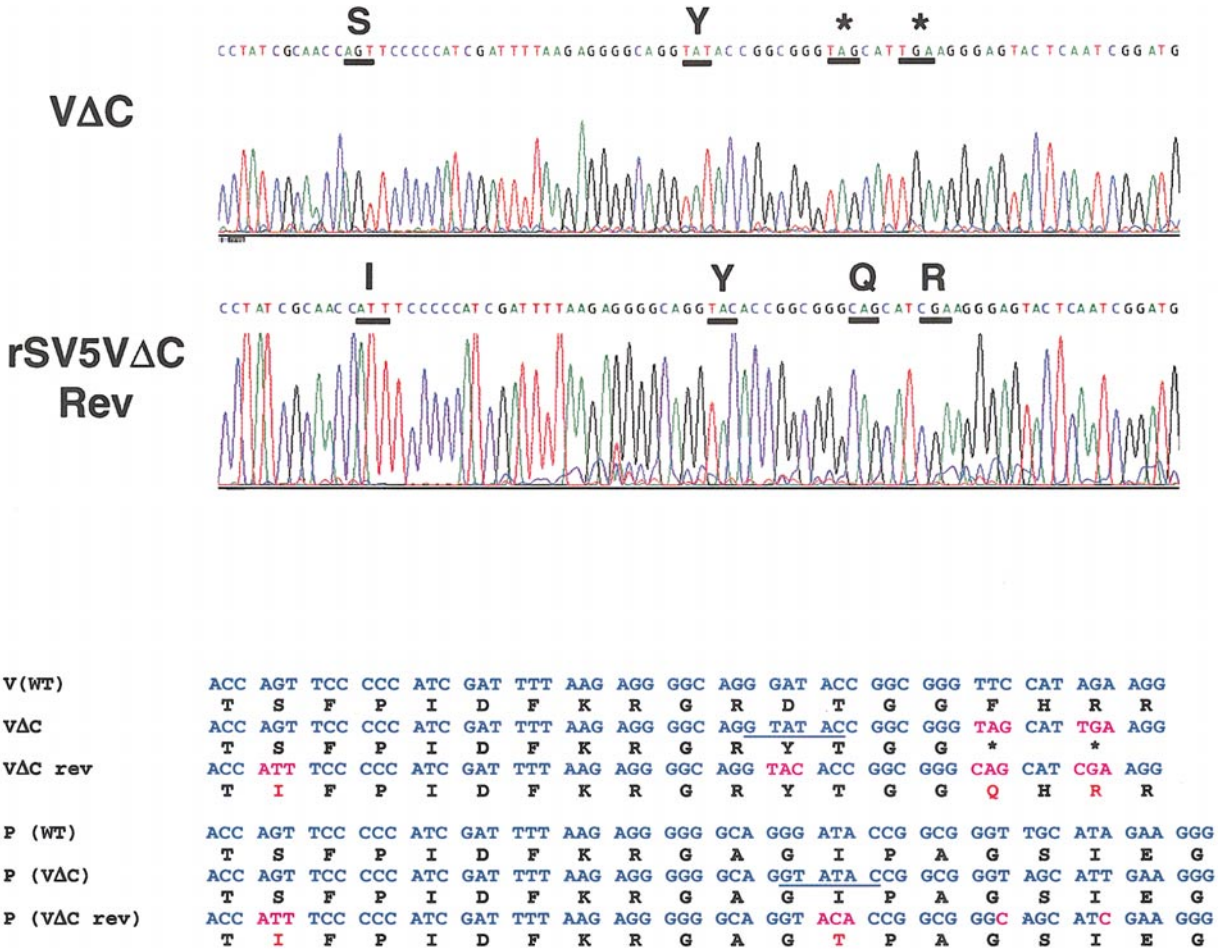


FIG. 1. Recovery of rSV5VΔC and rSV5VΔC revertant virus. (Top) RT-PCR sequencing of vRNA from rSV5VΔC (VΔC) and rSV5VΔC revertant (VΔC rev) virus-infected Vero cells. Mutated nucleotides and residues are indicated. (Bottom) Partial coding sequences of V protein and P protein. Nucleotide and codon changes are highlighted. Underline sequence confers a restriction enzyme site to facilitate construction of the parental genome plasmid.

were introduced into the VΔC protein, Q170 and R172. Interestingly, R172 is conserved among all V proteins of Paramyxoviruses (Lamb and Kolakofsky, 2001; Tidona *et al.,* 1999). In the RT-PCR sequencing sample shown (Fig. 1) a sporadic mutation (I156) in the revertant virus was also identified but this was not found in another revertant virus stock. Thus, the rapid accumulation of revertant virus suggests that deletion of the C-terminal unique domain of V protein is deleterious to the multiplication of SV5 and there is a strong selective pressure for the recovery of pseudo-wt virus.

To confirm that the rSV5VΔC virus did not express an intact V protein, immunofluorescent staining was performed by using an mAb 31C6 specific for the V protein C-terminal unique domain. In rSV5VΔC-infected cells no specific V staining was observed but in rSV5-infected cells V staining was readily detected in the cytoplasm of cells (Fig. 2). Staining for the surface glycoprotein HN is shown for comparison. Fewer cells were present on the coverslips at 24–36 h p.i. for rSV5VΔC-infected cells than rSV5-infected cells due to cytopathic effects (CPE) (see below). As is shown below (Fig. 8D) V protein is found localized predominantly in the nucleus in transfected

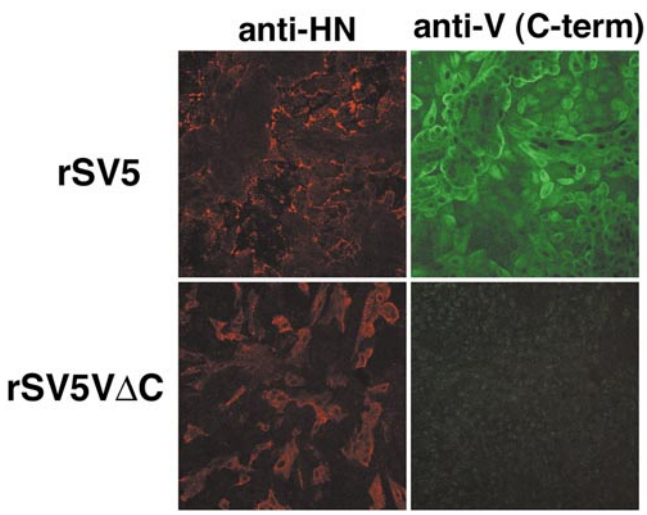


FIG. 2. Immunofluorescent staining patterns of rSV5- and rSV5VΔC-infected cells. CV-1 cells on glass coverslips were infected with rSV5 or rSV5VΔC and at 24 h p.i. The cells were fixed with 1% formaldehyde and permeabilized with 0.1% saponin. Cells were stained with an mAb specific for the C-terminal unique domain of V protein (31C6) (Paterson *et al.,* 1995) or rabbit polyclonal sera specific for HN and FITC-conjugated goat anti-mouse or Texas red conjugated goat anti-rabbit secondary antibody. Fluorescence was examined using a Zeiss 410 confocal microscope and appropriate filter sets.

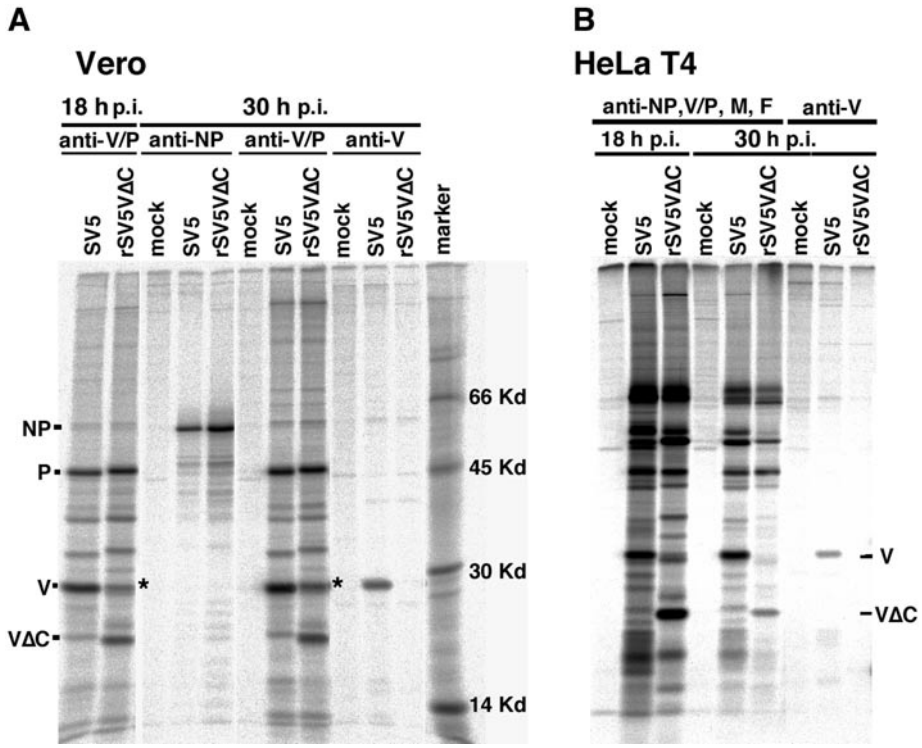


FIG. 3. Protein expression in rSV5- and rSV5VΔC-infected cells. Mock-, rSV5-, and rSV5VΔC-infected Vero cells (A) and HeLa T4 cells (B) were metabolically labeled with 100 μCi/ml ^{35}S-Promix for 2 h at times p.i. indicated. Polypeptides were immunoprecipitated using antibodies as follows: anti-V/P = mAb Pk; anti-NP = mAb NPd, anti-V = mAb clone 31C6; anti-NP, V/P, M, F = mixture of mAbs and polyclonal sera specific for NP, V/P, M, and F. The polypeptide band indicated by an asterisk is a fragment of P that closely migrates with V (Thomas *et al.,* 1988).

cells and in virus-infected cells when stained using an N-terminal-specific mAb (Pk). A possible explanation for this apparent discrepancy is the C-terminal-specific mAb does not detect V protein that is localized to the nucleus (data not shown), most likely because the V protein is complexed via its C-terminal domain with the cellular protein DDB1. In virus-infected cells a greater amount of V protein is expressed and the V protein localized in the cytoplasm is detected by the C-terminal-specific mAb.

Analysis of the viral proteins synthesized in Vero and HeLa T4 cells infected with rSV5 and rSV5VΔC showed the expression of V protein in rSV5-infected cells and the expression of the faster mobility VΔC protein in rSV5VΔC-infected cells (Fig. 3). The relative expression levels of the viral polypeptides examined (NP, P, M, F) for rSV5 and rSV5VΔC appeared to be similar. A polypeptide species of a similar, but not identical, mobility to the V protein was observed in rSV5VΔC-infected cells (asterisk, Fig. 3) and this species is known to be a degradation product of the P protein (Thomas *et al.,* 1988). Immunoprecipitation using an antibody specific for the C-terminal of the V protein did not detect a V-specific protein in rSV5VΔC-infected cells (Fig. 3). A pulse-label chase protocol in HeLa cells indicated that the VΔC protein is relatively unstable ($t_{1/2}$ ~ 45 min) (data not shown).

rSV5VΔC plaque size in BHK cells and Vero cells and growth curve in Vero cells

Plaque assay of rSV5 and rSV5VΔC in BHK cells (the conventional cell line used for plaquing SV5) indicated that SV5VΔC formed only pinpoint plaques in comparison to the irregularly sized plaques of rSV5 virus (Fig. 4A). A growth curve of rSV5VΔC in BHK cells indicated that the virus grew to ~2 logs lower titer than rSV5 (data not shown). In contrast to the plaques formed in BHK cells, in Vero cells rSV5VΔC formed discrete plaques with sharply defined edges that were more regular in shape and slightly larger than rSV5 plaques formed in Vero cells (Fig. 4B). The precise number of plaques between the two viruses and BHK cells and Vero cells cannot be compared as the data shown were derived from different experiments. A single-step growth curve of rSV5 and SV5VΔC in Vero cells indicated that the two viruses had similar growth rates and maximum titers, despite the differences in plaque size (Fig. 4B).

rSV5VΔC infection causes extensive CPE in many cell types

The cytopathic effect caused by SV5 in virus-infected cells ranges from very little in MDCK cells (Choppin, 1964) to massive syncytia formation in BHK cells (Holmes and Choppin, 1966). Recently we ob-

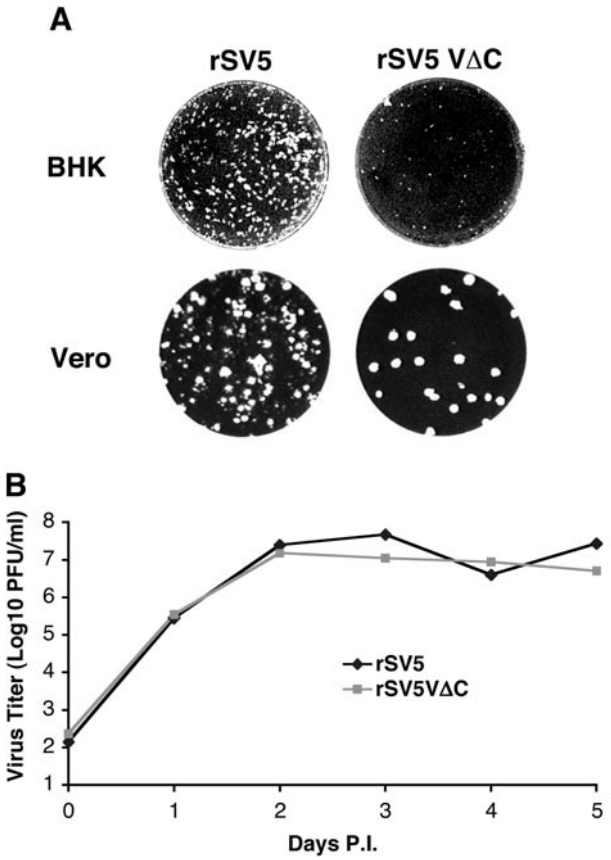


FIG. 4. Plaque sizes and single-step growth curves of rSV5 and rSV5VΔC. (A) Plaques of rSV5 and rSV5VΔC on BHK 21-F cells or Vero cells stained with Giemsa. (B) Vero cells were infected at an m.o.i. of 10 PFU/cell with rSV5 or rSV5VΔC and the media harvested at 1-day intervals up to 5 days p.i. The virus titers were determined by plaque assay on BHK cells.

served for a recombinant SV5 lacking the SH gene that this virus (rSV5ΔSH), unlike wt virus, caused apoptosis in MDCK cells (He *et al.,* 2001). We observed that rSV5VΔC infection of several cell types, e.g., HeLa, CV1 cells, Vero cells, and L929 cells, showed a severe CPE by 30 h p.i. Whereas for rSV5 infection of HeLa cells produced small foci of syncytia could be observed from 24 h p.i. onward, rSV5VΔC-infected cells at times $>$ 30 h p.i were detaching from the monolayer (Fig. 5). Data from propidium iodide staining analysis of cell DNA and from TUNEL assays suggested that the CPE was due to increased apoptotic cell populations in the rSV5VΔC-infected cells by 30 h p.i., whereas an apoptotic cell population was not detected in rSV5-infected HeLa cells (data not shown). Although CPE induced by rSV5VΔC in Vero and HeLa cells was evident by 30 h p.i, protein synthesis did not appear to be greatly decreased at this time (Fig. 3) nor was virus particle production (Fig. 4B). The formation of large discrete plaques in Vero cells by rSV5VΔC may be due to the induction of apoptosis.

rSV5VΔC infection does not cause degradation of STAT1 and does not block IFN signaling

To examine whether the V protein C-terminal domain was required for the degradation of STAT 1, lysates of mock, rSV5-, and rSV5VΔC-infected cells were prepared and an immunoblot for STAT 1 performed. As shown in Fig. 6A, whereas in rSV5-infected cells STAT 1 levels were very low, in rSV5VΔC-infected cells STAT 1 levels at 18 h p.i. were comparable to mock-treated cells and by 36 h p.i. were greater than mock-treated cells. Consistent with this observation, in a test for the presence of STAT 1 by measuring IFN signaling using a luciferase reporter assay in Vero cells infected with rSV5 or rSV5VΔC, it was found that whereas rSV5 largely abolished IFN signaling, rSV5VΔC infection of cells induced a strong IFN signaling response on treatment of cells with IFN-α that at 10 h p.i. was greater than in mock-treated cells (Fig. 6B). We did not examine times later in the infection cycle due to the increased CPE in the rSV5VΔC-infected cells. To examine further the differential effect of rSV5 and rSV5VΔC infection on IFN signaling, the assembly of the IFN responsive transcription complex ISGF3 was examined using an electrophoretic mobility shift assay (EMSA) with an oligonucleotide containing an ISGF3 promoter sequence. As shown in Fig. 6C a shift of labeled probe corresponding to binding of the assembled ISGF3 complex was only observed in rSV5VΔC-infected cell lysates and not in rSV5-infected cell lysates. Note that the IFN signaling experiment (Fig. 6B) was performed in Vero cells which are IFN-nonproducers and thus IFN was added to the cells to observe signaling on rSV5VΔC infection, whereas in the EMSA assay (Fig. 6C) 2fTGH cells (IFN producers) were used and ISGF3 formation occurred in SV5VΔC-infected cells in the absence of addition of exogenous IFN.

SV5 infection but not rSV5VΔC infection inhibits IFN-β production

The observation that the extent of IFN signaling and ISGF3 complex formation was greater in rSV5VΔC-infected cells than in mock-infected cells suggested that this stimulation might be due to the production of IFN. To examine further the effect of SV5 infection on IFN production, the amount of IFN-β released into the media of SV5- and rSV5VΔC-infected HeLa cells was measured using an ELISA assay. As shown in Fig. 7, very little IFN-β was detected from SV5-infected cells but by 36 h p.i. large amounts of IFN-β were detected from rSV5VΔC-infected cells. Thus, these data suggest that the SV5 V protein is involved in preventing IFN-β production in infected cells.

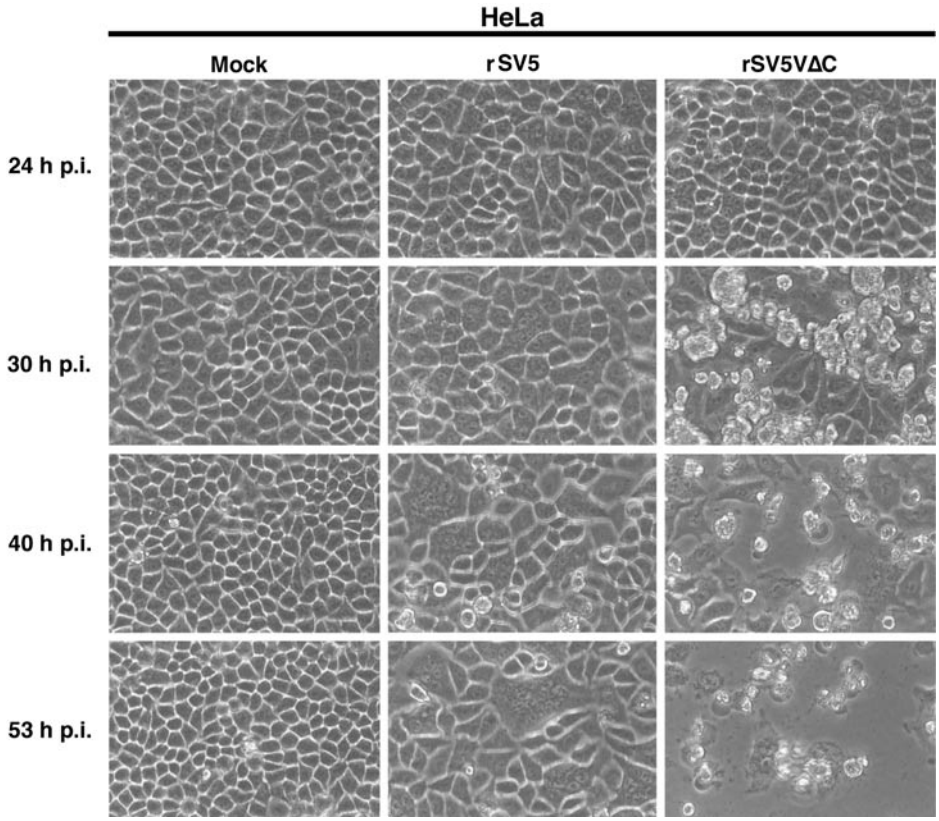


FIG. 5. CPE of rSV5 and rSV5VΔC in HeLa cells. HeLa T4 cells were infected at an m.o.i. of 10 PFU per cell. At varying times p.i. cells were photographed using a Nikon inverted microscope and a Kodak DCS 420 digital camera.

SV5 V protein blocks the nuclear localization of interferon regulatory factor 3 (IRF-3) on dsRNA stimulation

The transcription factor IRF-3 has been shown to be important for the transcription of the IFN-β gene (Sato *et al.,* 1998; Schafer *et al.,* 1998; Weaver *et al.,* 1998; Yoneyama *et al.,* 1998). Upon virus infection or exposure to dsRNA, IRF-3 which is constitutively expressed and cytoplasmically localized is phosphorylated on C-terminal serine and threonine residues (Lin *et al.,* 1998b). This activation by phosphorylation results in the relocalization of IRF-3 from the cytoplasm to the nucleus and the induction of transcription of host defense genes (Juang *et al.,* 1998; Sato *et al.,* 1998; Wathelet *et al.,* 1998; reviewed in Reich, 2002). Several viruses have evolved a means of interfering with IRF-3 functions and hence down-regulating the production of IFN. The E6 protein of human papillomavirus binds to IRF-3, inhibiting the transcriptional activation of the IFN-β gene (Ronco *et al.,* 1998) and expression of the influenza virus NS1 protein leads to a block in phosphorylation of IRF-3 and a cytoplasmic localization of IRF-3 in the presence of known inducers of IFN (Talon *et al.,* 2000). Thus, because SV5 infection prevents the production of IFN-β, we examined the subcellular localization of IRF-3 on expression of V protein in rSV5- and rSV5VΔC-infected cells.

To examine the localization of endogenous hIRF-3 in HeLa cells infected with rSV5 and rSV5VΔC, cells were stained with an mAb (SL-12) specific for hIRF-3 (Ronco *et al.,* 1998) and the cytoplasmic vs nuclear distribution of IRF-3 staining was examined by fluorescence microscopy for 400 randomly chosen cells (see Fig. 8A, for example). It was observed that whereas in rSV5-infected cells hIRF-3 was predominantly localized to the cytoplasm (12.0% of cells showed some nuclear staining), in rSV5VΔC-infected cells hIRF-3 was predominantly localized to the nucleus (67.7% cells showed strong nuclear staining).

To examine further the changes in the subcellular localization of hIRF-3 on SV5 infection, a biologically active green fluorescent protein (GFP) tagged version of IRF-3 was used and HeLa-CD4-LTR-β-gal cells were transfected, as these cells are highly transfectable. Cells were infected with rSV5 or rSV5VΔC and then transfected with the hIRF-3-GFP expression plasmid and fluorescence examined by confocal microscopy. In SV5-infected cells hIRF-3-GFP showed a cytoplasmic localization (Fig. 8B, panels A, D, C, and F), whereas in rSV5VΔC-infected cells hIRF-3-GFP showed a largely nuclear localization (Fig. 8B, panels G, J, I, and L). We also observed intensely staining small cytoplasmically localized granules when the bulk of hIRF-3-GFP was localized to the nucleus but the significance of these observations is unknown. In rSV5-infected cells and to a lesser extent

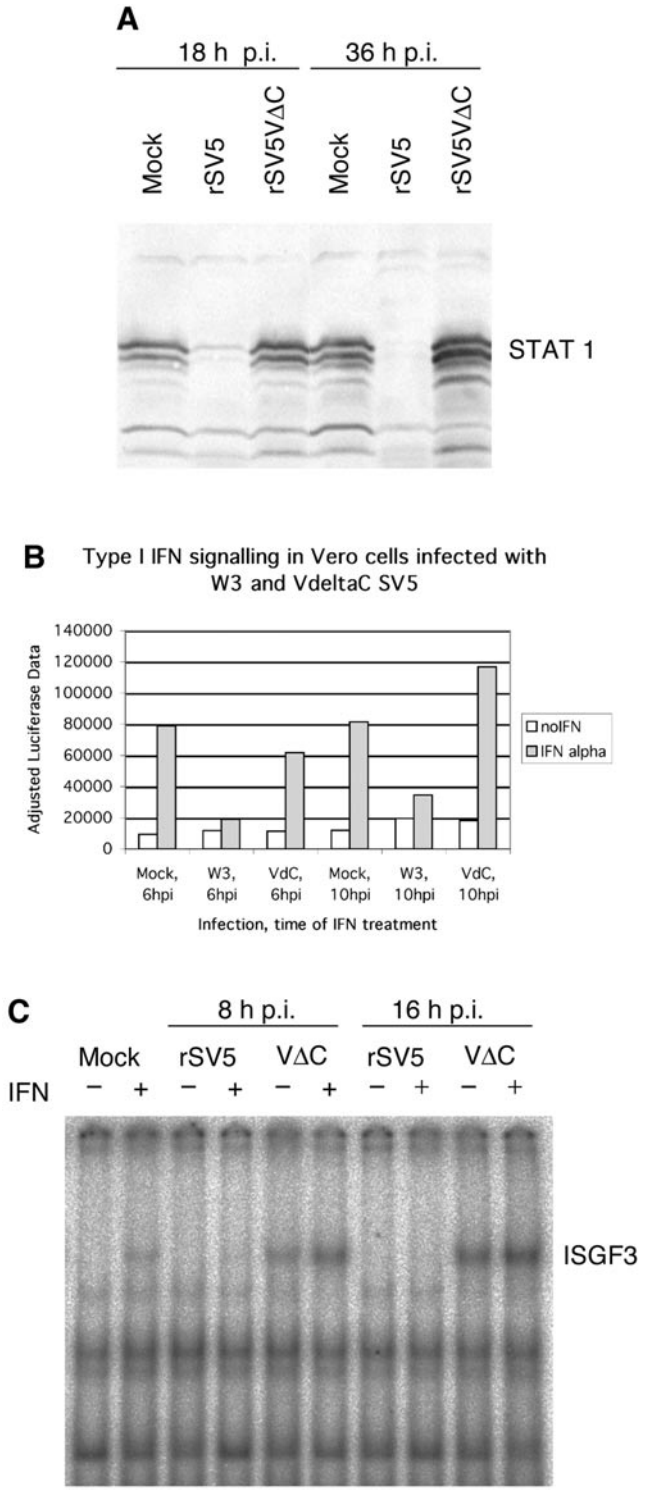


FIG. 6. The C-terminus of the V protein is required to block IFN signaling. (A) The C-terminal unique domain of V protein is required for STAT1 degradation. HeLa cells infected with rSV5 or SV5VΔC were lysed 18 and 36 h p.i. Lysates were subjected to SDS–PAGE on 10% gels and polypeptides transferred to an Immobilon filter and immunoblotted using antibodies specific for STAT1. (B) Comparison of the ability of mock-infected and rSV5 (W3)- and rSV5VΔC (VΔC)-infected cells to block IFN signaling. Cells were transfected with a plasmid that contained the luciferase gene under the control of an IFN-α/β-responsive promoter together with plasmid pJATlacZ, in which the *lac*Z gene is under the control of the rat β-actin promoter. At 6 or 10 h p.i. the culture medium was supplemented with IFN or left untreated. After a further 4 h the cells were lysed and luciferase activity was measured (relative light units) and normalized to β-galactosidase activity. (C). EMSA of IFN-activated transcription factor complex ISGF3. 2fTGH cells were mock-, rSV5-, or rSV5VΔC-infected, and at 8 or 16 h p.i. the culture medium was supplemented with IFN (+) or left untreated (−). After a further hour nuclear extracts were prepared and analyzed by EMSA using a probe specific for the 9-27 gene ISRE. The mobility of the transcription factor complex ISGF3 is indicated.

in rSV5VΔC-infected cells inclusion bodies were observed that stained with the Pk mAb specific for the shared V/P domain as have been observed previously (Randall and Bermingham, 1996). It was also observed that some of the hIRF-3-GFP colocalized with these inclusion bodies. For reasons we do not know we observed that it was very difficult to obtain >10% transfection efficiency in SV5-infected cells, whereas transfection of the cells without virus infection was possible in 85–90% of the cells. Conversely it was difficult to infect with SV5 cells that had been transfected with plasmid DNA.

To demonstrate more directly a role of the V protein in the altered subcellular localization of hIRF-3, we performed cotransfection experiments. Expression of hIRF-3-GFP, as expected, was observed to be predominantly cytoplasmic but on treatment of hIRF-3-GFP-expressing cells with poly(I)•poly(C), to induce IFN production, a large percentage of the cells exhibited nuclear staining of hIRF-3-GFP (Fig. 8C). In cells treated with poly(I)•poly(C) we again observed some intensely staining cytoplasmic granules. Expression of the SV5 V protein showed some cytoplasmic localization but also a strong nuclear staining as observed previously (Randall and Bermingham, 1996), whereas expression of the SV5 P protein (which shares 164 residues in common with V protein before the V protein C-terminal unique domain) exhibited a predominantly cytoplasmic staining pattern (Fig. 8C). When hIRF-3-GFP and V protein were coexpressed and the cells treated with poly(I)•poly(C), V protein showed a predominantly nuclear localization and hIRF-3-GFP exhibited a predominantly cytoplasmic localization (Fig. 8D). In contrast, when hIRF-3-GFP and P protein were coexpressed, hIRF-3-GFP showed a predominantly nuclear localization in the majority of the cells. Expression of the P protein was used as the control for expression of the V protein in this experiment because VΔC protein is unstable and could not be detected by fluorescent staining.

rSV5VΔC is less pathogenic in *STAT1*$^{-/-}$ mice than wt SV5

Recently we established a small animal model system for studying SV5 pathogenesis using 4- to 6-week-old BALB/c mutant mice homozygous for a targeted disruption of *STAT1* (He *et al.,* 2001). To examine for the replication of rSV5VΔC in mouse lungs, wt mice (BALB/c) and *STAT1*$^{-/-}$ mice were inoculated intranasally with 10^6 PFU

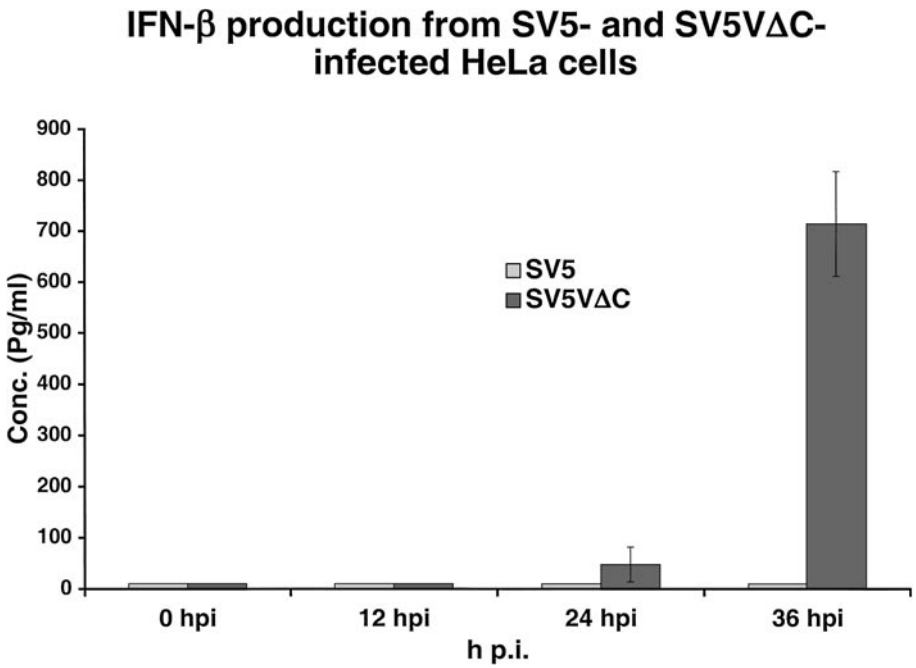


FIG. 7. IFN production in rSV5- and rSV5VΔV-infected cells. HeLa cells were infected with rSV5 or rSV5VΔV and media samples were taken at various times p.i. The amount of IFN-β was measured using an ELISA. Samples are triplicates. Values shown are average ± SEM.

rSV5 or rSV5VΔC (five mice per group). After 4 days the mice were sacrificed, the lungs were homogenized, and virus titers determined by plaque assay. As shown in the insert to Fig. 9, rSV5 and rSV5VΔC grew to equivalent titers in lungs of wt BALB/c mice (0.68×10^4 and 0.66×10^4 PFU/ml, respectively). This finding was not unexpected as it has been shown previously that rSV5 grows poorly in mice, likely because the V protein does not cause degradation of mSTAT1 protein in murine cells (Chatziandreou *et al.,* 2002; Didcock *et al.,* 1999a; Parisien *et al.,* 2002a; Young *et al.,* 2001). In *STAT1*$^{-/-}$ mice, rSV5 grew to a titer of 0.44×10^7 PFU/g of lung, whereas rSV5VΔC only grew to a titer of 0.14×10^6 PFU/g of lung tissue. The higher titer of rSV5 than rSV5VΔC in mice that lack a STAT1 signaling pathway suggests that the unique C-terminus of the V protein has another function in the virus lifecycle in addition to mediating the degradation of STAT1 protein. Examination of the body weight and survival of mice following inoculation with 10^6 PFU rSV5 (11 mice per group), rSV5VΔC (10 mice per group), or mock-infected mice (8 mice per group) showed that whereas mice infected with rSV5 lost weight and died by Day 10, mice infected with rSV5VΔC lost some weight from Day 4 to 10, typical with mild virus infection, but after Day 10 they regained their weight and gained weight slightly better than mock-infected mice for the next 10 days. These data suggest that rSV5VΔC is less pathogenic *in vivo* than wt rSV5, even though it causes vastly greater CPE in mouse and human cells, a finding consistent with the notion of virus clearance by apoptosis in infected cells in an infected animal.

DISCUSSION

The V/P/(C) genes of the Paramyxoviruses encode proteins that provide a rich diversity of distinct functions that affect both virus RNA transcription/replication and response of the host cell to viral infection. The V protein of the Rubulaviruses is encoded directly by the virus genome, whereas the V protein of the Respiro-, Morbilli-, and Henipaviruses results from the RNA editing process (reviewed in Lamb and Kolakofsky, 2001). However, whereas the V proteins of Rubulaviruses are small proteins and only contain ~230 amino acid residues, the V proteins of Respiro-, Morbilli-, and Henipaviruses are larger proteins and contain ~300–470 amino acid residues. Furthermore, the Rubulavirus V/P gene lacks the open reading frame encoding the C protein(s) found in Respiro-, Morbilli-, and Henipaviruses. There is a short stretch of relatedness between a region of Respirovirus C proteins and a region of Rubulavirus V proteins (Lamb and Kolakofsky, 2001), and although the significance of this homology is currently unknown, it is interesting to note that the C protein of Sendai virus leads to inactivation of STAT1 (Garcin *et al.,* 1999, 2000; Takeuchi *et al.,* 2001), whereas the Rubulavirus V proteins target STAT1 or STAT2 for proteasomal degradation (Andrejeva *et al.,* 2002; Didcock *et al.,* 1999b; Kubota *et al.,* 2001; Parisien *et al.,* 2001, 2002a,b). Thus, it has been suggested that the V protein N-terminal domain that is shared with the P protein has functional differences between the Rubulaviruses and the Respiro-, Morbilli-, and Henipaviruses (Lamb and Kolakofsky, 2001). Perhaps fitting with this conjecture is the fact that there is no report of recovery

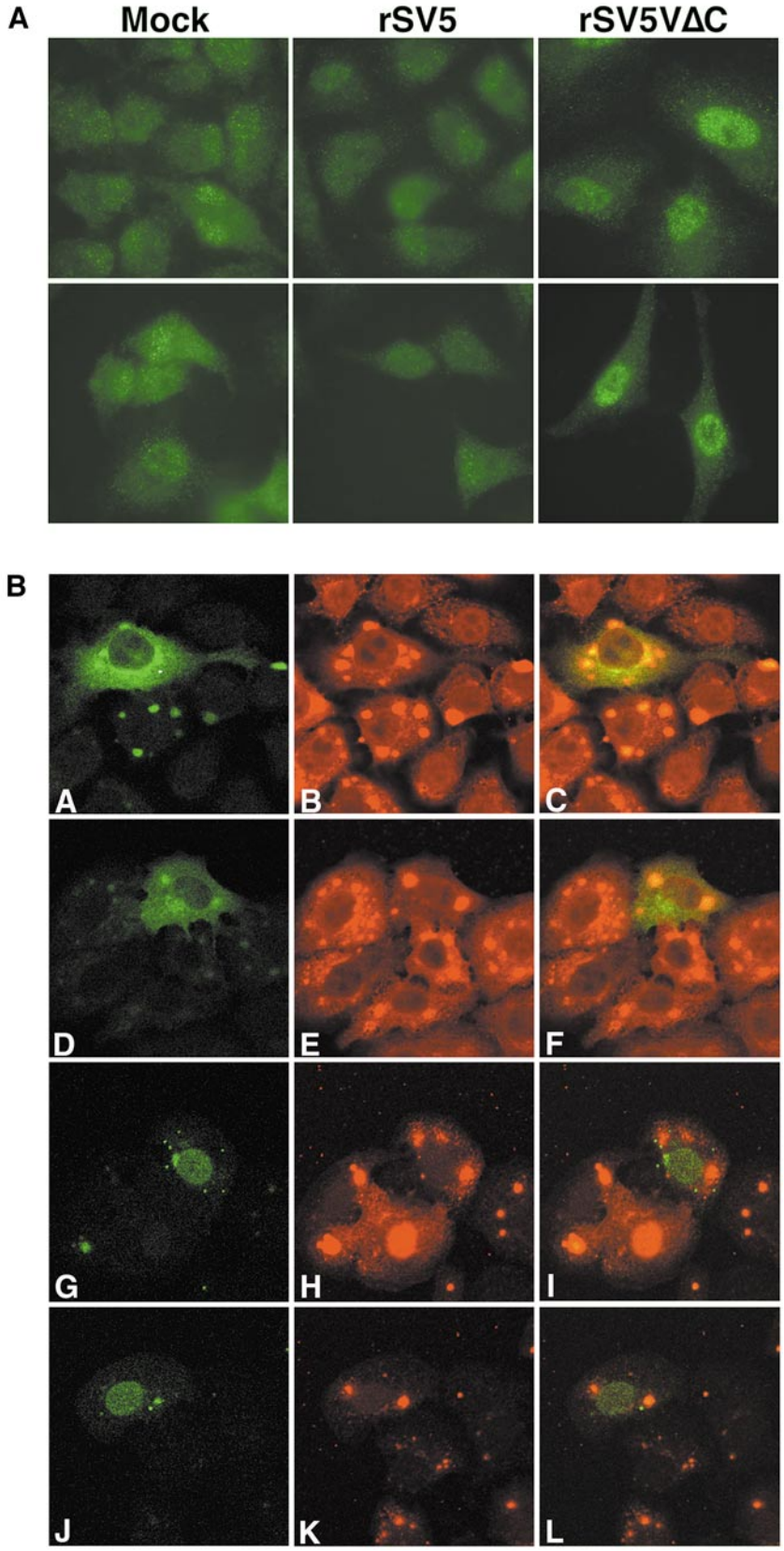
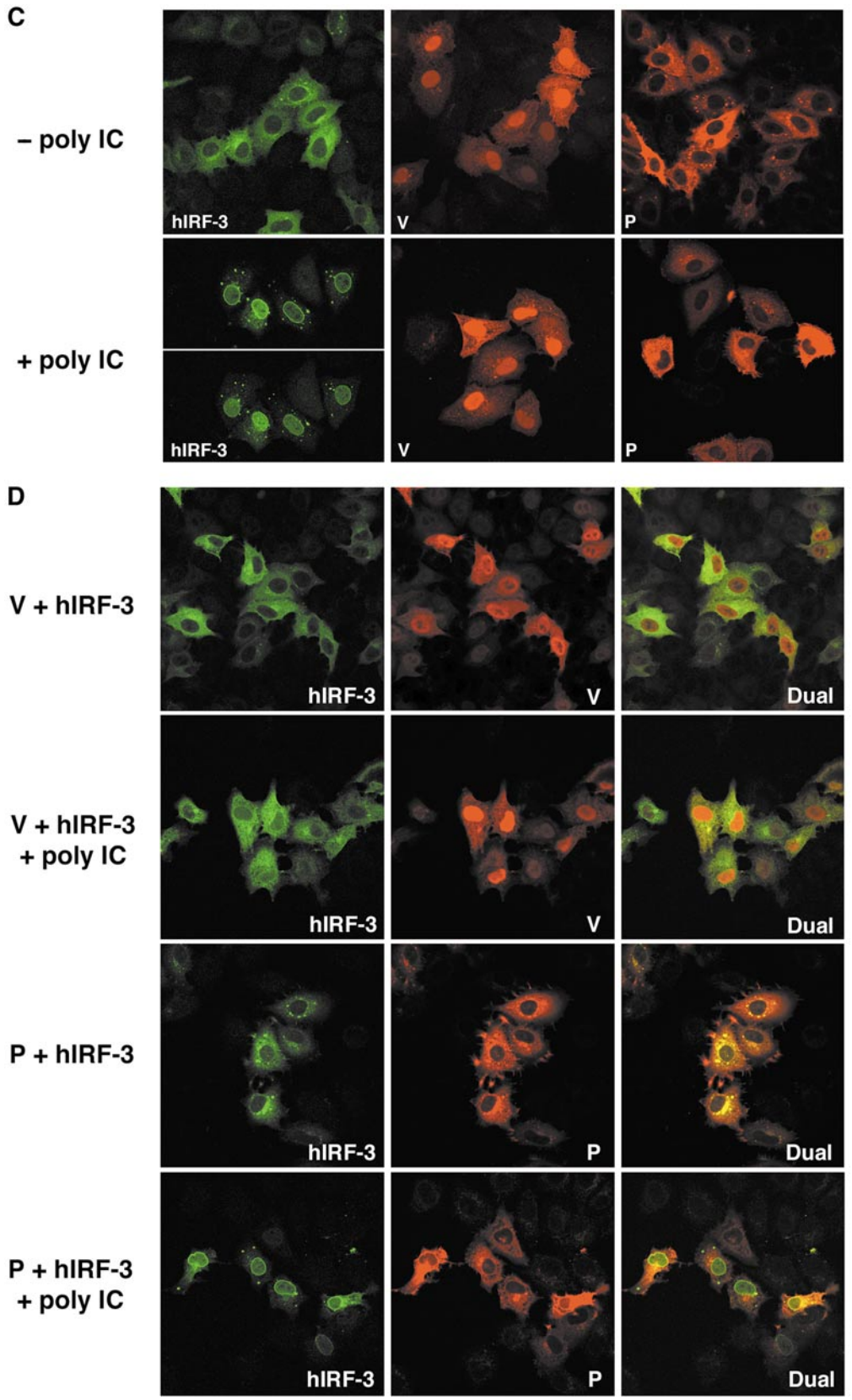


FIG. 8. Intracellular localization of hIRF-3 in cells treated with poly(IC). (A) Intracellular localization of hIRF-3 in SV5- and rSV5VΔC-infected cells. At 40 h p.i. HeLa cells were stained with mAb SL-12 specific for hIRF-3 and goat anti-mouse secondary antibody and fluorescence examined with a Nikon FXA fluorescent microscope. (B) HeLa-CD4 LTR-β-gal cells were infected with rSV5 or rSV5VΔC and transfected with phIRF-3-GFP as described under Materials and Methods. (A–F) rSV5-infected cells. (G–L) rSV5VΔC-infected cells. (A, D, G, J) hIRF-GFP fluorescence. (B, E, H, K) mAb Pk staining for P and V followed by Alexa Fluor 594 conjugated goat anti-mouse antibody (red). (C, F, I, L) Dual fluorescence. The panels in each row



show the same field of cells. (C) HeLa CD4-LTR-β-gal cells were transfected with plasmids expressing hIRF-3-GFP, V, or P and prepared for confocal microscopy as described under Materials and Methods. Cells expressing hIRF-3-GFP, V, or P individually. (+) Poly(IC) indicates cells treated with poly(I)•poly(C) (25 μg/ml 4–24 h p.t.). For hIRF-3 + poly(IC) two views of the same field of cells are shown at two focal planes to show different cross sections through the nuclei. (D) Poly(IC)-treated cells coexpressing hIRF-3-GFP and V or P. In C and D hIRF-3 was detected by the fluorescent signal of GFP (green) and P and V were detected by staining with mAb Pk followed by Alexa Fluor 594 conjugated goat anti-mouse antibody (red). Fluorescence in B, C, and D was examined using a Zeiss SLM410 confocal microscope.

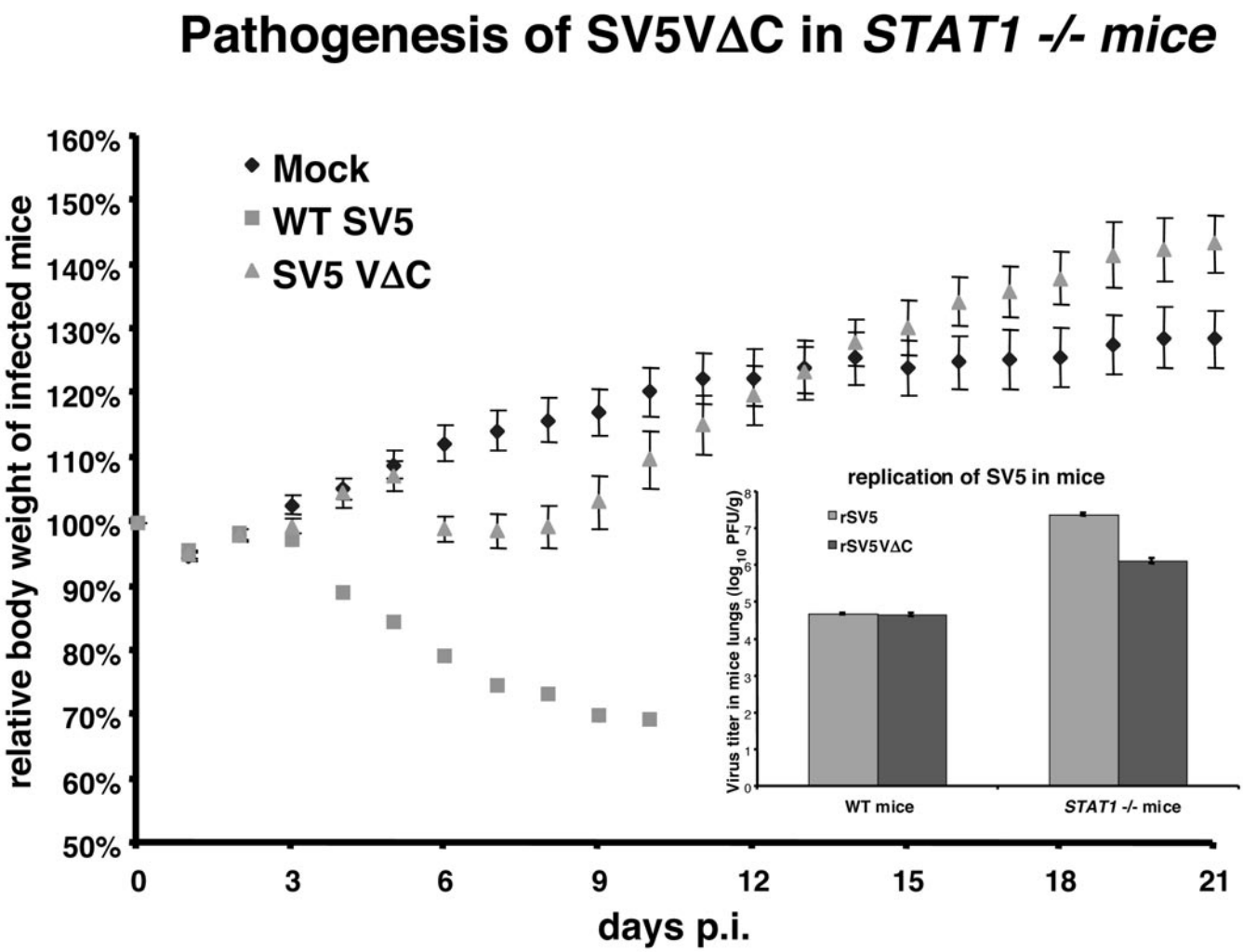


FIG. 9. Growth of rSV5VΔC in *STAT1*$^{-/-}$ mice. Six-week-old BALB/c *STAT1*$^{-/-}$ mice were inoculated intranasally with mock or 10^6 PFU of rSV5 or rSV5VΔC as described under Materials and Methods. Weights of the mice were monitored and graphed as a percentage of body weight of the mice (*y*-axis) at the date of inoculation. Note that error bars for wt SV5 are smaller than the size of the graph point. (Insert) Titer of rSV5 and rSV5VΔC in the lungs of wt BALB/c mice and BALB/c *STAT1*$^{-/-}$ mice.

of a V-minus Rubulavirus mutant, whereas infectious Sendai virus, measles virus, and rinderpest virus that are V-minus (caused by inactivation of RNA editing site) are viable viruses in tissue culture (Baron and Barrett, 2000; Delenda *et al.,* 1998; Kato *et al.,* 1997a; Schneider *et al.,* 1997). Nonetheless, although these latter V-minus viruses may be viable in tissue culture, in animal model systems both V-minus Sendai virus and V-minus measles virus are attenuated (Kato *et al.,* 1997a; Mrkic *et al.,* 2000; Patterson *et al.,* 2000; Tober *et al.,* 1998; Valsamakis *et al.,* 1998), a finding that suggests a luxury function of the V proteins of these viruses in interacting with the host organism. Recovery of hPIV2 that lacks the conserved C-terminal domain of the V protein has been reported (Kawano *et al.,* 2001). This hPIV2VΔC virus grew in Vero cells (IFN nonproducer cells) but would not grow in CV-1 or FL cells unless anti-IFN-β antibody was added, suggesting that the hPIV2 V protein C-terminal specific domain is related to IFN resistance (Kawano *et al.,* 2001). It is also known for SV5 and mumps virus, from *in vitro* studies, that the V protein C-terminal-specific domain is required for these proteins to mediate STAT1 degradation and thus block STAT1 signaling (Kawano *et al.,* 2001; J. Andrejeva, *et al.,* 2002a).

The recovery of rSV5VΔC from Vero cells, the formation by rSV5VΔC of large plaques in Vero cells, and the growth of rSV5VΔC in Vero cells at a similar rate to rSV5, but the formation only of small plaques by rSV5VΔC in BHK or CV-1 cells and the poor growth of rSV5VΔC in these latter cell types, is a finding consistent with rSV5VΔC failing to interdict interferon production and/or signaling. However, the observation that it did not prove possible to recover a recombinant SV5 containing a "V gene knock-out," together with the observation that rSV5VΔC reverted to pseudo-wt virus on multiple occasions even when rSV5VΔC was grown in Vero cells, suggests that either SV5 is imperfect at ablating the production of and/or response to IFN, or there is another

essential role of the V protein and its C-terminal domain in virus replication, e.g., the ability of the V protein to slow progression of the cell cycle (Lin and Lamb, 2000) or to affect RNA transcription/replication. Whereas SV5 is well known for causing minimal CPE in many cell types (Choppin, 1964), rSV5VΔC caused extensive CPE in many cells types and rSV5VΔC-infected HeLa cells were apoptotic (data not shown). However, because of the multiple functions of the V protein in the virus lifecycle, it is not clear at the present time if the observed CPE can be attributed to IFN production or signaling. As an *in vivo* model system for SV5 infection, 4- to 6-week-old BALB/c mutant mice homozygous for a targeted disruption of *STAT1* were used (He *et al.,* 2001) and it was found that rSV5VΔC was less pathogenic than wt rSV5, even though it causes vastly greater CPE in mouse and human cells. However, this finding is consistent with the notion of clearance of apoptotic cells in a host species.

Infection of HeLa cells with rSV5VΔC did not result in a loss of STAT1 protein, as occurs in wt SV5-infected cells, and there was not a loss of IFN signaling, as occurs in wt SV5-infected cells. As the extent of IFN signaling and ISGF3 complex formation in rSV5VΔC-infected cells was greater than that found in mock-infected cells, it suggested this stimulation might be due to the production of IFN. Related to this is an observation made by Purnell W. Choppin over 30 years ago. He found that wt SV5 infection of MDCK and HeLa cells resulted in very low to undetectable levels of production of type I IFN and that the SV5-infected cells did not respond to addition of exogenous IFN. He speculated that SV5 had evolved mechanisms to block IFN production and action (unpublished observations cited in Choppin and Compans, 1975). As shown in this and in the accompanying article (Poole *et al.,* 2002), such an effect may indeed be mediated by the V protein, specifically involving its C-terminus.

The transcription factor IRF-3 is important for transcription of the IFN-β gene on virus infection or exposure to dsRNA. IRF-3 is constitutively expressed and cytoplasmically localized and on stimulation is phosphorylated by an unidentified protein kinase and this phosphorylation results in the subcellular relocalization of IRF-3 from the cytoplasm to the nucleus, triggering induction of host defense genes (reviewed in Reich, 2002). As shown in the accompanying article infection of cells with wt SV5, but not with rSV5VΔC, diminishes dsRNA signaling using an IFN-β promoter reporter assay (Poole *et al.,* 2002). We show in this article that in SV5-infected cells IRF-3 is largely cytoplasmically localized, whereas in rSV5VΔC-infected cells IRF-3 is largely localized to the nucleus. Our expression experiments indicate that the V protein mediates the cytoplasmic localization of IRF-3 upon dsRNA treatment and the V protein C-terminal domain is required for this process. These findings correlate with the finding that mutations in the V protein C-terminus greatly reduce IFN-β induction (Poole *et al.,* 2002). The mechanism by which expression of the V protein mediates its effect in blocking translocation of IRF-3 into the nucleus remains to be determined. It is possible that the V protein interacts directly or indirectly with IRF-3 such that the activating phosphorylation of IRF-3 is prevented or that IRF-3 is in a complex such that it is physically retained in the cytoplasm. However, and perhaps more likely, the mechanism may affect a factor upstream in the pathway to IRF-3 activation as NF-κB, which is also required for IFN-β transcription and which also requires activation by phosphorylation, is activated in rSV5VΔC-infected cells but is not activated in rSV5(W3)-infected cells (see also accompanying article, Poole *et al.,* 2002). Interestingly, in the deletion mapping and mutagenesis experiments deletion of only the C-terminal domain of the V protein or mutation of its conserved cysteine residues affected dsRNA signaling using an IFN-β promoter reporter assay (see also accompanying article, Poole *et al.,* 2002), whereas deletions in the V protein C-terminus and in a V protein N-terminal region affected STAT1 signaling (Young *et al.,* 2001; Andrejeva *et al.,* 2002a), suggesting that the two processes are mediated by distinct processes both of which require the V protein C-terminal cysteine-rich domain.

The effect of SV5 V protein expression on the cytoplasmic localization of IRF-3 is consistent with the means by which other viruses inhibit the expression of host defense genes such as IFN-β. Human papilloma virus 16 (HPIV16) E6 protein binds to IRF-3 leading to a block in IRF-3 transcriptional activity (Ronco *et al.,* 1998). Influenza A virus NS1 protein binds to dsRNA, preventing activation of the dsRNA-activated protein kinase, PKR (Lu *et al.,* 1995). In addition the influenza virus NS1 protein inhibits the activation of IRF-3, which then fails to enter the nucleus (Talon *et al.,* 2000). Presumably these effects of the NS1 protein are not 100% efficient as IFN was initially discovered in experiments involving heat inactivated influenza virus (Issacs and Lindenmann, 1957). The influenza virus NS1 protein also inhibits host-cell mRNA polyadenylation and nonpolyadenylated mRNAs are not exported from the nucleus (Chen *et al.,* 1999). Perhaps influenza virus evolved this function of the NS1 protein to block the antiviral effect of IFN-induced proteins. For the Paramyxoviruses, in addition to SV5 it has been found that measles virus (Naniche *et al.,* 2000) and now Sendai virus and hPIV2 (see also accompanying article, Poole *et al.,* 2002) interfere with interferon production and although the mechanism by which this occurs has not been elucidated for Sendai virus and hPIV2, again the V protein is involved in the process (see also accompanying article, Poole *et al.,* 2002).

The ability of the SV5 V protein to block two distinct pathways of the innate immune response, IFN-β production and IFN signaling, is likely to be important in the pathogenicity and host range of the virus. This raises the question: how can SV5 V protein accommodate the multiple protein–protein interactions presumably required

for its multifunctionality? The conserved sequence of the V protein C-terminal domain binds two atoms of zinc (Paterson *et al.,* 1995), and although it is analogous to, it is different from, classic zinc fingers or RING fingers (Borden, 2000; Klug and Schwabe, 1995). Nonetheless the V protein C-terminal domain is likely to have some defined structure. However, it has been found recently that the shared N-terminal domain of the measles P and V proteins is natively unfolded (Karlin *et al.,* 2002), a property common to the acidic activation domains of transcription factors that also have to form multiple protein–protein interactions (Wright and Dyson, 1999). By using algorithms (Romero *et al.,* 2001) that predict unstructured regions of proteins, 54 residues (67–120) of the N-terminal region of SV5 V protein are predicted with high confidence to be intrinsically unstructured (K. Ratcliff and R. A. Lamb, unpublished results). Further biochemical work will be needed to support this hypothesis but a natively unfolded domain may provide the means of forming multiple protein–protein interactions.

MATERIALS AND METHODS

Viruses and cells

HeLa T4, CV-1, Vero, 2FTGH, MDCK, and BSR T7/5 (Buchholz *et al.,* 1999) cells were maintained in DMEM with 10% fetal calf serum (FCS). BHK 21-F cells were maintained in DMEM with 10% tryptose phosphate broth and 10% FCS. HeLa CD4-LTR-β-gal cells were grown and maintained in DMEM supplemented with 10% fetal bovine serum, 200 μg/ml G418 (Genetecin, Invitrogen Inc., San Diego, CA) and 100 μg/ml hygromycin B (Invitrogen). These cells were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH (HeLa-CD4-LTR-β-gal from Dr. Michael Emerman). All cells used were determined to be free of mycoplasma contamination by using a PCR-based assay and specific DNA primers (Boehringer Mannheim, Indianapolis, IN). For virus infection, cell monolayers were washed with phosphate-buffered saline (PBS) and then infected with viruses in DMEM-1% BSA at a multiplicity of infection (m.o.i.) of 0.1 to 10 PFU/cell for 1 to 2 h at 37°C. The monolayers were then washed and incubated with DMEM containing 2% fetal calf serum (FCS) at 37°C.

Recovery of mutant rSV5 by reverse genetics

rSV5VΔC was generated by using a reverse genetics system from an infectious clone of SV5 (plasmid pBH276) (He *et al.,* 1997) in which two in-frame tandem stop codons were made downstream of the RNA editing site of the V gene (plasmid pBH378) without affecting codons of the P mRNA (see Fig. 1). BSR T7/5 cells in 3.5-cm-diameter dishes (~60–90% confluent) were transfected with the genomic cDNA clone for wt SV5 or SV5VΔC together with the support plasmids pCAGGS-NP, pCAGGS-L, and pCAGGS-P using Lipofectamine-PLUS reagents following the manufacturer's recommendations (Invitrogen). Plasmid amounts were as follows: 1.0 μg SV5 genome plasmid, 100 ng pCAGGS-NP, 20 ng pCAGGS-P, and 500 ng pCAGGS-L. Cells were incubated with the transfection mix for 12–16 h at 37°C. Cells were then washed once with PBS and the medium was replaced with DMEM supplemented with 2% fetal bovine serum. Two to four days posttransfection (p.t.) the media were assayed for the presence of recombinant SV5 (rSV5) by monitoring syncytium formation on BHK-21F cells. The supernatant fluid from positive dishes was then used for plaque purification on Vero cells (Paterson and Lamb, 1993). Virus stocks of rSV5VΔC were grown in Vero cells.

Single-step growth rate

Monolayers of Vero cells in 35 mm plates were washed with PBS and then infected with rSV5 or rSV5VΔC in DMEM–1% BSA at an m.o.i. of 10 PFU/cell for 1 to 2 h at 37°C. The cells were then washed with PBS and maintained in DMEM–2% FCS. Medium was collected at 0, 1, 2, 3, 4 and 5 days p.i. for quantification of virus by plaque assay on BHK 21F cells.

Reverse transcriptase–PCR amplification and sequencing

Total RNAs from rSV5VΔC-infected Vero cells (6-cm-diameter plates) were purified using an RNeasy kit (Qiagen, Chatsworth, CA), according to the manufacturer's protocol. Total RNAs were dissolved in 50 μl H_2O and 19 μl was used in a reverse transcriptase (RT) reaction using appropriate oligonucleotide primers that annealed to the vRNA (−) strand. An aliquot of the cDNA was then amplified in a PCR reaction using appropriate oligonucleotide primer pairs. PCR was carried out at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min for 40 cycles. The PCR products were electrophoresed on a 1% agarose gel and bands were cut out and DNA was purified using a Qiaex II kit (Qiagen). The purified DNA was then amplified by PCR using fluorescent dye-labeled chain-terminating nucleotides and the nucleotide sequence was obtained using the ABI 310 sequencer and ABI Prism software.

Immunoprecipitation of polypeptides

Vero and HeLa T4 cells were infected with wt rSV5 or rSV5VΔC, and at 1 to 2 days p.i. were labeled for 2 h with ^{35}S-Promix label (100 μCi/ml) and the cells lysed in RIPA buffer and aliquots immunoprecipitated using monoclonal antibody HN4b (Ng *et al.,* 1989; Randall *et al.,* 1987), anti-F2 synthetic peptide rabbit sera (Dutch *et al.,* 2001), P-specific mAb 161 (Randall *et al.,* 1987), or anti-V synthetic peptide rabbit sera or V-specific mAb 31C6 (Paterson *et al.,* 1995). Polypeptides were analyzed by SDS–PAGE using 15 or 10% polyacrylamide gels (Paterson and Lamb, 1993) and radioactivity was analyzed using a Fuji

BioImager 1000 and MacBas software (Fuji Medical System, Stamford, CT).

IFN signaling and electrophoretic mobility shift assays

Details of the IFN signaling assay and electrophoretic mobility shift assays have been reported elsewhere (Didcock, 1999b). Briefly, in EMSA nuclear extracts were prepared from mock-infected or SV5-infected cells that had or had not been treated with rHuIFN-αA/D for 1 h prior to harvesting at 8 or 16 h p.i. Protein–DNA complexes were formed by incubating 10 μg protein for 15 min at 30°C with 1 ng probe (labeled with α-^{32}P by filling in the GATC 5′ overhangs with Klenow enzyme on the otherwise double-stranded oligonucleotides 5′ AGGAAATAGAAACTG 3′) in a 20-μl reaction mixture containing 20 mM Tris (pH 8.0), 12% glycerol, 2 mM $MgCl_2$, 0.6 mM dithiothreitol, and 375 ng poly(dI-dC). Complexes were resolved on 6% native polyacrylamide (1:30 bisacrylamide/acrylamide) gels in 0.5× Tris–borate–EDTA, and the dried gels were visualized by autoradiography.

In the IFN signaling assays, Vero cells were transfected with 1 μg DNA and 1.5 μl Fugene 6 (Roche) according to the manufacturer's instructions. After 24 h, the cells were infected with SV5 W3 or SV5VΔC at an m.o.i. of 5 and induced with 1000 U of rHuIFN-αA/D per milliliter at 6 or 10 h p.i. Cells were lysed 4 h after induction by IFN. Lysates were prepared and assayed for luciferase and β-galactosidase activity as described previously (King and Goodbourn, 1994). The relative expression levels were calculated by dividing the luciferase values by the β-galactosidase values.

Immunoblotting.

HeLa T4 cells in 6-cm plates were infected with rSV5VΔC or rSV5. Cells were lysed at 16 and 36 h p.i. in 0.5 ml of protein lysis buffer (2% SDS, 62.5 mM Tris–HCl, pH 6.8, 2% dithiothreitol) and lysates were sonicated briefly to shear DNA. Lysate (80 μl) was subjected to SDS–PAGE using a 10% gel (Paterson *et al.,* 1984). Polypeptides were transferred to Immobilon-P membrane (Millipore Corp., Bedford, MA) using a wet-gel transfer apparatus (Bio-Rad, Hercules, CA). The membrane was first incubated with primary antibodies against STAT1 (a mixture of A-2, C-136, and E-23; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and then a mixture of anti-mouse and anti-rabbit secondary antibodies conjugated to horseradish peroxidase. The proteins on the membrane were detected using the ECL+ kit (Amersham Pharmacia, Piscataway, NJ) and chemiluminescence was detected using a Storm System phosphorImager (Molecular Dynamics Inc., Sunnyvale, CA).

Fluorescent microscopy on rSV5- and rSV5VΔC-infected cells

CV-1 cells were grown on glass coverslips and infected with rSV5 or rSV5VΔC. At 24 h p.i. cells were washed with PBS and then fixed in 1% formaldehyde for 15 min at room temperature. In all further steps, 0.1% saponin was included in the PBS to permeabilize cells. The cells were washed with PBS/saponin solution and incubated for 30 min in a 1:500 dilution of rabbit anti-HN and mAb 31C6 specific for the C-terminus of the V protein. Cells were washed extensively in PBS/saponin and the appropriate secondary antibody [FITC-labeled goat anti-mouse IgG or Texas red labeled goat anti-rabbit IgG (Jackson Laboratory, Bar Harbor, ME)] was added to the cells for 30 min, and the cells were washed in PBS/saponin. Fluorescent staining was visualized using a Zeiss LSM 410 confocal microscope (Zeiss, Inc., Thornwood, NY).

Intracellular localization of hIRF-3

Endogenous hIRF-3 was examined by immunofluorescent staining of HeLa cells and mAb SL-12 (Ronco *et al.,* 1998). For hIRF-3-GFP localization, HeLa-CD4-LTR-β-gal cells seeded on coverslips in six-well dishes were transfected with 800 ng DNA consisting of 400 ng pCAGGS/P, 400 ng phIRF-3-GFP (Kumar *et al.,* 2000), or 40 ng pCAGGS/V either alone or in combination with the balance of the DNA made up of pCAGGS. Transfections were carried out using Lipofectamine-Plus (Invitrogen) according to the manufacturer's instructions. At 4 h p.t. poly(I)•poly(C) [poly(IC)] (Amersham Pharmacia Biotech Inc.) was added to a final concentration of 25 μg/ml and cells were examined by confocal microscopy at 24 h p.t. For virus-infection/transfection experiments HeLa-CD4-LTR-β-gal cells prepared as described for cotransfections were infected with either rSV5 virus or rSV5VΔC virus or were mock infected. At 1.5 h p.i. the cells were washed with PBS and transfected with 1 μg phIRF-3-GFP per well using ExGen 500 (Fermentas, Hanover, MD) according to the manufacturer's instructions. Infected/transfected cells were examined by confocal microscopy 24 h p.t. For fluorescence microscopy cells were fixed and permeabilized by incubating in 2.5% formaldehyde, 0.5% Triton X-100 for 20 min at room temperature and subsequently incubated with mAb Pk (specific for the SV5 P and V proteins) (Randall *et al.,* 1987) followed by Alexa Fluor 594 conjugated goat anti-mouse IgG (H+L) secondary antibody (Molecular Probes, Eugene, OR). hIRF-3-GFP was detected by GFP fluorescence. Fluorescence was visualized using either a Nikon FXA microscope (Fig. 8A) or a Zeiss LSM 410 confocal microscope.

Measurement IFN-β in media of virus-infected cells

HeLa cells were infected and media were collected at different time points postinfection. The amount of IFN-β

was measured using a human IFN-β detection kit purchased from PBL Biomedical Laboratories (Piscataway, NJ) according to the manufacturer's instruction. The amount of IFN-β was calculated using standard curves generated from known concentrations of IFN-β provided by the manufacturer.

Animal experiments

Six-week-old BALB/c mice or BALB/c *STAT1*$^{-/-}$ were first anesthetized and then inoculated intranasally with 50 μl PBS containing 10^6 PFU rSV5 or rSV5VΔC viruses. Body weights of the mice were recorded daily thereafter. Titers of viruses in mouse lungs were determined as described previously (He *et al.,* 2001).

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REFERENCES

Andrejeva, J., Poole, E., Young, D. F., Goodbourn, S., and Randall, R. E. (2002a). The p127 subunit (DDB1) of the UV-DNA damage repair binding protein is essential for the targeted degradation of STAT1 by the V protein of the paramyxovirus simian virus 5. *J. Virol.* **76,**(22) (in press).

Andrejeva, J., Young, D. F., Goodbourn, S., and Randall, R. E. (2002b). Degradation of STAT1 and STAT2 by the V proteins of simian virus 5 and human parainfluenza virus type 2, respectively: Consequences for virus replication in the presence of alpha/beta and gamma interferons. *J. Virol.* **76,** 2159–2167.

Baron, M. D., and Barrett, T. (2000). Rinderpest viruses lacking the C and V proteins show specific defects in growth and transcription of viral RNAs. *J. Virol.* **74,** 2603–2611.

Biron, C. A., and Sen, G. C. (2001). Interferons and other cytokines. *In* "Fields Virology" (B. Fields, D. Knipe, and P. Howley, Eds.), 4th ed., pp. 321–349. Lippincott, Williams, and Wilkins, Philadelphia.

Borden, K. L. (2000). RING domains: Master builders of molecular scaffolds. *J. Mol. Biol.* **295,** 1103–1112.

Buchholz, U. J., Finke, S., and Conzelmann, K. K. (1999). Generation of bovine respiratory syncytial virus (BRSV) from cDNA: BRSV NS2 is not essential for virus replication in tissue culture, and the human RSV leader region acts as a functional BRSV genome promoter. *J. Virol.* **73,** 251–259.

Calain, P., and Roux, L. (1993). The rule of six, a basic feature for efficient replication of Sendai virus defective interfering RNA. *J. Virol.* **67,** 4822–4830.

Chatziandreou, N., Young, D., Andrejeva, J., Goodbourn, S., and Randall, R. E. (2002). Differences in interferon sensitivity, and biological properties of two related isolates of simian virus 5: A model for virus persistence. *Virology* **293,** 234–242.

Chen, Z., Li, Y., and Krug, R. M. (1999). Influenza A virus NS1 protein targets poly(A)-binding protein II of the cellular 3′ end processing machinery. *EMBO J.* **18,** 2273–2283.

Choppin, P. W. (1964). Multiplication of a myxovirus (SV5) with minimal cytopathic effects and without interference. *Virology* **23,** 224–233.

Choppin, P. W., and Compans, R. W. (1975). Reproduction of paramyxoviruses. *In* "Comprehensive Virology" (H. Fraenkel-Conrat, and R. R. Wagner, Eds.), Vol. 4, pp. 95–178. Plenum Press, New York.

Curran, J., Boeck, R., and Kolakofsky, D. (1991). The Sendai virus P gene expresses both an essential protein and an inhibitor of RNA synthesis by shuffling modules via mRNA editing. *EMBO J.* **10,** 3079–3085.

Darnell, J. E., Jr. (1997). STATs and gene regulation. *Science* **277,** 1630–1635.

Delenda, C., Hausmann, S., Garcin, D., and Kolakofsky, D. (1997). Normal cellular replication of Sendai virus without the *trans*-frame, nonstructural V protein. *Virology* **228,** 55–62.

Delenda, C., Taylor, G., Hausmann, S., Garcin, D., and Kolakofsky, D. (1998). Sendai viruses with altered P, V, and W protein expression. *Virology* **242,** 327–337.

Didcock, L., Young, D. F., Goodbourn, S., and Randall, R. E. (1999a). Sendai virus and simian virus 5 block activation of interferon-responsive genes: Importance for virus pathogenesis. *J. Virol.* **73,** 3125–3133.

Didcock, L., Young, D. F., Goodbourn, S., and Randall, R. E. (1999b). The V protein of simian virus 5 inhibits interferon signalling by targeting STAT1 for proteasome-mediated degradation. *J. Virol.* **73,** 9928–9933.

Dutch, R. E., Hagglund, R. N., Nagel, M. A., Paterson, R. G., and Lamb, R. A. (2001). Paramyxovirus fusion (F) protein: A conformational change on cleavage activation. *Virology* **281**, 138–150.

Garcin, D., Curran, J., and Kolakofsky, D. (2000). Sendai virus C proteins must interact directly with cellular components to interfere with interferon action. *J. Virol.* **74,** 8823–8830.

Garcin, D., Latorre, P., and Kolakofsky, D. (1999). Sendai virus C proteins counteract the interferon-mediated induction of an antiviral state. *J. Virol.* **73,** 6559–6565.

Goodbourn, S., Didcock, L., and Randall, R. E. (2000). Interferons: Cell signalling, immune modulation, antiviral response and virus countermeasures. *J. Gen. Virol.* **81,** 2341–2364.

He, B., Leser, G. P., Paterson, R. G., and Lamb, R. A. (1998). The paramyxovirus SV5 small hydrophobic (SH) protein is not essential for virus growth in tissue culture cells. *Virology* **250,** 30–40.

He, B., Lin, G. Y., Durbin, J. E., Durbin, R. K., and Lamb, R. A. (2001). The SH integral membrane protein of the paramyxovirus simian virus 5 is required to block apoptosis in MDBK cells. *J. Virol.* **75,** 4068–4079.

He, B., Paterson, R. G., Ward, C. D., and Lamb, R. A. (1997). Recovery of infectious SV5 from cloned DNA and expression of a foreign gene. *Virology* **237,** 249–260.

Hiebert, S. W., Paterson, R. G., and Lamb, R. A. (1985). Identification and predicted sequence of a previously unrecognized small hydrophobic protein, SH, of the paramyxovirus simian virus 5. *J. Virol.* **55,** 744–751.

Hiebert, S. W., Richardson, C. D., and Lamb, R. A. (1988). Cell surface expression and orientation in membranes of the 44-amino-acid SH protein of simian virus 5. *J. Virol.* **62,** 2347–2357.

Holmes, K. V., and Choppin, P. W. (1966). On the role of the response of the cell membrane in determining virus virulence. Contrasting effects of the parainfluenza virus SV5 in two cell types. *J. Exp. Med.* **124,** 501–520.

Huang, C., Kiyotani, K., Fujii, Y., Fukuhara, N., Kato, A., Nagai, Y., Yoshida, T., and Sakaguchi, T. (2000). Involvement of the zinc-binding capacity of Sendai virus V protein in viral pathogenesis. *J. Virol.* **74,** 7834–7841.

Issacs, A., and Lindenmann, J. (1957). Virus interference. 1. The interferon. *Proc. R. Soc. Lond. Ser. B* **147,** 258–267.

Jacques, J. P., and Kolakofsky, D. (1991). Pseudo-templated transcription in prokaryotic and eukaryotic organisms. *Genes Dev.* **5,** 707–713.

Juang, Y. T., Lowther, W., Kellum, M., Au, W. C., Lin, R., Hiscott, J., and Pitha, P. M. (1998). Primary activation of interferon A, and interferon B gene transcription by interferon regulatory factor 3. *Proc. Natl. Acad. Sci. USA* **95,** 9837–9842.

Karlin, D., Longhi, S., Receveur, V., and Canard, B. (2002). The N-terminal domain of the phosphoprotein of morbilliviruses belongs to the natively unfolded class of proteins. *Virology* **296,** 251–262.

Kato, A., Kiyotani, K., Sakai, Y., Yoshida, T., and Nagai, Y. (1997a). The paramyxovirus, Sendai virus, V protein encodes a luxury function required for viral pathogenesis. *EMBO J.* **16,** 578–587.

Kato, A., Kiyotani, K., Sakai, Y., Yoshida, T., Shioda, T., and Nagai, Y.

(1997b). Importance of the cysteine-rich carboxyl-terminal half of V protein for Sendai virus pathogenesis. *J. Virol.* **71,** 7266–7272.

Kawano, M., Kaito, M., Kozuka, Y., Komada, H., Noda, N., Nanba, K., Tsurudome, M., Ito, M., Nishio, M., and Ito, Y. (2001). Recovery of infectious human parainfluenza type 2 virus from cDNA clones and properties of the defective virus without V-specific cysteine-rich domain. *Virology* **284,** 99–112.

King, P., and Goodbourn, S. (1994). The beta-interferon promoter responds to priming through multiple independent regulatory elements. *J. Biol. Chem.* **269,** 30609–30615.

Klug, A., and Rhodes, D. (1987). Zinc fingers: A novel protein fold for nucleic acid recognition. *Cold Spring Harb. Quant. Biol.* **52,** 473–482.

Klug, A., and Schwabe, J. W. (1995). Protein motifs 5. Zinc fingers. *FASEB J.* **9,** 597–604.

Kubota, T., Yokosawa, N., Yokota, S., and Fujii, N. (2001). C terminal CYS-RICH region of mumps virus structural V protein correlates with block of interferon alpha and gamma signal transduction pathway through decrease of STAT 1-alpha. *Biochem. Biophys. Res. Commun.* **283,** 255–259.

Kumar, K. P., McBride, K. M., Weaver, B. K., Dingwall, C., and Reich, N. C. (2000). Regulated nuclear-cytoplasmic localization of interferon regulatory factor 3, a subunit of double-stranded RNA-activated factor 1. *Mol. Cell. Biol.* **20,** 4159–4168.

Lamb, R. A., and Kolakofsky, D. (2001). *Paramyxoviridae*: The viruses and their replication. *In* "Fields Virology" (D. M. Knipe, and P. M. Howley, Eds.), 4th ed., pp. 1305–1340. Lippincott, Williams and Wilkins, Philadelphia.

Lin, G. Y., and Lamb, R. A. (2000). The paramyxovirus simian virus 5 V protein slows progression of the cell cycle. *J. Virol.* **74,** 9152–9166.

Lin, G. Y., Paterson, R. G., and Lamb, R. A. (1997). The RNA binding region of the paramyxovirus SV5 V and P proteins. *Virology* **238,** 460–469.

Lin, G. Y., Paterson, R. G., Richardson, C. D., and Lamb, R. A. (1998a). The V protein of the paramyxovirus SV5 interacts with damage-specific DNA binding protein. *Virology* **249,** 189–200.

Lin, R., Heylbroeck, C., Pitha, P. M., and Hiscott, J. (1998b). Virus-dependent phosphorylation of the IRF-3 transcription factor regulates nuclear translocation, transactivation potential, and proteasome-mediated degradation. *Mol. Cell. Biol.* **18,** 2986–2996.

Liston, P., and Briedis, D. J. (1994). Measles virus V protein binds zinc. *Virology* **198,** 399–404.

Lu, Y., Wambach, M., Katze, M. G., and Krug, R. M. (1995). Binding of the influenza virus NS1 protein to double-stranded RNA inhibits the activation of the protein kinase that phosphorylates the eIF-2 translation initiation factor. *Virology* **214,** 222–228.

Mrkic, B., Odermatt, B., Klein, M. A., Billeter, M. A., Pavlovic, J., and Cattaneo, R. (2000). Lymphatic dissemination and comparative pathology of recombinant measles viruses in genetically modified mice. *J. Virol.* **74,** 1364–1372.

Naniche, D., Yeh, A., Eto, D., Manchester, M., Friedman, R. M., and Oldstone, M. B. (2000). Evasion of host defenses by measles virus: Wild-type measles virus infection interferes with induction of Alpha/Beta interferon production. *J. Virol.* **74,** 7478–7484.

Ng, D. T., Randall, R. E., and Lamb, R. A. (1989). Intracellular maturation and transport of the SV5 type II glycoprotein hemagglutinin-neuraminidase: Specific and transient association with GRP78-BiP in the endoplasmic reticulum and extensive internalization from the cell surface. *J. Cell Biol.* **109,** 3273–3289.

Nishio, M., Tsurudome, M., Ito, M., Kawano, M., Komada, H., and Ito, Y. (2001). High resistance of human parainfluenza type 2 virus protein-expressing cells to the antiviral and anti-cell proliferative activities of alpha/beta interferons: Cysteine-rich V-specific domain is required for high resistance to the interferons. *J. Virol.* **75,** 9165–9176.

Parisien, J. P., Lau, J. F., and Horvath, C. M. (2002a). STAT2 acts as a host range determinant for species-specific paramyxovirus interferon antagonism and simian virus 5 replication. *J. Virol.* **76,** 6435–6441.

Parisien, J. P., Lau, J. F., Rodriguez, J. J., Sullivan, B. M., Moscona, A., Parks, G. D., Lamb, R. A., and Horvath, C. M. (2001). The V protein of human parainfluenza virus 2 antagonizes type I interferon responses by destabilizing signal transducer and activator of transcription 2. *Virology* **283,** 230–239.

Parisien, J. P., Lau, J. F., Rodriguez, J. J., Ulane, C. M., and Horvath, C. M. (2002b). Selective STAT protein degradation induced by paramyxoviruses requires both STAT1 and STAT2 but is independent of alpha/beta interferon signal transduction. *J. Virol.* **76,** 4190–4198.

Paterson, R. G., Harris, T. J. R., and Lamb, R. A. (1984). Analysis and gene assignment of mRNAs of a paramyxovirus, simian virus 5. *Virology* **138,** 310–323.

Paterson, R. G., and Lamb, R. A. (1993). The molecular biology of influenza viruses and paramyxoviruses. *In* "Molecular Virology: A Practical Approach" (A. Davidson, and R. M. Elliott, Eds.), pp. 35–73. IRL Oxford Univ. Press, Oxford.

Paterson, R. G., Leser, G. P., Shaughnessy, M. A., and Lamb, R. A. (1995). The paramyxovirus SV5 V protein binds two atoms of zinc and is a structural component of virions. *Virology* **208,** 121–131.

Paterson, R. G., Thomas, S. M., and Lamb, R. A. (1989). Specific non-templated nucleotide addition to a simian virus 5 mRNA: Prediction of a common mechanism by which unrecognized hybrid P-cysteine-rich proteins are encoded by paramyxovirus "P" genes. *In* "Genetics and Pathogenicity of Negative Strand Viruses" (D. Kolakofsky, and B. W. J. Mahy, Eds.), pp. 232–245. Elsevier, London.

Patterson, J. B., Thomas, D., Lewicki, H., Billeter, M. A., and Oldstone, M. B. (2000). V and C proteins of measles virus function as virulence factors in vivo. *Virology* **267,** 80–89.

Peluso, R. W., Lamb, R. A., and Choppin, P. W. (1977). Polypeptide synthesis in simian virus 5-infected cells. *J. Virol.* **23,** 177–187.

Poole, E. L., He, B., Lamb, R. A., Randall, R. E., and Goodbourne, S. (2002). The V proteins of simian virus 5 and other paramyxoviruses inhibit induction of interferon-beta. *Virology* **303,** 33–46.

Randall, R. E., and Bermingham, A. (1996). NP:P and NP:V interactions of the paramyxovirus simian virus 5 examined using a novel protein: Protein capture assay. *Virology* **224,** 121–129.

Randall, R. E., Young, D. F., Goswami, K. K. A., and Russell, W. C. (1987). Isolation and characterization of monoclonal antibodies to simian virus 5 and their use in revealing antigenic differences between human, canine and simian isolates. *J. Gen. Virol.* **68,** 2769–2780.

Reich, N. C. (2002). Review: Nuclear/cytoplasmic localization of IRFs in response to viral infection or interferon stimulation. *J. Interferon Cytokine Res.* **22,** 103–109.

Romero, P., Obradovic, Z., Li, X., Garner, E. C., Brown, C. J., and Dunker, A. K. (2001). Sequence complexity of disordered proteins. *Proteins* **42,** 38–48.

Ronco, L. V., Karpova, A. Y., Vidal, M., and Howley, P. M. (1998). Human papillomavirus 16 E6 oncoprotein binds to interferon regulatory factor-3 and inhibits its transcriptional activity. *Genes Dev.* **12,** 2061–2072.

Sato, M., Tanaka, N., Hata, N., Oda, E., and Taniguchi, T. (1998). Involvement of the IRF family transcription factor IRF-3 in virus-induced activation of the IFN-beta gene. *FEBS Lett.* **425,** 112–116.

Schafer, S. L., Lin, R., Moore, P. A., Hiscott, J., and Pitha, P. M. (1998). Regulation of type I interferon gene expression by interferon regulatory factor-3. *J. Biol. Chem.* **273,** 2714–2720.

Schneider, H., Kaelin, K., and Billeter, M. A. (1997). Recombinant measles viruses defective for RNA editing and V protein synthesis are viable in cultured cells. *Virology* **227,** 314–322.

Shiyanov, P., Nag, A., and Raychaudhuri, P. (1999). Cullin 4A associates with the UV-damaged DNA-binding protein DDB. *J. Biol. Chem.* **274,** 35309–35312.

Stark, G. R., Kerr, I. M., Williams, B. R., Silverman, R. H., and Schreiber, R. D. (1998). How cells respond to interferons. *Annu. Rev. Biochem.* **67,** 227–264.

Steward, M., Samson, A. C. R., Errington, W., and Emmerson, P. T. (1995). The Newcastle disease virus V protein binds zinc. *Arch. Virol.* **140,** 1321–1328.

Takeuchi, K., Komatsu, T., Yokoo, J., Kato, A., Shioda, T., Nagai, Y., and

Gotoh, B. (2001). Sendai virus C protein physically associates with Stat1. *Genes Cells* **6,** 545–557.

Talon, J., Horvath, C. M., Polley, R., Basler, C. F., Muster, T., Palese, P., and Garcia-Sastre, A. (2000). Activation of interferon regulatory factor 3 is inhibited by the influenza A virus NS1 protein. *J. Virol.* **74,** 7989–7996.

Taniguchi, T., Ogasawara, K., Takaoka, A., and Tanaka, N. (2001). IRF family of transcription factors as regulators of host defense. *Annu. Rev. Immunol.* **19,** 623–655.

Thomas, S. M., Lamb, R. A., and Paterson, R. G. (1988). Two mRNAs that differ by two nontemplated nucleotides encode the amino coterminal proteins P and V of the paramyxovirus SV5. *Cell* **54,** 891–902.

Tidona, C. A., Kurz, H. W., Gelderblom, H. R., and Darai, G. (1999). Isolation and molecular characterization of a novel cytopathogenic paramyxovirus from tree shrews. *Virology* **258,** 425–434.

Tober, C., Seufert, M., Schneider, H., Billeter, M. A., Johnston, I. C., Niewiesk, S., ter Meulen, V., and Schneider-Schaulies, S. (1998). Expression of measles virus V protein is associated with pathogenicity and control of viral RNA synthesis. *J. Virol.* **72,** 8124–8132.

Valsamakis, A., Schneider, H., Auwaerter, P. G., Kaneshima, H., Billeter, M. A., and Griffin, D. E. (1998). Recombinant measles viruses with mutations in the C, V, or F gene have altered growth phenotypes in vivo. *J. Virol.* **72,** 7754–7761.

Vidal, S., Curran, J., and Kolakofsky, D. (1990). A stuttering model for paramyxovirus P mRNA editing. *EMBO J.* **9,** 2017–2022.

Waning, D. L., Schmitt, A. P., Leser, G. P., and Lamb, R. A. (2002). Roles for the cytoplasmic tails of the fusion (F) and hemagglutinin-neuraminidase (HN) proteins in budding of the paramyxovirus simian virus 5. *J. Virol.* **76,** 9284–9297.

Wathelet, M. G., Lin, C. H., Parekh, B. S., Ronco, L. V., Howley, P. M., and Maniatis, T. (1998). Virus infection induces the assembly of coordinately activated transcription factors on the IFN-beta enhancer in vivo. *Mol. Cell* **1,** 507–518.

Weaver, B. K., Kumar, K. P., and Reich, N. C. (1998). Interferon regulatory factor 3 and CREB- binding protein/p300 are subunits of double-stranded RNA-activated transcription factor DRAF1. *Mol. Cell. Biol.* **18,** 1359–1368.

Winston, J. T., Chu, C., and Harper, J. W. (1999). Culprits in the degradation of cyclin E apprehended. *Genes Dev.* **13,** 2751–2757.

Wright, P. E., and Dyson, H. J. (1999). Intrinsically unstructured proteins: Re-assessing the protein structure- function paradigm. *J. Mol. Biol.* **293,** 321–331.

Yoneyama, M., Suhara, W., Fukuhara, Y., Fukuda, M., Nishida, E., and Fujita, T. (1998). Direct triggering of the type I interferon system by virus infection: Activation of a transcription factor complex containing IRF-3 and CBP/p300. *EMBO J.* **17,** 1087–1095.

Young, D. F., Chatziandreou, N., He, B., Goodbourn, S., Lamb, R. A., and Randall, R. E. (2001). Single amino acid substitution in the V protein of simian virus 5 differentiates its ability to block interferon signaling in human and murine cells. *J. Virol.* **75,** 3363–3370.

Young, D. F., Didcock, L., Goodbourn, S., and Randall, R. E. (2000). Paramyxoviridae use distinct virus-specific mechanisms to circumvent the interferon response. *Virology* **269,** 383–390.

Yu, Z. K., Gervais, J. L., and Zhang, H. (1998). Human CUL-1 associates with the SKP1/SKP2 complex and regulates p21(CIP1/WAF1) and cyclin D proteins. *Proc. Natl. Acad. Sci. USA* **95,** 11324–11329.