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SCIENCE DIRECT

Virology 338 (2005) 270 – 280

VIROLOGY

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The role of simian virus 5 V protein on viral RNA synthesis

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Received 26 January 2005; returned to author for revision 10 March 2005; accepted 6 May 2005
Available online 13 June 2005

Abstract

The paramyxovirus simian virus 5 (SV5) has seven genes but encodes eight known viral proteins. The V/P gene is transcribed into two mRNA species: V mRNA from a faithful transcription of the gene and P mRNA from transcription with addition of two G residues at a specific site of the gene. V, a 222-amino acid (AA) residue protein, and P, a 392 AA residue protein, share an identical N-terminus domain of 164 amino acid residues. P is essential for SV5 RNA replication and transcription. Whereas it is known that V plays important roles in virus pathogenesis, the role of V in SV5 replication and transcription is not clear. A mini-genome system, free of vaccinia virus gene expression system, consisting of plasmids expressing NP, P, and L, as well as a plasmid encoding a reporter gene, chloramphenicol acetyltransferase (CAT) flanked by SV5 trailer and leader sequences under control of a bacteriophage T7 RNA polymerase promoter, has been established to examine the role of V in SV5 RNA transcription and replication. Addition of V-expressing plasmid in the mini-genome system caused inhibition of the reporter gene expression, suggesting that V plays a role in regulating SV5 gene expression. By examining the amount of encapsidated viral RNA genome using reverse transcription with primer annealing to viral anti-genome RNA and PCR, it was found that expression of V reduced the amount of viral RNA genome in the mini-genome system, suggesting that V inhibits viral RNA replication. To examine whether the V protein inhibits viral RNA transcription as well, a mini-genome system with a defective anti-genome promoter (AGP) such that a reporter gene (luciferase, Luc) expression is only derived from transcription of newly produced mini-genome and not from de novo replicated viral genome due to the defect in replication element has been utilized. The V protein inhibited luciferase expression from the mini-genome with a defective AGP, suggesting V inhibits SV5 transcription. Thus, SV5 V inhibits both SV5 RNA replication and transcription.

Keywords: Simian virus 5; RNA synthesis; V/P gene; V

Introduction

Simian virus 5 (SV5) is a prototypical member of the *Rubulavirus* genus of the family Paramyxoviridae, which includes many well known human and animal pathogens such as mumps virus, human parainfluenza virus type 2 (HPIV2) and type 4 (HPIV4), Newcastle disease virus (NDV), Sendai virus, HPIV3, measles virus, canine distemper virus, rinderpest virus, and respiratory syncytial (RS) virus, as well as important emerging viruses such as Hendra virus and Nipah virus (Lamb and Kolakofsky, 2001). Although SV5 was originally isolated from cultured primary monkey cells its natural host is the dog in which it causes kennel cough (McCandlish et al., 1978). SV5 is believed to infect humans (Cohn et al., 1996) and isolates have been obtained from human sources, but no known symptoms or diseases in humans have been associated with SV5 (Hsiung et al., 1965).

SV5's negative-stranded RNA genome is 15264 nucleotides long and has seven genes but encodes eight known viral proteins (Lamb and Kolakofsky, 2001). Nucleocapsid protein (NP), phosphoprotein (P), and large RNA polymerase (L)

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doi:10.1016/j.virol.2005.05.014

protein are essential for transcription and replication of the viral RNA genome. The functional template for transcription and replication of SV5, like other non-segmented negative sense RNA viruses, is the nucleocapsid (NC) which consists of nucleocapsid protein (NP)-encapsidated RNA (Lamb and Kolakofsky, 2001). The viral RNA-dependent RNA polymerase (vRNAP) consists of two proteins, P and L. The P–L complex transcribes the nucleocapsid protein (NP)-encapsidated RNA into 5′ capped and 3′ polyadenylated mRNAs and replicates genome RNA in cytoplasm (Emerson and Yu, 1975). The V protein plays important roles in viral pathogenesis (details will be discussed below). The fusion (F) protein, a glycoprotein, mediates virus entry into cells by promoting both cell-to-cell and virus-to-cell fusion in a pH-independent manner (Paterson et al., 1984, 1985). The hemagglutinin–neuraminidase (HN), another viral glycoprotein, is also involved in virus entry and release from the host cells (Paterson et al., 1985; Schmitt et al., 1999, 2002). The matrix (M) protein plays an important role in virus egress (Schmitt et al., 1999, 2002). The small hydrophobic (SH) protein is a 44-AA residue hydrophobic integral membrane protein and plays a role in blocking a tumor necrosis factor (TNF)-α-mediated extrinsic apoptotic pathway (He et al., 1998, 2001; Lin et al., 2003).

The V/P gene of SV5 is transcribed into the V mRNA by faithfully transcribing the gene and the P mRNA through a process of pseudo-templated addition of nucleotides, commonly called "RNA editing" (Thomas et al., 1988). It is thought that during transcription the viral RNA polymerase complex recognizes a specific RNA sequence in the V/P gene and inserts two non-templated G residues at the site to generate the P mRNA. As a result, the V/P gene is transcribed into two mRNAs and translated into two proteins, which share identical N-termini but different C-termini. Insertion of the G residues occurs about 50% of the time, resulting in about an equal amount of V and P mRNA being produced. The process of inserting non-templated residues occurs for almost all viruses in the subfamily Paramyxovirinae (Lamb and Kolakofsky, 2001). Interestingly, only in *Rubulaviruses* is the V mRNA faithfully transcribed from the genome RNA; for the *Respiroviruses* and the *Morbilliviruses* the P mRNA is faithfully transcribed from the genome RNA and the V mRNA is the result of the additional pseudo-templated G nucleotide(s) (Jacques and Kolakofsky, 1991). Although the V proteins are produced differently in the Paramyxovirinae, the sequences of the C-terminal domain of the V proteins are highly conserved among the paramyxoviruses.

The V protein of SV5 is a multifunctional protein and plays important roles in viral pathogenesis. The V protein is essential to counter host interferon action. The V protein blocks interferon (IFN) signaling in infected cells by causing degradation of STAT1 protein in human cells (Didcock et al., 1999) and inhibits interferon-β production by sequestering IRF-3 in the cytoplasm (He et al., 2002; Poole et al., 2002). V interacts with MDA-5, an IFN-inducible RNA helicase, which plays an important role in inhibiting IFN-β promoter (Andrejeva et al., 2004). The V protein C-terminal domain contains seven cysteine residues, which are very well conserved among all paramyxoviruses, resembles a zinc finger domain, and binds atomic zinc (Liston and Briedis, 1994; Paterson et al., 1995; Steward et al., 1995; Thomas et al., 1988). SV5 lacking the C-terminus of the V protein (rSV5VΔC) causes apoptosis in infected cells and the V protein can block cell death induced by rSV5VΔC, suggesting V can inhibit apoptotic signal pathways (Sun et al., 2004). The SV5 V protein, via its cysteine-rich C-terminus, interacts with a cellular protein (DDB1), the 127-kDa subunit of the damage-specific DNA-binding protein (DDB) that is involved in damaged DNA repair. Deletion of the C-terminus from the V protein interrupts the interaction (Lin and Lamb, 2000). Overexpression of the SV5 V protein in cells slows down the cell cycle (Lin and Lamb, 2000). Co-expression of DDB1 can partially restore the changes in cell cycle caused by V expression (Lin and Lamb, 2000). V, DDB1, Cul4A, STAT1, and STAT2 form a complex, which is essential for V-mediated STAT1 degradation and V has an E3 ubiquitin ligase activity (Ulane and Horvath, 2002).

The role of SV5 V in viral RNA transcription and replication is not clear. It is known that the V protein of SV5 interacts with soluble NP (Randall and Bermingham, 1996) and the N-terminal domain of V binds RNA through a basic region (Lin et al., 1997b). Since V shares an identical N-terminus of 164 AA with P, which is essential for SV5 RNA transcription and replication, it is thought that V may play a role in SV5 RNA transcription and replication. To investigate this putative role played by V in SV5 RNA transcription and replication, a mini-genome system, free of vaccinia virus (VV) gene expression, consisting of plasmids expressing NP, P, and L, as well as a plasmid encoding a reporter gene, CAT flanked by SV5 trailer and leader sequences under control of a bacteriophage T7 RNA polymerase promoter has been established in this work. In addition, a mini-genome system with a defective anti-genome promoter (AGP) that cannot be replicated but still can be transcribed has been constructed. Using the mini-genome systems, the role of SV5 V in transcription and replication has been investigated.

Results

Establishing a mini-genome system free of vaccinia virus

To study the role of V in viral transcription and replication, it is desirable to have a mini-genome system that is free of vaccinia virus. We constructed a mini-genome plasmid, containing the SV5 leader (Le), trailer (Tr) ,and a reporter gene, CAT (Fig. 1A), under the control of a T7 RNAP promoter. Transcription from the T7 promoter results in a negative strand mini-genome. In the presence of NP, P, and L, this template is used for transcription to give rise to