RNA editing-like phenomenon in paramyxovirus V gene mRNA observed in insect cells infected with a recombinant baculovirus

Haruo Matsumura,1 Norihisa Ikemura,2† Yasuhiko Ito2 and Kagemasa Kuribayashi3

1 Department of Immunology, Kinki University School of Medicine, 377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589, Japan
2,3 Departments of Microbiology2 and Bioregulation3, Mie University School of Medicine, 2-174 Edobashi, Tsu-shi, Mie 514, Japan

The V gene of the paramyxovirus human parainfluenza virus type 2 (hPIV2) is transcribed into both V and P mRNA. The V mRNA is a faithful transcript of the V gene; however, the P mRNA is transcribed by an RNA-editing mechanism in hPIV2-infected mammalian cells. Recombinant baculoviruses (rBV) were constructed containing the wild-type V gene, which has seven G residues at its editing site, and a manipulated V gene with ten G residues at its editing site. A small amount of the P protein was synthesized, in addition to the V protein, when the wild-type V gene was expressed in rBV-infected insect cells. Furthermore, synthesis of the P protein increased when rBV containing the manipulated V gene was used to infect insect cells. Both the P and V proteins were detected after in vitro translation of mRNA from rBV-infected cells. Moreover, G-residue insertions and a deletion were detected in mRNA. Since the P protein was not detected after in vitro translation of V RNA that had been transcribed in vitro by T7 RNA polymerase, these results suggest that the non-encoded G residues were inserted and deleted during transcription in insect cells. This RNA editing-like phenomenon and the implications of the length of the G cluster are discussed.

Introduction

Human parainfluenza virus type 2 (hPIV2) is a member of the paramyxovirus family of non-segmented negative-strand RNA viruses and is one of the major human respiratory pathogens (Kingsbury et al., 1978). The genome encodes seven proteins, the nucleoprotein (NP), phosphoprotein (P), V protein, matrix protein (M), fusion protein (F), haemagglutinin–neuraminidase protein (HN) and large polymerase protein (L). In related paramyxoviruses, it has been shown that the P protein is required, in cooperation with the L protein, for viral RNA synthesis (Curran et al., 1993; Deshpande & Portner, 1985; Hamaguchi et al., 1983; Horikami et al., 1992). The C-terminal sequence of the V protein is conserved in paramyxoviruses (Thomas et al., 1988) and contains conserved cysteine residues in a motif that is homologous to the zinc-finger domains that have been identified in many transcription factors and nucleic acid-binding proteins (Jacques et al., 1994). A recent report showed that the V protein is required for pathogenesis of the virus (Kato et al., 1997). A faithful transcript of the hPIV2 P/V gene, with a seven G-residue (G7) stretch at its editing site, encodes the V protein, while the mRNA for the P protein, which has a G9 stretch at its editing site, is generated by RNA editing (Ohgimoto et al., 1990; Thomas et al., 1988). Two non-encoded G residues are co-transcriptionally inserted in the middle of the coding region, and the resulting frame-shift results in production of the P protein. The P and V proteins therefore share the same N-terminal amino acid sequence upstream of the G-residue insertion site and both proteins are detected in virus-infected cells. It has been reported that paramyxovirus-encoded proteins are essential for paramyxovirus RNA editing, based on observations that neither T7, T3 or SP6 RNA polymerase nor a vaccinia virus vector system was able to edit the virus P gene (Galinski et al., 1992; Horikami & Moyer, 1991; Thomas et al., 1988; Vidal et al., 1990a). The mechanism of RNA editing has not yet been clarified. However, it has been suggested that the G insertion

Author for correspondence: Haruo Matsumura.
Fax +81 723 67 7660. e-mail matumura@med.kindai.ac.jp
† Present address: Third Department of Internal Medicine, Mie University School of Medicine, Mie, Japan.
results from slippage of the polymerase, or so-called polymerase stuttering (Vidal et al., 1990b).

The subfamily Paramyxovirinae is organized into three genera, the rubulaviruses (including hPIV2 and mumps virus), paramyxoviruses (including Sendai virus) and morbilliviruses (including measles virus) (Rima et al., 1995). In the rubulaviruses, except for Newcastle disease virus (Steward et al., 1993), the P/V gene encodes the V protein and has a long G stretch (6–7 Gs) at its editing site. RNA editing is therefore essential for synthesis of the P protein, which is a structural component and is necessary for replication (Curran et al., 1993; Deshpande & Portner, 1985; Hamaguchi et al., 1983; Horikami et al., 1992). In contrast, in the paramyxoviruses and morbilliviruses, with the exception of hPIV1 (Matsuoka et al., 1991), the P/V gene encodes the P protein and has a short G stretch (3–5 Gs) at its editing site. Thus, RNA editing is not necessary for synthesis of the P protein in these viruses.

In this study, we have characterized this RNA editing-like phenomenon in recombinant baculovirus (rBV)-infected insect cells and examined the possibility that the long G-residue stretch at the editing site of hPIV2 contributes to the generation of P mRNA.

**Methods**

**Cells, viruses and antibodies.** Spodoptera frugiperda (Sf9) insect cells were cultured in TNM-FH medium (Gibco) containing 10% foetal calf serum and were infected with rBV at an m.o.i. of 1–3. Primary monkey kidney (PMK) cells were cultured in Dulbecco’s modified Eagle’s medium (Nissui) containing 10% foetal calf serum and were infected with hPIV2 Toshiba strain at an m.o.i. of 3–5. An hPIV2 P-specific MAb, 211A, a mixture of the anti-hPIV2 P/V MAb5 315-1 and 202A (Tsurudome et al., 1989) and an hPIV2 V-specific rabbit polyclonal antibody were used for Western blotting and immunoprecipitation (Ohgimoto et al., 1990). The polyclonal antibody was purified from anti-V peptide serum by pre-adsorption to immobilized BSA and Protein G-Sepharose CL4B (Pharmacia), and was used at 10 μg/ml in the presence of 1 mg/ml BSA.

**Construction of recombinant baculoviruses.** The full-length V gene in pUC119 was constructed from pSP10M and pSP31M (Ohgimoto et al., 1990), which cover the entire P/V gene coding region, by a combination of restriction digestion, PCR and linker ligation. The nucleotide sequence of the entire P/V gene was confirmed after construction of the V gene in pUC119. The resulting plasmid, pP2P7G (Fig. 1A), was cut with BamHI and a 500 bp fragment containing the 5’ end of the V gene was recovered and ligated into the BglII site of a linker-ligated transfer vector, pAcYM1 (Matsuura et al., 1987). The orientation of the fragment was confirmed by nucleotide sequencing. The large XbaI–PstI fragment of this plasmid was ligated with an XbaI–EcoRI 2 kb fragment (1.1 kbp) of pP2P7G. The resulting plasmid was designated pAcP2P27G (Fig. 1B). Oligonucleotides for linker ligation to pAcYM1 were 5’ GATCGTGACCAATGACGATGCAGTCA 3’ and 5’ GATCTGCAGTCTGAGGTGATACC 3’. These oligonucleotides were annealed and then ligated into the BamHI site of pAcYM1.

PCR mutagenesis was performed as described previously (Ito et al., 1991) to construct pAcP2P210G, which has a G10 cluster at the editing site of the V gene. pP2P7G was used as a template for mutagenesis. Primers V10G (5’ CAACATTAAAGGGGGGGGGGACCTAATTAG 3’) and M4 (5’ GTTTTCCCATGTCAGCAG 3’) were used to generate the mutation, and reverse primer (5’ CAGGATGCGTACGATGCAGTATG 3’) and MUTR3 (5’ ACGGCCGCTAGTCTCTAGCT 3’) were used to disrupt an EcoRI site.

Methods for the preparation of an infectious baculovirus DNA, AcRP23.lacZ (Possee & Howard, 1987), have been described previously (Summers & Smith, 1988). AcRP23.lacZ and lipofectin (Gibco) were used for co-transfection with the transfer vector. The rBV were screened by Western blotting and plaque-cloned three times. The cloned rBV, AcP2P27G and AcP2P210G, have G6 and G10 clusters, respectively, at the editing site of the hPIV2 V gene.

**SDS–PAGE and Western blotting.** Cells were inoculated with rBV at an m.o.i. of 3. Infected cells were harvested 3 days after infection, washed twice with TNM-FH medium, solubilized with SDS sample buffer (62.5 mM Tris–HCl, pH 6.8, 2% SDS, 15% glycerol, 0.05% bromophenol blue), sonicated for 5 s to shear chromosomal DNA and then subjected to SDS–PAGE (Laemmli, 1970) under non-reducing conditions. The ratio of acrylamide to bis-acrylamide was 39:1 and 12.5% acrylamide gels were used. After electrophoresis, gels were stained with Coomassie brilliant blue R-250 or blotted onto nitrocellulose filters (Schleicher & Schuell) as described previously (Towbin et al., 1979). Blots were blocked with 5% skimmed milk powder in 0.01% antifoam A (Sigma) (pH 7.2) for 1 h, incubated with various antibodies for 1 h and washed with 20 mM Tris–HCl (pH 7.5), 0.15 M NaCl. Next, blots were incubated with alkaline phosphatase-conjugated goat anti-mouse immunoglobulins (IgG + IgM, Cappel) or alkaline phosphatase-conjugated goat anti-rabbit IgG (Cappel), washed as described above and developed with BCIP/NBT (Cappel).

**Radiolabelling and immunoprecipitation.** PMK cells, as a monolayer in 2 cm² culture wells, were pulse-labelled with 10 μCi [35S]methionine or 50 μCi [32P]orthophosphate (Amersham) in methionine-free minimal essential medium (MEM) or phosphate-free MEM (Gibco), as appropriate, at 8 h post-infection (p.i.). At 24 h p.i., cell
monolayers were solubilized on ice for 10 min with 200 µl extraction buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1 mM PMSE) containing 0.5% NP40. After centrifugation of the extract at 10000 g for 10 min, supernatants were mixed with antibody-coupled Protein G–Sepharose CL-4B (Pharmacia) and incubated on a rocking platform at 4 °C for 2 h. The beads were washed three times with extraction buffer containing 0.1% NP40 before being mixed with 15 µl 2 × SDS sample buffer. After SDS–PAGE, the gels were treated with ENHANCE (NEN) for fluorography. Sf9 cells (1 × 10^7 cells) in 1 cm^2 culture wells were pulse-labelled with 15 Ci [35S]methionine in a volume of 25 µl at 30 °C for 1 h. The products were subjected to SDS–PAGE or immunoprecipitation after dilution with extraction buffer containing 0.1% NP40. Treatment of RNA and baculovirus DNA, cDNA synthesis and PCR. Total RNA was prepared by the AGPC method (Chomczynski & Sacchi, 1987) and baculovirus DNA was prepared as described previously (Summers & Smith, 1988) from rBV-infected Sf9 cells at 72 h p.i. To eliminate any contaminating baculovirus DNA from RNA samples, 20 µg of the RNA sample was treated with 70 U DNase I (Takara) for 10 min at 37 °C, followed by phenol–chloroform extraction. Oligo(dT)~30~ super (Nippon Rosche) was used for preparation of mRNA. Elimination of DNA from RNA samples was confirmed by PCR method described below. Oligo(dT)~18~–primed, single-stranded cDNA was synthesized from 2 µg DNase-treated RNA by reverse transcriptase (Superscript RT, Gibco) in a final volume of 20 µl, according to the manufacturer’s protocol. An aliquot of cDNA (5 µl) or viral DNA (20 ng) and P gene-specific primers (20 pmol each), spanning nucleotides 518–536 and 717–737 (Ohgimoto et al., 1990), were used for PCR. PCR was carried out for 30 cycles of 30 s at 94 °C, 30 s at 59 °C and 30 s at 72 °C, in a final volume of 50 µl containing 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 0.001% gelatin, 200 µM dNTP and 2.5 U Taq polymerase (Kurabo Biomedical). Amplified DNA fragments were digested with BamHI and subcloned into puCC119 for nucleotide sequencing with Sequenase (USB) or colony hybridization.

**In vitro translation and in vitro transcription.** T7 RNA polymerase was used for *in vitro* transcription (Megascript kit, Ambion) as described in manufacturer’s protocol. Briefly, a SalI–EcoT22I fragment (540 bp) of pP2P7G or pP2P10G and a KpnI–SalI fragment (680 bp) of pAcP2P7G or pAcP2P10G were ligated into KpnI–SstI-cut pBluescript II SK(–) (Stratagene). The expected sequence of the 5’ end of the transcribed RNA was 5’ GGGCGGATTTGCGTACCAGATCCATG 3’. The initiation codon of the V gene is underlined. These plasmids were linearized for run-off transcription by restriction digestion at the T7 MAbs and anti-hPIV2 V-specific antibody (Fig. 2B, C) (Ohgimoto et al., 1990; Tsurudome et al., 1989). Furthermore, the apparent molecular mass of the protein was identical to that in hPIV2-infected PMK cells (data not shown). Interestingly, a small amount of P protein was detected in addition to the large amount of V protein (Fig. 2B, lane 3). We then constructed the rBV AcP2P10G, which has a G19 stretch at the editing site of the hPIV2 V gene (V^19G-gene), in order to clarify the mechanism of synthesis of the P protein and to examine the effects of an extended G stretch on generation of the P protein. Proteins expressed in rBV-infected Sf9 cells were analysed as described above and compared with those in AcP2P7G-infected cells (Fig. 2). Both the P and V^19G-proteins were synthesized in AcP2P10G-infected cells and the ratio of P to V^19G-protein was higher than the ratio of P to V protein in AcP2P7G-infected cells. The identity of the P protein was confirmed by Western blotting with anti-hPIV2 P/V-specific MAbs and anti-hPIV2 V-specific antibody (Fig. 2B, C) (Ohgimoto et al., 1990; Tsurudome et al., 1989). Furthermore, the apparent molecular mass of the P protein was identical to that in hPIV2-infected PMK cells. Kinetic synthesis of P/V proteins in rBV-infected cells showed that P/V proteins were detected 36 h p.i. and increased

**Results.** It has been confirmed that RNA editing does not occur when the P/V gene alone is expressed in mammalian cells or translated *in vitro*. However, we found that an RNA editing-like phenomenon is seen when the hPIV2 V gene is expressed in rBV-infected insect cells. We therefore characterized this RNA editing-like phenomenon and examined the effects of the length of the G stretch on the frequency of G insertion at the editing site.

**Expression of wild-type and mutated V genes in Sf9 cells.** We first prepared the rBV AcP2P7G (Fig. 1B), which contains the hPIV2 P/V cDNA, covering the entire region encoding the P/V proteins, under the control of the polyhedrin promoter, in order to characterize the V protein of hPIV2. The RNA-editing site of the P/V gene in AcP2P7G contains a G19 stretch, which is a faithful copy of the hPIV2 genomic P/V gene. Proteins expressed in infected Sf9 insect cells were then examined by SDS–PAGE and Western blotting with the anti-hPIV2 P/V MAb mixture and the anti-hPIV2 V-specific serum. A large amount of V protein was synthesized in these cells (Fig. 2A). Identification of the V protein was confirmed by Western blotting with anti-hPIV2 P/V-specific MAbs and anti-hPIV2 V-specific antibody (Fig. 2B, C) (Ohgimoto et al., 1990; Tsurudome et al., 1989). Furthermore, the apparent molecular mass of the protein was identical to that in hPIV2-infected PMK cells (data not shown). Interestingly, a small amount of P protein was detected in addition to the large amount of V protein (Fig. 2B, lane 3). We then constructed the rBV AcP2P10G, which has a G19 stretch at the editing site of the hPIV2 V gene (V^19G-gene), in order to clarify the mechanism of synthesis of the P protein and to examine the effects of an extended G stretch on generation of the P protein. Proteins expressed in rBV-infected Sf9 cells were analysed as described above and compared with those in AcP2P7G-infected cells (Fig. 2). Both the P and V^19G-proteins were synthesized in AcP2P10G-infected cells and the ratio of P to V^19G-protein was higher than the ratio of P to V protein in AcP2P7G-infected cells. The identity of the P protein was confirmed by Western blotting with P/V-specific MAbs and also with a P-specific MAb, clone 211A (Tsurudome et al., 1989) (Fig. 2B). Furthermore, the apparent molecular mass of the P protein was identical to that in hPIV2-infected PMK cells.
Phosphorylation of the V protein

It is known that the P protein is phosphorylated in paramyxovirus-infected cells (Curran et al., 1991; Vidal et al., 1988). We examined the phosphorylation of V protein expressed in Sf9 cells. Both P and 30 kDa proteins, corresponding to the V protein, were phosphorylated in hPIV2-infected PMK cells. While the V protein was phosphorylated in rBV-infected Sf9 cells, it was not clear whether the P protein was also phosphorylated (data not shown).

In vitro translation of RNA

We analysed mRNA from rBV-infected Sf9 cells by in vitro translation to clarify the mechanism of generation of the P protein. Fig. 3 shows that both the V and P proteins were synthesized from mRNA isolated from either AcP2P7G- or AcP2P10G-infected cells. We quantified the radioactivity in each band (Fig. 3, lanes 2 and 3) by bioimage analyser (BAS 2000, Fuji). We then estimated the molar ratio of each protein, based on the number of methionine residues in each protein (2 in V and 9 in P). The percentage ratio of P protein to V protein was 0.35% (AcP2P7G) and 0.78% (AcP2P10G). These results support the notion that P mRNA is transcribed from the V gene. Moreover, the ratio of P mRNA to V mRNA in AcP2P10G-infected cells was higher than that in AcP2P7G-infected cells. However, the possibility that P protein was generated by ribosomal frame-shifting in the reticulocyte lysate cannot be excluded. Thus, we next used in vitro-transcribed RNA for in vitro translation, since it has been reported that T7, T3 and SP6 RNA polymerases do not edit transcription site and the first ATG of the V gene, the existence of two bands cannot be clearly explained. Both normal and aberrant initiation of translation might occur. Fig. 4 shows that only V or V106G protein was detected and that no P protein was detected. These results indicate that very little or no ribosomal frame-shifting occurred in the reticulocyte lysate when V mRNA was translated. Thus, it is most likely that the P protein is translated from P mRNA in rBV-infected Sf9 cells (Fig. 3).

Detection of P mRNA

Next, we tried to detect P mRNA by PCR subcloning of cDNA, as described in Methods, to prove that P mRNA was...
Table 1. Detection of G insertions and a deletion

The table shows the number of clones analysed that contained the insertions or deletions listed. P mRNA clones are shown in bold.

<table>
<thead>
<tr>
<th>Template for PCR</th>
<th>No. of G residues inserted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−2</td>
</tr>
<tr>
<td>AcP2P7G DNA</td>
<td>0</td>
</tr>
<tr>
<td>AcP2P10G DNA</td>
<td>0</td>
</tr>
<tr>
<td>AcP2P7G mRNA</td>
<td>0</td>
</tr>
<tr>
<td>AcP2P10G mRNA</td>
<td>0</td>
</tr>
</tbody>
</table>

generated (Table 1). About 100 clones were picked randomly and sequenced. The remainder were screened by colony hybridization with deoxyoligonucleotides as probes. Over 96% of colonies harboured PCR products. The nucleotide sequences of the cloned V gene fragments in all clones were identical, except for the number of G residues at the editing site. No insertions or deletions were detected in the viral DNA. In AcP2P7G mRNA, all clones were faithful transcripts of the V gene, while in AcP2P10G mRNA, 11 of 428 clones showed deletions or insertions of G residues. The number of deletions and insertions in AcP2P10G mRNA was significantly higher than that in AcP2P10G viral DNA (P < 0.01) or in AcP2P7G mRNA (P < 0.02). Among these 11 clones, two with a single deletion and one with two G insertions encoded the P protein. Thus, the frequency of P mRNA is estimated as 0.35% or one hundred colonies would need to be screened in order to detect P mRNA clone.

Table 1. Detection of G insertions and a deletion

We show in this report that both V and P proteins were synthesized when the V gene of hPIV2 was expressed in rBV-infected insect cells. Furthermore, P mRNA was detected in rBV-infected cells by two methods, in vitro translation of mRNA and PCR subcloning. From our results, we conclude that the P protein was translated from P mRNAs and that these P mRNAs were transcribed from the V cDNA in rBV by deletions or addition of G residues at the editing site. Although, as already mentioned, ribosomal frame-shifting in the reticuloocyte lysate was undetectable, the following explanations of our results are possible. (i) Deletions and insertions in the 11 cDNA clones shown in Table 1 may be artefacts of Taq polymerase or reverse transcriptase. (ii) mRNAs with deletions and insertions might have been transcribed from rBV clones with deletions or insertions in the editing site. (iii) P protein detected in Sf9 cells might be generated from V mRNA by ribosomal frame-shifting. However, these explanations can be excluded for the following reasons. In the case of explanation (i), since the frequency of deletions or insertions in AcP2P10G viral DNA clones was statistically lower than that in AcP2P10G mRNA clones (Table 1), it is unlikely that clones with deletions or insertions were generated by errors of Taq polymerase or reverse transcriptase. Although only insertions of G residues into the editing site of hPIV2 have been reported (Ohgimoto et al., 1990; Southern et al., 1990), deletions as well as the insertion of G residues have been reported as a result of RNA editing in bovine parainfluenza virus type 3 and Sendai virus (Jacques et al., 1994). Our observations are consistent with these results, in that both deletions and insertions were detected. Furthermore, the number of G residues inserted into the mRNA is imprecise (Cattaneo et al., 1989; Elliott et al., 1990; Galinski et al., 1992; Kondo et al., 1990; Pelet et al., 1991; Vidal et al., 1990). Considering explanation (ii), it is unlikely that mRNAs with deletions or insertions were transcribed from rBV clones with deletions or insertions in the editing site. As described above, the frequency of deletions or insertions in AcP2P10G DNA clones was statistically lower than that in AcP2P10G mRNA clones (Table 1). We confirmed the clonality of AcP2P7G and AcP2P10G by three rounds of plaque purification. In addition, when another five clones each of AcP2P7G and AcP2P10G were used for infection of Sf9 cells, patterns of protein synthesis identical to those reported in Fig. 2 were obtained (data not shown). In the case of explanation (iii), it is possible that some of the P protein detected in rBV-infected cells resulted from ribosomal frame-shifting in Sf9 cells. However, since mRNAs were detected encoding the P protein and at a frequency, three out of 428 (0.70%), that correlates well with the molar ratio of P protein to V protein (0.78%), any effects of ribosomal frame-shifting on our results must have been small.

RNA editing in paramyxoviruses has been recognized as the insertion of non-encoded G residues at a specific editing site of the P/V gene mRNA (Thomas et al., 1988; Vidal et al., 1990b). According to this criterion, our observations indicate that P mRNA resulted from editing. The mechanism of deletion and insertion of G residues in our system is possibly similar to that in paramyxovirus-infected cells. However, there are differences between RNA editing in our system and that in paramyxovirus-infected cells. Firstly, the frequency of insertions and deletions in our system was lower than that in paramyxovirus-infected cells (Ohgimoto et al., 1990; Southern
et al., 1990). Also, the distribution of sizes of deletions and insertions (−1, +1 or +2) was different to that in hPIV2-infected mammalian cells (Southern et al., 1990; Oghimoto et al., 1990). Furthermore, the polymerase that is responsible for transcription of the V gene in rBV-infected Sf9 cells is different from that in paramyxovirus-infected cells. The template for transcription of the V and P mRNA in our system was DNA, whereas in paramyxovirus-infected cells the template is RNA. L protein, the predicted RNA-dependent RNA polymerase, is thought to be responsible for RNA editing in paramyxovirus-infected cells (Thomas et al., 1988; Vidal et al., 1990a), whereas the only paramyxovirus proteins present in the rBV-infected cells were the P/V proteins. The polymerase responsible for transcription of genes under the control of the polyhedrin promoter appears to be the baculovirus-encoded α-amanitin-resistant RNA polymerase (Rohrmann, 1992; Yang et al., 1991). This polymerase is presumably responsible for the insertion and deletion of G residues in our system.

Since we showed that more P mRNAs were transcribed from the V gene when a G cluster longer than the wild-type one was present, the number of G residues at the editing site seems to affect the RNA editing-like phenomenon in rBV-infected cells. Moreover, when we constructed an rBV to express the P gene of Sendai virus (three G residues at the editing site) in Sf9 cells, only P protein and no V protein was detected (data not shown). It is not known whether the frequency of RNA editing in cells infected with wild-type hPIV2 is higher than that in cells infected with hPIV2 that has fewer G residues at its editing site. The number of G residues at the editing site may ensure the generation of P mRNA in rubulaviruses. Interestingly, in the rubulaviruses, including hPIV2 but excluding Newcastle disease virus, the P/V gene encodes V protein and has a long G stretch (6–7 G residues) at the editing site. In these viruses, RNA editing is essential for synthesis of the P protein, which is a structural component and is necessary for replication (Curran et al., 1993; Deshpande & Portner, 1985; Hamaguchi et al., 1983; Horikami et al., 1992). In the morbilliviruses and paramyxoviruses, including measles virus (Cattaneo et al., 1989) and Sendai virus (Vidal et al., 1990a), the P/V gene encodes P protein and has a short G stretch (3–5 G residues) at its editing site. RNA editing is not necessary for synthesis of the P protein in these viruses. Recent reports indicate that the V protein is not essential for replication of Sendai virus (Delenda et al., 1997) or measles virus (Schneider et al., 1997). It can therefore be speculated that rubulaviruses have an additional mechanism to accelerate or to ensure RNA editing. The observation that the frequency of RNA editing in rubulavirus-infected mammalian cells is not higher than that in paramyxovirus- or morbillivirus-infected cells may be explained by the different mechanisms of RNA editing in the three genera. In fact, the nucleotide sequences at the editing sites of these viruses are different. Further analysis by reverse genetics may clarify the mechanisms of RNA editing and the implications of length of the G cluster at the editing site.

This research was supported by a grant-in-aid from the Ministry of Education, Science and Culture in Japan. We thank Drs Y. Matsuura and S. Morikawa at the NIH of Japan for providing the baculovirus expression vector system and technical advice. We also thank Dr M. Tsurudome for monoclonal antibodies, Drs Y. Chinzei, M. Kohase and S. Saito for encouragement and financial support.

References


Kondo, K., Bando, H., Tsurudome, M., Kawamo, N., Nishio, M. & Ito, Y. (1990). Sequence analysis of the phosphoprotein (P) genes of human...
parainfluenza type 4A and 4B viruses and RNA editing at transcript of the P genes: the number of G residues added is imprecise. Virology 178, 321–326.


Received 5 May 1998; Accepted 9 September 1998