cDNA Cloning of the Nucleocapsid and Nucleocapsid-associated Protein Genes of Mumps Virus

By J. A. CURRAN, J. P. QUINN, E. M. HOEY, S. J. MARTIN and B. K. RIMA*

Department of Biochemistry, The Queen's University of Belfast, Belfast BT9 7BL, U.K.

(Accepted 14 January 1985)

SUMMARY

cDNA clones of the mumps virus N and P messenger RNAs were isolated from an infected cell cDNA library. The N and P clones selected the two predominant polyadenylated RNAs found in mumps virus-infected cells with mol. wt. of $0.69 \times 10^6$ and $0.51 \times 10^6$, respectively. In addition, clones of the P gene hybridized to and selected mRNAs of higher mol. wt. probably representing polycistronic transcripts of the mumps genome. Hybrid-select translation experiments confirmed the specificity of the clones as representing the nucleocapsid (N) and nucleocapsid-associated protein (P) genes.

INTRODUCTION

Mumps virus is a human paramyxovirus of considerable clinical importance, yet its biochemical characterization has taken place only in the last few years since virus strains, adapted to growth in mammalian tissue culture cells, became available. Apart from one early study on RNAs of the virus (East & Kingsbury, 1971), little information is available on the nucleic acids and replication of mumps virus. In the past 5 years, studies on the proteins of mumps virus have led to a description of the virus proteins (Orvell, 1978; McCarthy & Johnson, 1980) and those induced in infected cells (Rima et al., 1980; Herrler & Compans, 1982), which appear to confirm that the virus has the usual protein complement for a member of the Paramyxoviridae. It contains a phosphorylated nucleocapsid protein (N) of mol. wt. 70K, a nucleocapsid-associated protein (P) of mol. wt. 45K, a large protein (L) of mol. wt. 160K, a haemagglutinin-neuraminidase protein (HN) of mol. wt. 80K, a fusion protein (F) of mol. wt. 71K which is cleaved into two glycopeptides, F$_1$ (61K) and F$_2$ (10 to 14K), and a membrane protein (M) of mol. wt. 40K (Örvell, 1978; McCarthy & Johnson, 1980; Rima et al., 1980; Herrler & Compans, 1982, 1983; Merz et al., 1983). Furthermore, the virus induces at least two non-structural proteins of mol. wt. 24K and 16K (Rima et al., 1980; Herrler & Compans, 1982) which have been shown to be related to the P protein (Herrler & Compans, 1982) and appear to be synthesized from the P mRNA (E. J. B. Simpson, J. A. Curran, E. M. Hoey, S. J. Martin & B. K. Rima, unpublished results).

In order to study the replication and expression of the viral genome in a variety of cell lines and in lytic and persistent infections, we decided that cDNA cloning was necessary to monitor gene expression which generally has been found to reach only low levels in infected cells. Such clones would also be useful in pathogenicity studies of the virus. Here we report a description of mumps virus messenger RNA induced in infected cells and the generation and characterization of cDNA clones from the N and P genes of mumps virus.

METHODS

Cells and viruses. Vero cells (African green monkey kidney cells) were obtained from Flow Laboratories and grown in Eagle's medium (Glasgow modification) supplemented with 8% newborn calf serum. The origin and method of propagation of the two mumps virus strains used, i.e. the Enders vaccine strain and the Belfast (BF) strain, have been discussed earlier (Rima et al., 1980) except that the present stocks were never passaged more than three times after plaque purification.
**Protein analysis and in vitro translation.** Labelling of infected cells in vivo with \(^{[35}S\)methionine and subsequent analysis of protein profiles were as described earlier (Rima et al., 1980). In vitro translation of polyadenylated (pA\(^+\)) RNA was carried out by dissolving RNA pellets from ethanol precipitates in 4 \(\mu\)l sterile water and 1 \(\mu\)l Rnasin solution (40 U/\(\mu\)l, P & S Biochemicals Liverpool, U.K.) and mixing this with 25 \(\mu\)Ci \(^{[35}S\)methionine (sp. act. 1200 Ci/mmol; Amersham) and 20 \(\mu\)l rabbit reticulocyte lysate (Amersham). The mixture was incubated for 90 min at 30 °C and 20 \(\mu\)l solubilizing buffer for SDS-PAGE was added prior to heating at 100 °C for 2 min. Subsequently, samples were analysed on 8 to 15%, polyacrylamide gels.

**Limited proteolysis.** The analysis of viral proteins by limited proteolysis with *Staphylococcus aureus* V8 protease was carried out as described by Rima et al. (1981).

**Labelling of RNA in infected cells.** Vero cells infected at m.o.i. > 1 with the Enders or BF strains of mumps virus were transferred to phosphate-free medium (Gibco) containing 2 \(\mu\)g/ml actinomycin D and 2\% dialysed newborn calf serum for 1 h prior to the addition of fresh phosphate-free medium containing actinomycin D and dialysed serum to which 200 \(\mu\)Ci/ml \(^{[32}P\) orthophosphate (carrier-free; Amersham) had been added. The infection was allowed to continue for 2 h (18 to 20 h post-infection) before RNA was extracted.

**Extraction of pA\(^+\) RNA from infected cells.** Radioactively labelled RNA or unlabelled RNA were extracted from Vero cells infected with mumps virus at an m.o.i. of > 1 by the method of Glisin et al. (1974) and Martin & ter Meulen (1976). Infected cell monolayers were solubilized in 4\% (w/v) sodium lauryl sarcosinate in 0·1 M-Tris–HCl pH 8·0. After reduction of the viscosity of the sample by force-pipetting three times through a narrow-gauge syringe needle, ultrapure cesium chloride (Bethesda Research Laboratories) was added to a concentration of 1 g/ml and then the sample was overlaid on a 5.7 r~-CsCl cushion prepared in 0.1 M-EDTA pH 6.7 and centrifuged for 91 rain at 30°C and 20 ral solubilizing buffer for SDS PAGE was added prior to heating at 100 °C for 2 rain. The mixture was used to transform Escherichia coli ED8767 cells made competent by the method of Dagerl & Ehrlich (1979). Tetracycline-resistant, ampicillin-sensitive colonies were screened further by colony hybridization performed according to the method of Grunstein and Hogness (1975) using \(^{32}P\)cDNA probes (2 \(\times\) 10\(^6\) c.p.m./\(\mu\)g cDNA) prepared by reverse transcription of pA\(^+\) RNA from infected and mock-infected cells as described above in the presence of \(^{[2}P\)dCTP (410 Ci/mmol).

**Selection of RNA by recombinant plasmids.** Plasmid DNAs were extracted from the various clones by the method of Marko et al. (1982). Recombinant plasmid DNA (20 to 50 \(\mu\)g) was linearized by treatment with EcoRI restriction enzyme and bound to nitrocellulose discs (1 cm\(^2\)). The discs were baked, cut, prehybridized and hybridized with radioactive pA\(^+\) RNA (100000 c.p.m.) or unlabelled pA\(^+\) RNA (20 \(\mu\)g), washed and eluted under the conditions described by Barrett & Mahy (1984). The eluted RNA was coprecipitated with 10 \(\mu\)g glycogen by addition of 2 vol. ethanol. The resulting cDNA/mRNA hybrid molecules were directly extended with dCMP by the homopolymeric tailing procedure of Roychoudhury et al. (1976) using terminal transferase (P-L Biochemicals), phenol-extracted and passed over a Sephadex G-50 column. PstI-cleaved plasmid pAT153 DNA was extended with dGMP homopolymer tails by the same procedure. For transformations, equimolar amounts of tailed plasmid DNA and cDNA/mRNA hybrid molecules were coprecipitated with ethanol; the pellet was resuspended in 20 \(\mu\)l 100 mM-NaCl, 10 mM-dithiothreitol, 100 mM-NaCl, 1 mM-dATP, 1 mM-dTTP, 1 mM-dGTP and 0·4 mM \(^{[3}H\)dCTP (sp. act. 1 \(\mu\)Ci/\(\mu\)mol), 100 \(\mu\)g/ml actinomycin D, 20 \(\mu\)g/ml oligo (dT)\(_{12-18}\), 20 \(\mu\)g/ml pA\(^+\) RNA and 400 units/ml RNasin. Electrophoresis was continued until the marker dye migrated out of the gel, after which the gels were soaked twice for 15 min in 0·5 M-ammonium acetate solution to destroy the toxic methylmercuric hydroxide.
Further soaking for 1 h in 10 × SSC (SSC = 0.15 M-NaCl, 0.015 M-sodium citrate) prepared the gel for blotting onto nitrocellulose. The transfer was carried out in 20 × SSC for 16 h, the filter was then blotted dry and baked for 2 h at 80 °C. Northern blots on nitrocellulose were prehybridized for a minimum of 4 h at 42 °C in a solution of 50% (v/v) deionized formamide, 5 × Denhardt's solution, 2 × SSC and 100 µg/ml denatured salmon sperm DNA. The filters were hybridized in the same solution to which 10% (w/v) dextran sulphate and 5 × 10⁶ c.p.m. of denatured cDNA probe were added. Hybridization was carried out for 16 h at 42 °C. Filters were then washed twice in 2 × SSC, 0.1% (v/v) SDS at room temperature for 10 min and twice in 0.1 × SSC, 0.1% (v/v) SDS at 50 °C for 10 min. The filters were then blotted dry and exposed to X-ray film with intensifying screens.

RESULTS

In order to characterize mumps virus-induced RNAs, infected cells were labelled in vivo in the presence of actinomycin D with either 32P, or [3H]uridine and RNA was extracted as described. Polyadenylated RNA was selected by oligo(dT)-cellulose chromatography and resolved on gels under denaturing conditions. Fig. 1 shows the result of such an experiment in which pA⁺ RNAs induced by two strains of mumps virus, the Enders vaccine strain and the BF strain, were

Fig. 1. Analysis of in vivo labelled pA⁺ RNA on formaldehyde-agarose gels. Lane 1, BF mumps virus-infected cell pA⁺ RNA; lane 2, Enders mumps virus-infected cell pA⁺ RNA; lane 3, CDV (Onderstepoort strain)-infected cell pA⁺ RNA; lane 4, measles virus (human 2 strain)-infected cell pA⁺ RNA. The position of rRNA markers and virus-specific bands is indicated.

Fig. 2. In vitro synthesis of proteins using pA⁺ RNA of Enders virus-infected cells. (a) Translation products analysed on an 8 to 15% polyacrylamide gradient gel. Lane 1, in vivo labelled Enders mumps virus-infected cell polypeptides; lane 2, control: no RNA added to in vitro translation system; lane 3, in vitro translated Enders mumps pA⁺ RNA harvested at 18 h; lane 4, in vitro translated Enders mumps pA⁺ RNA harvested at 24 h. (b) Analysis of limited proteolysis digests produced by S. aureus V8 protease on a 15% polyacrylamide gel. Lane 1, in vivo labelled N polypeptide; lane 2, in vitro labelled N polypeptide; lane 3, in vivo labelled P polypeptide; lane 4, in vitro labelled P polypeptide.
compared with pA+ RNAs from measles virus- and canine distemper virus (CDV)-infected Vero cells by analysis on formaldehyde–agarose gels. This comparison revealed that the two major and six minor species of RNA in BF and Enders virus-infected cells were mumps virus-specific. Size estimates of the RNA bands induced by Enders virus were made from methylmercuric hydroxide–agarose gels, because it was found that formaldehyde–agarose gels were not fully denaturing the mumps virus RNAs: glyoxalated RNA migrated more slowly in these gels than non-glyoxalated samples whereas in contrast, on methylmercuric hydroxide–agarose gels there was no migration difference between the glyoxalated and non-glyoxalated RNA samples (data not shown). However, we used the formaldehyde–agarose gel system throughout, since the bands were more discrete and well-separated in this system. The major bands had mol. wt. of $0.51 \times 10^6$ and $0.69 \times 10^6$. Minor virus-specific bands had mol. wt. of $0.41, 0.66$ and $0.85$, each $\times 10^6$.

A number of high mol. wt. pA+ RNAs were also identified as minor bands in the pA+ RNA induced by the Enders strain of mumps virus. These had mol. wt. of $1.18 \times 10^6, 1.55 \times 10^6$ and $2.0 \times 10^6$, similar to the polycistronic transcripts of measles virus- and canine distemper virus-infected cells (Barrett & Mahy, 1984; Russell et al., 1985). The precise designation of these bands is described below after cDNA clones became available.

**Characterization of pA+ RNA used for cDNA cloning experiments**

In order to characterize the starting material for cDNA cloning experiments we extracted pA+ RNA from infected Vero cells and analysed its ability to direct protein synthesis in vitro in rabbit reticulocyte lysates. The products labelled with $[^{35}S]$methionine were resolved by SDS-PAGE (Fig. 2a) to see whether virus-specific protein bands were detectable. These experiments showed the synthesis of protein comigrating with the N and P proteins in infected cells. Gel analysis of the limited proteolysis products obtained by digestion of isolated protein bands with *S. aureus* V8 protease showed that the N and P proteins synthesized in vitro were identical to those synthesized in vivo (Fig. 2b).

It appeared from cell-free translation studies (Fig. 2a) that RNA extracted from mumps virus-infected cells at 24 h post-infection was not enriched in virus-specific mRNA as compared to the 18 h sample. This agreed with the results of time course studies of polypeptide synthesis by labelling with $[^{35}S]$methionine or of RNA by labelling with $^{32}P$, in the presence of actinomycin D, which indicated that maximal virus genome expression occurred 4 to 6 h before the formation of complete syncytia at 18 to 20 h after infection and then remained steady until the end of infection (24 h) when the cells fell off the glass substrate. Cell-free translation studies and polypeptide labelling with $[^{35}S]$methionine also indicated that treatment of infected cells with actinomycin D at 2 μg/ml for 2 h prior to RNA extraction did not produce the enrichment for virus-specific mRNA which has been observed with respiratory syncytial virus (Collins & Wertz, 1983).

**Preparation of cDNA clones**

Infected cell pA+ RNA was reverse-transcribed after priming with oligo(dT)$_{12-18}$. Yields of 10 to 20% for cDNA were routinely obtained. The resulting cDNA/mRNA hybrids were directly extended with 8 to 20 residues of dCMP and these were annealed with PstI-cut pAT153 DNA extended with homopolymeric dGMP tails (10 to 20 residues per 3' end). Aliquots (10 ng) of cDNA/mRNA annealed to equimolar amounts of tailed plasmid DNA were used to transform *E. coli* ED8767. Clones carrying plasmids of the correct antibiotic resistance phenotype were selected. In several experiments about 10 to 20 ampicillin-sensitive, tetracycline-resistant colonies were obtained per nanogram of hybrid. Virus-specific clones were identified by colony hybridizations according to Grunstein & Hogness (1975) with probes prepared by reverse transcription of pA+ RNA from infected cells (+) or mock-infected cells (−) in the presence of $[^{32}P]$dCTP or alternatively by colony hybridization with a $^{32}P$-labelled probe prepared by reverse transcription of pA+ RNA from infected cells followed by hybridization of the resulting cDNA to at least a 100-fold excess of pA+ RNA from mock-infected cells. Of the 800 colonies obtained from Enders virus-infected cell pA+ RNA, only about 3% appeared to be virus-specific. More colonies were judged to be virus-specific by the
Table 1. Description of mumps virus-specific clones obtained

<table>
<thead>
<tr>
<th>Source of pA + RNA</th>
<th>Clone number</th>
<th>Size of PstI-released insert (bp)</th>
<th>Designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enders</td>
<td>pMuE64</td>
<td>340</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>pMuE163</td>
<td>200</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>pMuE182</td>
<td>300</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>pMuE187</td>
<td>350</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>pMuE190</td>
<td>850</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>pMuE217</td>
<td>150</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>pMuE225</td>
<td>590</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>pMuE315</td>
<td>620</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>pMuE324</td>
<td>800</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>pMuE408</td>
<td>200</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>pMuE438</td>
<td>850</td>
<td>P</td>
</tr>
<tr>
<td>Belfast</td>
<td>pMuBF77</td>
<td>630 + 370</td>
<td>N</td>
</tr>
</tbody>
</table>

plus/minus probing system than with the 'blocked' probe. The latter appeared thus more discriminating but false negatives have been observed. In addition to Enders virus clones, 125 recombinant clones were generated from pA + RNA from BF virus-infected cells of which only three appeared to be virus-specific with the plus/minus probing system. One of these, pMuBF77, which was also judged virus-specific with a blocked probe was characterized. Eleven clones of Enders origin have also been characterized (see Table 1).

Characterization of cDNA clones

The clones were analysed by sizing the inserts, released after digestion with PstI, on polyacrylamide gels. Those with inserts > 150 bp were further characterized by hybridization experiments. Northern blots of pA + RNA extracted from Enders-, BF- or mock-infected Vero cells, resolved on denaturing agarose gels, were hybridized with recombinant plasmid DNAs labelled by nick translation (Rigby et al., 1977) in the presence of [32P]dCTP. Probes prepared from pMuE187, pMuE190 and pMuBF77 hybridized strongly to BF- and Enders-infected cell pA + RNA. No hybridization signals were observed in mock-infected cell RNAs, confirming the virus specificity of the clones. Experiments in which radioactive pA + RNA labelled in vivo with 32P, in the presence of actinomycin D was hybridized to nitrocellulose-bound plasmid DNA (Fig. 3), indicated that all clones tested so far either selected an mRNA of mol. wt. 0-69 x 10^6 or an mRNA of mol. wt. 0-51 x 10^6, the suggested N and P mRNAs from in vitro translation experiments carried out on separated mRNA bands (E. J. B. Simpson, J. A. Curran, E. M. Hoey, S. J. Martin & B. K. Rima, unpublished results). However all these clones also selected an RNA with approximate mol. wt. of 1·18 x 10^6, probably representing polycistrionic transcripts including the P gene sequence. In addition, these clones also consistently selected the N mRNA band whereas N gene-specific clones did not appear to select the P mRNA band. Recombinant clones of the N gene did not appear to select the higher mol. wt. bands.

Unlabelled RNA species were also allowed to hybridize to the filter-bound plasmid DNAs and after release were used in in vitro translation. Such hybrid-select translations of selected clones (Fig. 4) indicated that those selecting the 0-69 x 10^6 mol. wt. RNA encoded the N protein and those selecting the 0-5 x 10^6 mol. wt. RNA the P protein of mumps virus. These experiments thus linked six clones to the N protein of Enders virus, and pMuBF77 to the N protein of BF virus, and to the mRNA band of mol. wt. 0-69 x 10^6. Five clones were linked to the P gene of mumps virus and the mRNA of 0-51 x 10^6 mol. wt.

DISCUSSION

This paper describes the first characterization of cDNA clones specific for the N and P genes of mumps virus. These clones have allowed the identification of the N and P mRNAs in mumps virus-infected cells as species of mol. wt. 0-69 x 10^6 and 0-51 x 10^6 respectively. Other minor
bands of pA+ RNAs were found to be virus-induced but their specificity is as yet unknown. The specificity of the characterized cDNA clones has been established from their ability to bind to pA+ RNA in Northern blotting experiments, by their ability to select radioactive messenger RNAs of the appropriate mol. wt. and by their ability in hybrid-select translation experiments to direct the synthesis of the N and P proteins. The size of the N and P mRNAs induced in the infected cells are 2100 and 1500 nucleotides respectively and they are comparable in this respect to those reported for the N and P mRNAs of Newcastle disease virus, which are 2280 and 1600 nucleotides, respectively (Thomas et al., 1978), but not to those for Sendai virus which have been reported to be 2100 and 2400 nucleotides for the N and P mRNAs (Gupta et al., 1983). This observation, and the similarity between mumps virus and Newcastle disease virus in the generation of non-structural proteins in contrast to Sendai virus (Collins et al., 1982; Giorgi et al., 1983; E. J. B. Simpson, J. A. Curran, E. M. Hoey, S. J. Martin & B. K. Rima, unpublished results) indicate that mumps virus appears to be more closely related to Newcastle disease virus than to Sendai virus amongst the paramyxoviruses.

In addition to selecting P mRNA, P gene-specific clones also selected two higher mol. wt. pA+ RNAs (1.18 × 10^6 and 1.55 × 10^6) which may represent bi- and tricistronic transcripts of the viral genome. The RNA of mol. wt. 1.18 × 10^6 probably represents a P–M bicistronic RNA, since none of the N-specific clones and all of the P-specific cDNA clones in several experiments selected this band. The weak selection of the N mRNA by the P clones is difficult to explain. Hybrid-select experiments with different clones of this type all confirm that they select primarily a mRNA encoding the P protein. It is unlikely that all these clones represent cDNAs originating from N–P bicistronic mRNAs which have not been identified in Enders mumps
Fig. 4. Hybrid-select translations. Non-labelled RNA from virus-infected cells, selected by recombinant plasmid DNA bound to nitrocellulose was translated in vitro in rabbit reticulocyte lysates in the presence of $^{[35]}$S)methionine. Products were analysed on a 10% polyacrylamide gel. (a) Lane 1, in vivo labelled Enders mumps virus polypeptides; lane 2, in vivo labelled BF mumps virus polypeptides; lane 3, in vitro translated BF mumps virus pA+ RNA; lane 4, in vitro translated BF mumps virus pA+ RNA selected by pAT153 plasmid DNA; lane 5, in vitro translated BF mumps virus pA+ RNA selected by pMuE190 plasmid DNA; lane 6, control: no RNA added to translation mixture. (b) Lane 1, in vitro translated Enders mumps virus pA+ RNA; lane 2, in vitro translated Enders mumps virus pA+ RNA selected by pMuE182; lane 3, in vitro translated Enders mumps virus pA+ RNA selected by pAT153.

virus-infected cells in these experiments. Polycistronic mRNAs have now been reported in a number of non-segmented negative-stranded virus-infected cells such as vesicular stomatitis virus (Herman et al., 1980), Newcastle disease virus (Collins et al., 1982), respiratory syncytial virus (Collins & Wertz, 1983), measles virus (Udem & Cook, 1984) and canine distemper virus (Barrett & Mahy, 1984; Russell et al., 1985).

Clones specific for the M gene or the HN and F genes have not been identified in this investigation. The level of M protein and probably therefore M mRNA has been found to be low in cells infected with our present stocks of Enders and BF mumps virus as judged from in vivo and in vitro labelled polypeptide profiles. It is also possible that because of the low level of M gene expression, M-specific cDNA clones would not have been identified by the colony hybridization techniques used, as these plus/minus systems are only found suitable for mRNAs representing more than 0.1% of the total mRNA population (Williams & Lloyd, 1979; Dworkin & Dawid, 1980). Since we found less than 3% of our cDNA clones to be virus-specific and since most of the radioactivity in pA+ RNA preparations appeared to be in the N and P mRNAs, the detection of M-specific cDNA clones may be difficult with this technique. The low levels of the M mRNA and protein which are exemplified by the comparison of the pA+ RNA induced by mumps virus strains with those of measles and canine distemper virus may be partly explained by the presence of a large amount of the P–M bicistronic RNA. Such an RNA molecule is probably able to direct the translation of only the P protein in vivo, as has been suggested by Herman et al. (1980) for vesicular stomatitis virus. The aberrant expression of M protein by paramyxoviruses has been suggested to be related to persistent infection in animal models,
subacute sclerosing panencephalitis patients and in tissue culture systems. In this respect, it is notable that our virus stocks established persistent infection very readily (unpublished observations). Also, it has recently been shown that whereas in mumps virus-infected hamster brain, the virus-specific N, P, M, HN and F antigens are readily detectable, in a more restricted infection in mouse brain only the N and P antigens are detectable (Kristensson et al., 1984).

In conclusion, we have obtained gene-specific probes for the mumps virus nucleocapsid and nucleocapsid-associated protein genes and these cloned cDNAs can now be applied to pathogenesis studies as well as to studies of gene expression in abortive, lytic and persistent infections by this virus.

We would like to thank the U.K. Medical Research Council for grant support during the course of this study. J. A. Curran was supported by a DENI postgraduate scholarship and the MRC and J. P. Quinn was supported by an MRC recombinant DNA training fellowship. We thank Miss C. Lyons for the tissue culture work associated with this project, the Secretarial Centre for typing the manuscript and Dr S. E. H. Russell and Dr J. D. Hull for pA+ RNAs of canine distemper virus- and measles virus-infected cells respectively.

REFERENCES


cDNA cloning of mumps virus 985


(Received 16 October 1984)