Virus and host cell-dependent variation in transcription of the mumps virus genome

M. A. Afzal, G. D. Elliott, B. K. Rima* and C. Örvell

1Northern Ireland Centre for Genetic Engineering, The Queen's University of Belfast, Belfast BT9 7BL, Northern Ireland and 2Statens Bakteriologiska Laboratoriet, S-105 21 Stockholm, Sweden

Evidence has been presented that generation of polycistronic readthrough RNAs in mumps virus-infected cells is not a simple stochastic process with strain-dependent variations in the generation of certain readthrough products, but that this process is affected by host as well as viral factors. RNAs extracted from infected Vero cells or chicken embryo fibroblast (CEF) cells have been analysed by Northern blotting with virus-specific probes for the nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), small hydrophobic (SH) and haemagglutinin-neuraminidase (HN) genes. Vero cells infected with tissue culture cell-adapted virus strains generate monocistronic as well as polycistronic RNAs. Transcription analysis of Vero cells infected with an egg-adapted strain reveal the absence of monocistronic M and F transcripts, with a concomitant increase in readthrough transcripts involving these genes. When the same virus infects CEF cells monocistronic RNAs accumulate. The presence of viral proteins in the various virus/host cell combinations assessed by immunofluorescence with mumps virus-specific monoclonal antibodies for the N, P, M, F and HN proteins correlates well with the patterns of transcription.

Introduction

Much is now known about paramyxovirus transcription and replication. The negative-stranded genome of these viruses is transcribed into six or seven transcripts, each of which encodes one protein, with the exception of the second gene from the 3' end [the phosphoprotein (P) gene], which generally encodes two or more proteins (Pringle, 1987). Occasionally, by a stochastic process and with a constant frequency the intergenic signals between the genes are not recognized by the transcriptase complex, leading to the synthesis of so-called bicistronic or polycistronic transcripts. These readthrough transcripts have been described in all non-segmented negative strand viruses analysed so far. Their function, if any, is unclear, but the accumulation of a tandem P-matrix (M) gene transcript in subacute sclerosing panencephalitis (SSPE) brains (Cattaneo et al., 1986) probably leads to the reduced expression of the M protein, because the single 5' start site for eukaryotic translation leads to lack of translation of the M-encoding sequences from such transcripts (Wong & Hirano, 1987). The gene order, a physical map and a transcription map have recently been described for mumps virus (Elango et al., 1988; Elliott et al., 1989), which show that mumps virus is similar to other paramyxoviruses, particularly to simian virus 5 and that its gene order is 3' N-P-M-F-SH-HN-L 5', where N, F, SH, HN and L represent the genes for the nucleocapsid, fusion, small hydrophobic, haemagglutinin-neuraminidase and large proteins, respectively. Analysis of transcription by Northern blots showed that in mumps virus-infected Vero cells bi- and polycistronic RNA species are numerous (Elliott et al., 1989). These are more prevalent in cells infected with our mumps virus strains than in other morbillivirus- or paramyxovirus-infected cells. In this report we show that the generation of such polycistronic RNA species is variable and depends on host and viral factors and that their accumulation affects the expression of some proteins of the virus in infected cells.

Methods

Viruses and cells. The following mumps virus strains were used in this study. The egg- and Vero cell-adapted SBL1 strains, denoted SBL/E and SBL/V, respectively, were obtained from the collection of the State Bacteriological Laboratory in Stockholm (Sweden). The SBL/E strain was propagated as described earlier (Elliott et al., 1989). The Enders strain adapted to Vero cells (End/V) was grown up from plaques picked from the Enders strain, as described by Curran et al. (1985). Vero cells were obtained from Flow Laboratories and grown in Eagle's minimal essential medium (Glasgow modification) supplemented with 2% newborn calf serum and antibiotics, as described before (Rima et al., 1980). Primary chick embryo fibroblast (CEF) cells were prepared as described by Purchase (1980).
cDNA clones, probe preparation and Northern blot analysis. The cDNA clones for the various genes of mumps virus were as described earlier by Elliott et al. (1989) and Northern blot analysis was carried out as described in the same paper. None of the probes used in this paper hybridized to RNA samples extracted from mock-infected Vero or CEF cells.

Immunofluorescence and monoclonal antibodies. Monoclonal antibodies used to study the expression levels of various mumps virus proteins have been designated (Orvell, 1984) as clone 2.142 against the N protein, 2.186 against the M protein, 5.525 against the F protein and 2.048 against the HN protein. For immunofluorescence studies confluent Vero cell monolayers were grown on microscope slides and infected with mumps virus at an m.o.i. of 0.1 p.f.u. per cell. After 27 to 30 h post-infection, when c.p.e. were pronounced, the slides were fixed in cold (−20 °C) acetone for 5 min, then air-dried and monoclonal antibodies were used as 1:50 dilutions of ascites fluids. After a 1 h incubation at 37 °C slides were washed thoroughly with phosphate-buffered saline and further incubated for 1 h with 1:20 dilutions of goat anti-mouse fluorescein-labelled immunoglobulin. After washing and mounting, the slides were examined using a fluorescence microscope at 40 × magnification, photographed and printed at a final magnification of 800 ×.

Results

Analysis of transcription of various mumps virus strains

Transcription of certain strains of mumps virus in Vero cells is characterized by the generation of substantial amounts and numbers of polycistronic transcripts. We have been able to detect bicistronic readthrough transcripts as well as the N–P–M, P–M–F–SH, M–F–SH, M–F–SH–HN and F–SH–HN transcripts in mumps virus (Enders strain)-infected Vero cells (Elliott et al., 1989). During studies comparing transcription of the End/V and SBL/E strains of mumps virus we observed that the generation of readthrough transcripts was different for both strains when poly(A)⁺ RNA extracted from infected cells was probed in Northern blots with probes for the M, N and F genes of mumps virus (Fig. 1). The results showed that whereas the Enders strain allowed accumulation of the viral monocistronic mRNAs for the N, M and the bicistronic F–SH genes, the SBL/E strain did not allow accumulation of the monocistronic N, but not the F and M gene transcripts. Bands with higher Mr were observed prominently in the SBL/E-infected Vero cells and these were likely to represent polycistronic RNAs. In particular the M–F readthrough transcript is present in very high amounts. The band labelled g in this blot was considered to be representative of the genome, as it hybridized with both positive and negative strand probes derived from in vitro transcription (data not shown). We have often observed that oligo(dT) selection of paramyxovirus RNAs does not lead to complete removal of genomic RNA species.

Because of the differences in transcription observed between the SBL/E and End/V strains we analysed the patterns of transcription with not only these viruses but also an SBL strain derivative that was adapted to Vero cells (SBL/V), and in SBL/E-infected primary CEF cells. Northern blots of total RNA were hybridized with probes specific for the N, P, M, F, SH and HN genes of mumps virus.

Fig. 2 shows Northern blots of total RNA extracted from cells infected with the various mumps virus strains described above. The RNAs were hybridized with probes specific for each of the mumps virus genes, except that encoding the L protein. The data indicate that the End/V strain (lane 1) allowed the accumulation of all monocistronic mRNA transcripts in Vero cells in substantial amounts, except for the F gene, which is mostly transcribed into an F–SH readthrough transcript. The polycistronic RNAs are also abundant. The SBL/V strain (lane 2) also allows a similar transcription pattern to be generated in Vero cells. However, the SBL/E strain (lane 3) does not generate monocistronic mRNA transcripts in the infected Vero cells. Besides the genomic-size RNA band one can observe only polycistronic RNA species representing the readthrough transcripts of three or more genes. Particularly notable for
their absence are the M and F gene monocistronic transcripts and the reduced amounts of the N, P, SH–HN and HN gene transcripts. When the same strain, SBL/E, was used to infect CEF cells (lane 4) monocistronic transcripts of the M and F genes were present, indicating that the efficiency of recognition of intergenic sequences is influenced by the host cell. Thus we concluded that transcription patterns of mumps virus are dependent on both host- and virus-specific parameters. In the past we have observed that the patterns obtained
with SBL/V and End/V virus-infected Vero cells vary greatly with different virus pools analysed (data not shown).

These Northern blots thus showed that transcription of certain genes, particularly the M and F genes of SBL/E, was altered and led to the accumulation of large amounts of bi- and polycistronic readthrough transcripts in infected Vero cells.

**Protein synthesis**

In order to attempt to correlate these transcription patterns with the synthesis of mumps proteins we analysed, by immunofluorescence, the ability of Vero cells infected with the End/V, SBL/V and SBL/E strains of mumps virus to bind monoclonal antibodies specific for the N, P, M, F and HN proteins (Örvell, 1984). The data obtained (Fig. 3) indicate that indeed the expression levels of the F and M proteins are very much reduced in the case of the SBL/E-infected Vero cells and this correlated well with the transcription patterns. No fluorescence was observed with any of the monoclonal antibodies in mock-infected Vero cells. The fluorescence in the SBL/E-infected Vero cells was somewhat more granular and indicated the restricted replication of the virus in the cells, as compared to SBL/V and End/V, which gave fluorescence in some large syncytia. Similar data were obtained with several monoclonal antibodies and the results thus indicate that probably very little M and F protein is made in SBL/E-infected Vero cells.

**Discussion**

The data presented in this paper show that the generation of polycistronic mRNAs, which until recently, had been considered to be a stochastic process with possible strain-dependent variations in the generation of certain readthrough RNAs, is also dependent on host cell factors. The consequent reduction in the
amounts of some of the viral proteins have been demonstrated with monoclonal antibodies. So far in no other negative strand, non-segmented RNA virus has the generation of polycistronic RNA species been described to be variable. Recently we have obtained evidence in the measles virus system that mixed infections of standard and defective interfering (DI) virus will not only suppress virus gene transcription and increase the gradient of gene expression (Cattaneo et al., 1986), but also increase the level of one (the F-H) of the bicistronic mRNA species (M. J. Welsh & B. K. Rima, unpublished results). Hence approximately 50% of the F gene transcripts are in the tandem F–H readthrough product in the DI/standard virus coinfection, whereas in the standard virus infection alone only 10% of the H transcripts are in the bicistronic species. Of course the possible importance of bicistronic RNAs has already been suggested to play a role in the reduced expression of the M gene in the case of SSPE virus (Cattaneo et al., 1986). Recently, Wong & Hirano (1987) showed that the second genes cannot be translated from bicistronic RNAs. In the SSPE case described (Cattaneo et al., 1986) no sequence variation was observed in the P–M intergenic region of the SSPE virus genome. Thus the authors suggested that other viral genes (presumably the polymerase) was responsible for the faulty recognition of the P–M intergenic start/stop and polyadenylation signals. On the basis of the evidence given here for mumps virus, host cell factors should also be considered to be of importance in the generation of such bicistronic RNA species.

In conclusion we have demonstrated that the generation of polycistronic transcripts in mumps virus-infected cells and probably in cells infected with other paramyxoviruses is not a stochastic process, but may be under the control of viral and host-specific factors. It will be possible in future to study these factors by the analysis of in vitro transcription assays as well by gene complementation assays.

We thank Dr A. Trudgett for help with the immunofluorescence experiments and the Medical Research Council of the United Kingdom for support under grant G8801654CA.

References


(Received 6 July 1989; Accepted 6 November 1989)