Restricted Synthesis of the Fusion Protein of Measles Virus at Elevated Temperatures

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SUMMARY

The elevation of culture temperatures from 35 °C to 39 °C led to the cessation of the synthesis of the fusion (F) protein of measles virus. This effect was caused by inhibition of the translation of the corresponding mRNA rather than by a decrease in the synthesis or stability of the mRNA or by increased degradation of the F protein at elevated temperatures. The haemagglutinin (H) and F mRNAs were distributed differently in gradients on which polysomes were fractionated. The H mRNA was present almost exclusively in the largest polysomes whereas the F mRNA was more evenly distributed over large and small polysomes. The distribution was not affected by a temperature shift. The inhibition of F protein synthesis thus appeared to be related to a cessation of elongation of the nascent polypeptide chain rather than to a defect in initiation of the translation of the F mRNA at 39 °C.

Recent findings suggest that the synthesis of the membrane (M) protein is affected by temperature elevation in measles virus- (MV) (Ogura et al., 1987) and in Sendai virus- (Ogura et al., 1984) infected cells. In MV, the restriction in protein synthesis is selective for the M protein, because the syntheses of the nucleocapsid (N) protein, phospho- (P) protein and haemagglutinin (H) protein are not affected (Ogura et al., 1987). Furthermore, it is clear that this restriction occurs at the translational rather than at the transcriptional level of gene expression, since elevation of the temperature does not affect the rate of transcription nor the relative stability of the mRNA encoding the M protein. The restricted synthesis of M protein is also not related to rapid degradation of this protein at elevated temperatures and the effect is independent of the virus strain and can be demonstrated in vivo in several host cell lines in which MV can be propagated. It was however, impossible to demonstrate a selective inhibition of the synthesis of M protein when in vitro translation was carried out at different incubation temperatures (Ogura et al., 1987).

In our previous study it had been difficult to assess the effect of temperature elevation on the synthesis of the fusion protein (F) of MV, because we failed to detect this protein reliably by immunoprecipitation. The present paper describes experiments in which we used a set of monoclonal antibodies to the F protein for immunoprecipitations. These allowed reliable detection of the F1 protein so that we could study the effect of temperature elevation. The results indicate that the synthesis of F protein is restricted in a similar way to that of the M protein. We also determined the effect of temperature elevation on the synthesis and stability of the F mRNA and its distribution in different polysome fractions.

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Fig. 1. (a) Mock-infected or Edm-infected Vero cells were labelled at either 35 °C (odd-numbered lanes) or immediately after shift up to 39 °C (even-numbered lanes) with [3H]leucine for 2 h. Lanes 1, 2, 5, 6, 9 and 10, mock-infected cells; lanes 3, 4, 7, 8, 11 and 12, Edm-infected cells. Lysates were immunoprecipitated with hyperimmune serum (lanes 1 to 4), a mixture of monoclonal antibodies to the H and M proteins (lanes 5 to 8) or a mixture of monoclonal antibodies to the H and F proteins (lanes 9 to 12). Analysis on a 10% polyacrylamide gel was carried out as described before (Ogura et al., 1987). The position of the F1 protein in lane 4 is indicated by an open triangle. (b) Mock-infected cells (lanes 1, 9) or Edm-infected Vero cells (all other lanes) were labelled with [35S]methionine for 1 h at 26 h p.i. at 35 °C (lanes 2, 10) and chased for either 1 h (lanes 3, 6, 11, 14), 3 h (lanes 4, 7, 12, 15) or 6 h (lanes 5, 8, 13, 16) at 35 °C (lanes 3 to 5 and 11 to 13) or at 39 °C (lanes 6 to 8 and 14 to 16). The lysates were immunoprecipitated with hyperimmune serum (lanes 1 to 8) or monoclonal antibodies to the F protein of MV (lanes 9 to 16). 14C-labelled marker proteins (lane M) were myosin (200K), phosphorylase (92.5K), bovine serum albumin (69K), ovalbumin (46K) and carbonic anhydrase (30K). Analysis was on a 10% polyacrylamide gel.

In preliminary experiments, in which Vero cells infected with the Edmonston strain (Edm) of MV were labelled at temperatures ranging from 33 °C to 45 °C (2 °C intervals), we demonstrated that while synthesis of the N and H proteins was detectable at temperatures over 41 °C, that of the M and F proteins was markedly reduced at 37 °C and absent at 39 °C. Further experiments were carried out by shifting incubation temperatures from the highest permissive (35 °C) to the lowest restrictive (39 °C) temperature. Mock-infected and Edm-infected Vero cells were kept at 35 °C until 26 h post-infection (p.i.) at which time they were either further incubated at that temperature or shifted to 39 °C and labelled immediately with [3H]leucine (sp. act. 160 Ci/mmol, 100 μCi/ml). The lysates were then immunoprecipitated with hyperimmune serum, a mixture of monoclonal antibodies to the H and M proteins, or a mixture of monoclonal antibodies to the H and F proteins (Fig. 1a). These experiments indicated that immediately after temperature shift the synthesis of the M and F proteins became undetectable, whereas the synthesis of the N protein and the H protein was unaffected. The H protein was preferred as a reference protein in these experiments since the susceptibility of the N and P proteins to proteolysis (Rima, 1983) might have given rise to bands comigrating in the gels with the M and F1 proteins. The observed restriction in the synthesis of the F protein could not be explained by an increased rate of degradation, since as demonstrated in Fig. 1(b) the turnover of [35S]methionine-labelled F protein was not found to be significantly different at 35 °C and at 39 °C and did not differ from the rates of turnover of the H, N and M proteins.

In order to determine whether the reduction in the rate of synthesis of the F protein was related to the synthesis or stability of the viral mRNA encoding this protein, we assessed the synthesis of viral RNA from incorporation of 32P into RNA in the presence of actinomycin D at the two temperatures (Fig. 2a). There was no quantitative difference in the relative amounts of each of the viral mRNAs, bicistronic RNAs and genomic RNA at both temperatures. The designation of the mRNAs has been described earlier (Ogura et al., 1987). The absolute amount
Fig. 2. (a) Mock-infected cells (lanes 1, 3) and Edm-infected Vero cells (lanes 2, 4) were incubated at 35 °C until 24 h p.i. and then incubated in phosphate-free medium supplemented with 5% dialysed foetal calf serum for 30 min. The medium was then replaced by phosphate-free medium containing 5% dialysed calf serum and 10 μg/ml actinomycin D. After 30 min incubation the cells were labelled with 500 μCi/ml 32P (carrier-free) in the presence of 10 μg/ml actinomycin D at either 35 °C (lanes 1, 2) or after shift up to 39 °C (lanes 3, 4) for 6 h. RNA was extracted as described before (Ogura et al., 1987) and electrophoresed on 1% agarose-formaldehyde gels. The positions of some host bands (host), the viral genome (gen.), bicistronic mRNAs (bic.) and the L, F, H, N, P and M mRNAs are indicated. (b) Northern blot analysis of RNA extracted from mock-infected cells (lanes 1, 2, 5, 6, 9 and 10) or Edm-infected Vero cells (lanes 3, 4, 7, 8, 11 and 12) incubated at 35 °C (odd-numbered lanes) or 2 h after shift up to 39 °C (even-numbered lanes). Equal amounts of RNA extracted from infected cells incubated at 35 °C or 39 °C were electrophoresed on 1% agarose-formaldehyde gels and Northern blots were prepared as described before (Ogura et al., 1987) using mixed probes (lanes 9 to 12), an F gene-specific probe (lanes 5 to 8) or an M gene-specific probe (lanes 1 to 4). Labelling of bands was as in (a).

of all viral RNAs appeared slightly reduced after temperature shift but this could certainly not explain the immediate and selective inhibition of the synthesis of the F and M proteins. Furthermore, Northern blot analysis (Fig. 2b) of RNAs extracted from mock-infected and Edm-infected Vero cells kept at 35 °C or shifted for 2 h to 39 °C indicated no differences in the total amounts of the mRNAs when the blots were probed with a mixed probe containing sequences from the N, P, M, F and H genes described by Ogura et al. (1987). It was particularly clear that the amounts of F and M mRNAs did not differ significantly when the Northern blots were incubated with cDNA probes specific for the M and F genes.

The previous experiments indicated that the concentration or synthesis of the mRNAs encoding the F protein were not affected specifically by elevation of the incubation temperature. We attempted to assess whether the shift of temperature coincided with a release of the ribosomes from the F mRNA by comparing polysomes extracted from infected cells maintained at 35 °C or shifted for 30 min to 39 °C. Polysomes were extracted from infected Vero cells according to the method of Ballinger & Pardue (1983). Fig. 3(a) shows the absorbance profiles at
Fig. 3. (a) Absorbance profiles of sucrose gradients. Extracts from Edm-infected Vero cells incubated at 35 °C and 39 °C for 30 min were layered onto 12% to 50% isokinetic sucrose gradients made up in 0.02 M-HEPES pH 7.3, 0.5 M-KCl, 0.005 M-MgCl₂ and 0.1% Triton X-100. Centrifugation was for 135 min at 36000 r.p.m. in a Beckman SW41 rotor at 4 °C. Absorbance at 254 nm was monitored with an Isco type 4 optical unit connected to a recorder. The direction of sedimentation is from left (top, T) to right (bottom, B). (b) Autoradiograms of RNA in different gradient fractions. Fractions were collected from the bottom of the sucrose gradients. Total RNA from each fraction was obtained by phenol extraction and analysed on a 1% agarose-formaldehyde gel, transferred to nitrocellulose, and hybridized to nick-translated insert cDNA representing parts of the F and H genes of MV. The position of the bicistronic RNAs (bic.) and the F and H mRNAs are indicated. Lanes C contained poly(A)+ RNA extracted from Edm-infected Vero cells.

254 nm of gradients on which polysomes were fractionated. Except for the small increase in monosomes at 39 °C known to be associated with a mild heat shock response (McCormick & Penman, 1968) there was no significant difference in the overall profiles at either temperature. When the RNA was extracted from each of the gradient fractions and analysed by electrophoresis and Northern blotting followed by hybridization to the radiolabelled insert of a clone spanning the F–H boundary, containing 326 nucleotides from the F gene of MV and 88 nucleotides from the H gene as well as the intergenic sequence (Fig. 3b) (Billeter et al., 1984), it became clear that, whereas the largest amount of mRNA encoding the H protein was found in
the fractions containing the largest polysomes at both temperatures, the F mRNA appeared to be more evenly distributed over the whole gradient at 35 °C and at 39 °C indicating that this mRNA was present in smaller polysomes at both temperatures. This may be related to the observed low efficiency of translation of the F mRNA since the F protein was synthesized in very small amounts compared to the H protein even though more F mRNA is present in the cells than H mRNA. However, the polysome experiments did not show the dramatic shift of the F mRNA from polysomal to monosomal fractions expected if the mechanism of restriction of protein synthesis was to be at the level of initiation of protein synthesis and if run-off could occur at the elevated temperature. On the contrary, these experiments would suggest that the ribosomes remain attached to the mRNA. Similar results were obtained with probes for the M-specific mRNA (data not shown).

These experiments also indicate that in MV as well as in Newcastle disease virus (Wilde & Morrison, 1984) the bicistronic mRNAs are in the polysomal fractions and, therefore, presumably translated. Recently, Wong & Hirano (1987) have shown that probably only the 5' proximal coding sequence is translated from MV bicistronic mRNAs in vivo. A number of F gene-specific degradation products have also been identified in these Northern blots, but it is difficult to determine whether these are extraction artefacts or coupled to the translation of this mRNA.

In conclusion we have demonstrated that the synthesis of the F protein in MV-infected cells is restricted in a similar way to that of the M protein and that this defect occurs at the level of translation of these mRNAs. We are not able to assess the temperature effect on the F protein in in vitro translation experiments as was done for the synthesis of the M protein, because F protein-related products or precursors have not yet been reliably identified in in vitro translations directed by poly(A)+ RNA extracted from MV-infected cells. The mechanism of the selective inhibition of the synthesis of these two proteins is not clear at present and a simple failure of the ribosomes to attach to the mRNAs at higher temperatures is not indicated. The ribosomes appear to remain attached to the mRNA encoding the M and F proteins and this could also explain the rapid onset of the synthesis of the M protein after reduction of the temperature from 39 °C to 35 °C (Ogura et al., 1987). We are attempting to study these phenomena in a eukaryotic expression system directed by M and F gene-containing plasmids. The specific restriction of the synthesis of the M and F proteins could be important in the restriction of virus budding and cell-to-cell spread of MV which occurs at the elevated temperatures associated with natural measles infection. This might provide a mechanism by which the virus may be driven into a persistent state during periods of fever in the affected individual.

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REFERENCES


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