Synthesis of a Unique Polypeptide in Measles Virus-infected BGM Cells

(Accepted 25 May 1982)

SUMMARY

In measles virus-infected BGM (African green monkey kidney) cells, a unique polypeptide (84K) was identified. This polypeptide was not found in other cell lines examined and could be immunoprecipitated with anti-NP monoclonal antibody. Peptide mapping studies confirmed that it contained all the peptides of the 60K NP plus several additional ones. Pulse–chase experiments failed to establish it as a precursor of the 60K polypeptide. The possible origin of this protein is discussed.

Measles virus, a member of the morbillivirus subgroup of the genus paramyxovirus, contains six structural polypeptides. The L protein is probably, by analogy with other viruses, associated with transcription, whereas the nuclear protein (NP), the phosphoprotein (P), and possibly the matrix (M) proteins play an ancillary role (Seifreid et al., 1978). There are two membrane-associated antigens, the haemagglutinin (HA) and the haemolysin or fusion polypeptide (F), both of which are glycosylated (Hardwick & Bussell, 1978), but the F protein is cleaved into a non-glycosylated (F1) and a highly glycosylated (F2) portion. No non-structural proteins have been described for measles virus infections.

Measles virus replicates in a number of cell types (Mottet & Szanton, 1961; Joseph et al., 1975). The host cell may regulate or modify the course of the virus infection and this may act on transcription, protein synthesis or processing. Recently, Fujinami & Oldstone (1981) have shown that certain lymphoblastoid cell lines lack the protease necessary for the cleavage of the F protein of measles virus. During our study of measles virus infections in a number of cell lines, we found that infection of BGM (African green monkey kidney) cells with the Hallé strain of measles virus produced an additional polypeptide that was not found in the other cell lines investigated (Vero, Quokka, HeLa, HEp2 and MRC5). In the present communication, we have characterized this polypeptide.

Monolayer cultures of BGM cells (2 × 10⁶ cells) were infected with the Hallé strain of measles virus (3 p.f.u./cell) and incubated in Eagle’s medium containing 2% foetal calf serum at 37 °C. Twenty-six h after infection, cells were labelled with 50 μCi/ml [35S]methionine (1000 mCi/ mmol, Amersham International) for 2 h in methionine-free medium and then solubilized in RIPA buffer (0.1 M-NaCl, 10⁻³ M-EDTA, 0.02 M-Tris pH 7.2, 1% Triton X-100, 1% DOC, 0.1% SDS) containing 0.28 trypsin-inhibiting units/ml aprotinin. The extracts were subjected to immunoprecipitation as described previously (Giraudon & Wild, 1981), and analysed by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) according to the method of Laemmli (1970) (Fig. 1). Immunoprecipitation with polyclonal anti-measles virus serum (lane 1) showed that besides the normal measles virus-induced polypeptides, there was an additional band at 84K. When immunoprecipitations were performed with measles virus monoclonal antibodies (Giraudon & Wild, 1981), the NP monoclonal antibodies precipitated both the normal 60K polypeptide and also the 84K protein. None of the other monoclonal antibodies available (anti-HA, M, L and P) precipitated the 84K band (data not shown).

The relationship between the two polypeptides was confirmed by peptide mapping. The [35S]-methionine-labelled polypeptides (84K, 60K and also the P polypeptide, 70K) were excised from the gels and, after digestion with trypsin, analysed in a two-dimensional system (Fig. 2). The 84K polypeptide contained all the peptides found in the 60K protein plus a number of additional ones.

To examine the possibility that the 84K polypeptide is a precursor of the 60K NP, cells were labelled 24 h after infection with [35S]methionine for 10 min and subsequently chased with an excess of unlabelled methionine for 2 h. Cell lysates were prepared from the ‘pulsed’ and the
Fig. 1. Immunoprecipitation and SDS–PAGE of 35S-labelled measles virus-induced proteins in BGM cells. Cells were labelled with [35S]methionine for either 2 h (lanes 1 to 4), 10 min (lane 5), or 10 min followed by a 2 h chase with unlabelled methionine (lanes 7 and 8). The extracts were immunoprecipitated with either polyclonal antibody (lanes 1, 5 and 8), anti-NP monoclonal antibody (lanes 2 and 4), anti-HA monoclonal antibody (lane 3) or a negative serum (lane 7). Lane 6 shows the 14C-labelled mol. wt. standard proteins: myosin (200K), phosphorylase b (92.5K), bovine serum albumin (69K), ovalbumin (46K) and carbonic anhydrase (30K).

'chased' samples, and subsequently immunoprecipitated and analysed by SDS–PAGE (Fig. 1, lanes 5, 7, 8). Densitometer tracing (not shown) of Fig. 1 (lanes 5 and 8) showed that the relative proportions of the 35S in the 84K and 60K bands remained constant during the chase period (1:2.2). This would suggest that in BGM cells, the 84K polypeptide is not a precursor of the 60K NP. The non-cleavage of the 84K polypeptide may arise from the presence of only limited amounts of a specific protease, as has been observed in measles-infected Ramos and Daudi cells for the cleavage of the F protein (Fujinami & Oldstone, 1981). This was checked by mixing cell lysates from 35S-labelled measles-infected BGM cells prepared in the absence of aprotinin with
Fig. 2. Two-dimensional peptide map analysis of ^35S-labelled measles virus-induced polypeptides isolated from BGM cells. Peptide mapping was performed according to Gibson (1974). The polypeptide bands radiolabelled with ^35S)methionine were excised from polyacrylamide gels in 0.5 M-ammonium bicarbonate pH 8, containing 50 μg/ml trypsin (Worthington) at 37 °C for 18 h. The supernatants were lyophilized, resuspended in 10 μl acetic acid : formic acid : water (15 : 5 : 80, by vol.) and electrophoresed at 800 V in the same buffer on cellulose plates (10 × 10 cm, Merck). The oligopeptides were further separated in a second dimension by chromatography in butanol : pyridine : acetic acid : water (32.5 : 25 : 5 : 20, by vol.) plus 7% (w/w) PPO. After drying, the ^35S-labelled oligopeptides were detected by autoradiography. (a) 60K NP; (b) 84K polypeptide; (c) P polypeptide.
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those from unlabelled infected HeLa cells. After incubation at 37 °C for 1 h, the virus proteins were immunoprecipitated and analysed by SDS–PAGE (gels not shown). The 84K polypeptide was not cleaved.

The relationship between the 84K and 60K NP proteins is obscure. However, Zweig et al. (1980) have described an analogous system for herpes simplex viruses 1 and 2. In their system, anti-NP serum (monoclonal and polyclonal) precipitates a 40K and an 80K protein, and as in the measles–BGM system, the larger protein does not act as a precursor. Stallcup & Fields (1981) have described a measles virus-induced protein of 90K in CV-1 cells but were unable to establish its identity.

It is not clear how the 84K measles virus-induced protein arises but several possibilities can be suggested. (i) The 84K protein corresponds to the full potential of the NP mRNA and so the normal 60K protein could represent a pre-terminated state. (ii) There is a ‘read-through’ during transcription. This could occur either directly or by the process of ‘chattering’ (Herman et al., 1980). (iii) The anti-genome (+) strand could act as an mRNA.

It is evident that the virus–cell interaction can be modified at several levels. The result may determine the infection produced. It is obvious that the study of host cell factors in measles virus replication is important in the understanding of the various types of infection that the virus can produce.

This work was supported by a grant from I.N.S.E.R.M., C.R.L. no. 80 1041. We would like to thank Mme Jacquier for the photography and Mme Perret and Miss Mary for typing the article.

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REFERENCES


(Received 18 March 1982)