Temperature elevation enhances cell surface expression of measles virus fusion protein in infected cells

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Cell fusion proceeded gradually in measles virus-infected cells incubated at 35 °C. Shift-up of incubation temperature to 39 °C induced rapidly increased cell fusion in spite of the cessation of *de novo* synthesis of the fusion (F) protein. Pulse–chase experiments showed that there was little difference in the acquisition of immunoreactivity by haemagglutinin (H) and F proteins between the two temperatures. H protein was detected on the cell surface 60 min after the chase at either temperature. However, appearance of F protein on the cell surface took less than 3 h at 39 °C whereas it took 5 h at 35 °C. These data indicate that temperature elevation induces more efficient expression of F protein on the cell surface accompanied by marked syncytium formation in measles virus-infected cells.

The major cytopathic effect in measles virus (MV) infection *in vitro* is cell fusion. This membrane fusion occurs via cell-to-cell contact of infected cells with infected or uninfected cells. In natural hosts, such syncytium formation by MV is known as giant cell pneumonia (Enders *et al.*, 1959). The fusion (F) protein is responsible for this phenomenon (Graves *et al.*, 1978) and also directs virus penetration into the host cell. Thus, the F protein of MV appears to have crucial roles for its pathogenesis, similar to those of other paramyxoviruses. Recently we reported that the elevation of culture temperature from 35 °C to 39 °C led to the selective cessation of synthesis of the F protein of MV (Ogura *et al.*, 1988) along with that of the membrane (M) protein (Ogura *et al.*, 1987). It was concluded that this restriction occurs at the translational level of gene expression because elevation of the temperature does not affect the rate of transcription nor the relative stability of either F or M mRNAs or proteins. In the course of these studies we observed that after temperature elevation of MV-infected cells, syncytium formation increased drastically. In the present study we analysed the intracellular processing of glycoproteins F and haemagglutinin (H) by pulse–chase experiments after temperature elevation.

HeLa cells used in this work were cultured in Dulbecco's modified Eagle's medium supplemented with 5% foetal calf serum at 35 °C. The cells were infected with MV (Edmonston strain) at an m.o.i. of 2 and incubated at 35 °C for 24 h. After labelling with 200 µCi/ml of [35S]methionine (1154 Ci/mmol; American Radiolabeled Chemicals) for appropriate periods at 35 °C, the cells were chased in the presence of 2 mM unlabelled methionine at 35 °C and 39 °C and viral proteins were analysed by immunoprecipitation. For total cell immunoprecipitation, the chased cells were solubilized in radioimmune precipitation assay (RIPA) detergent buffer (Ogura *et al.*, 1987) and immunoprecipitated with anti-H or anti-F monoclonal antibody (kindly provided by Professor V. ter Meulen, Würzburg, F.R.G.) and the immunoprecipitates were subjected to SDS–PAGE. For cell surface immunoprecipitation the chased cells were washed with cold phosphate-buffered saline (PBS). Monoclonal antibodies were added and the cells were incubated at 4 °C for 15 min. The cells were washed with ice-cold PBS five times to remove excess antibody and solubilized in RIPA detergent buffer. The lysates were precipitated by Protein A–Sepharose CL-4B and analysed by SDS–PAGE. The fusion index was defined as the ratio of the number of nuclei present in polykaryocytes to the total number of nuclei in a given microscopic field according to the method of Reeve & Poste (1971).

Monolayers of HeLa cells infected with MV were shifted from 35 °C to 39 °C at 24 h post-infection (p.i.). A kinetic study of syncytium formation was performed. As shown in Fig. 1, the fusion index at 24 h p.i. was 10% and gradually increased to 23% at 29 h p.i. at 35 °C, whereas temperature elevation resulted in a fusion index of 75% 5 h after shift-up at 24 h p.i. This phenomenon was also observed in cells infected with other strains of MV (Braxator and Woodfolk strains) and productive subacute sclerosing panencephalitis virus (Halle,
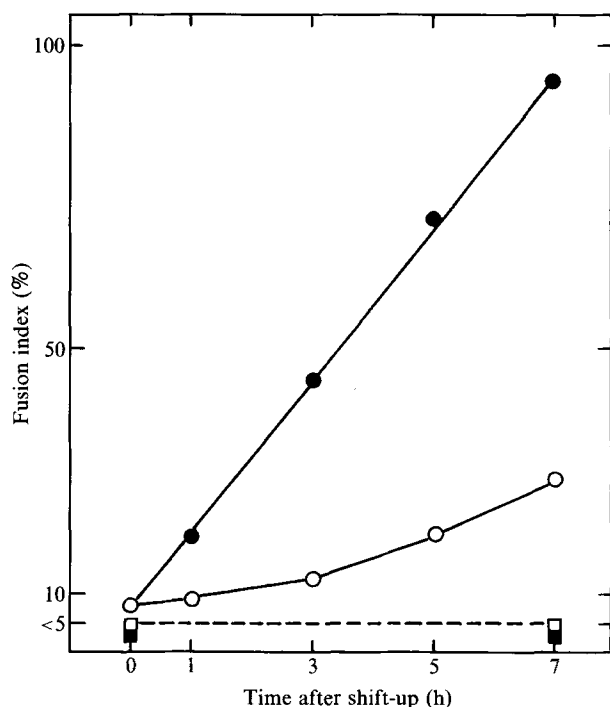
Fig. 1. Cell fusion of MV-infected HeLa cells after shift-up of incubation temperature from 35 °C to 39 °C. Monolayers of HeLa cells on coverslips were infected with MV at an m.o.i. of 2 and incubated at 35 °C. At 24 h p.i., one part was further incubated at 35 °C (O) and the other was shifted to 39 °C (●). At the times (h) indicated the percentage of polykaryocytosis was determined. Mock infection was at 35 °C (□) or 39 °C (■).

Mantooth and Lec strains) (data not shown). The synthesis of F protein was inhibited immediately after shift-up (Ogura *et al.*, 1988) so F protein synthesized before shift-up appeared to be responsible for the above phenomenon.

To analyse the intracellular processing of F protein at both temperatures, MV-infected HeLa cells were pulse-labelled with [^{35}S]methionine for 20 min at 35 °C and then chased for increasing periods of time at 35 °C or 39 °C. At either temperature, F_1 and F_2 proteins were first detected after 30 and 60 min of chase, respectively (Fig. 2). Their amounts increased gradually during subsequent incubation at 35 °C and 39 °C, suggesting that the acquisition of immunoreactivity by the F protein was gradual and similar at both temperatures. On the other hand, H protein appeared at the end of pulse-labelling and reached a maximum level by 60 min of the chase at either temperature (Fig. 2). After 60 min of chase, the H protein band shifted toward a slower electrophoretic mobility, reflecting the processing pathway from high mannose to complex type sugars. There seemed to be no significant difference in glycosylation of H protein between the two temperatures.

In order to examine the effect of temperature elevation on the cell surface expression of F and H proteins, pulse-
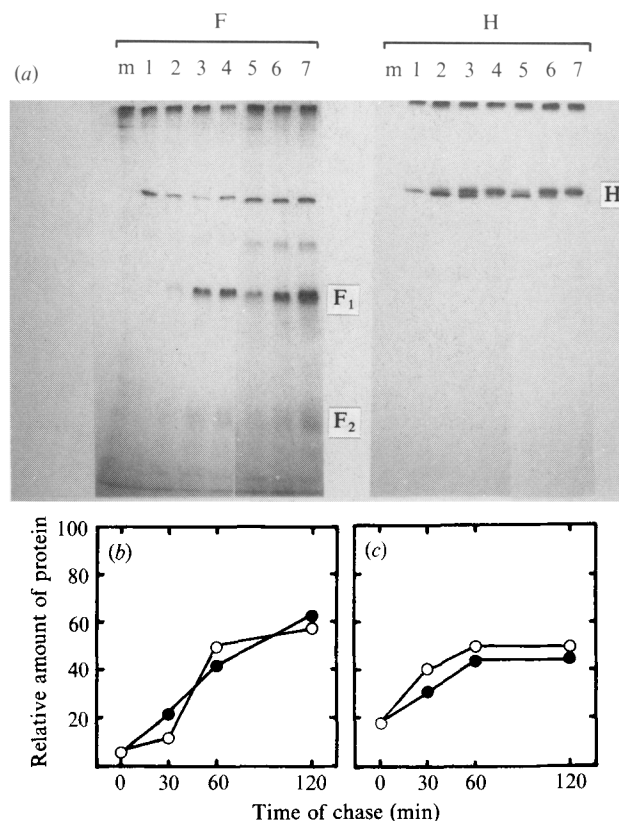

Fig. 2. Total cell immunoprecipitation of pulse-labelled and chased F and H proteins. HeLa cells in 3·5 cm diameter Petri dishes were infected with MV as described in the legend to Fig. 1. At 24 h p.i., the cells were pulse-labelled with [^{35}S]methionine at 35 °C for 20 min, chased with unlabelled methionine (2 mM) at 35 °C or 39 °C for various times and then subjected to total cell immunoprecipitation using anti-F or anti-H monoclonal antibody followed by SDS-PAGE. (*a*) Fluorograph of the gel. Lane m, mock infection, 35 °C; lane 1, 35 °C, 0 min; lane 2, 35 °C, 30 min; lane 3, 35 °C, 60 min; lane 4, 35 °C, 120 min; lane 5, 39 °C, 30 min; lane 6, 39 °C, 60 min; lane 7, 39 °C, 120 min. (*b*, *c*) Scanning of the fluorograph of total cell immunoprecipitation to show the amount of (*b*) F_1 and (*c*) H proteins recovered. Cells were chased with unlabelled methionine at 35 °C (O) or 39 °C (●).

labelled cells were chased at 35 °C or 39 °C and analysed by cell surface immunoprecipitation. At 35 °C, F_1 protein was barely detectable after 5 h of chase whereas it appeared clearly at the cell surface after 3 h of chase at 39 °C and increased afterwards (Fig. 3). About 15% and 63% of the total F_1 protein was expressed on the surface of infected cells after 5 h of chase at 35 °C and 39 °C, respectively. In contrast to F protein, H protein pulse-labelled for 20 min at 35 °C was expressed on the cell surface after 60 min of chase at both temperatures and did not increase afterwards (data not shown). About 30% of total H protein was expressed on the surface of infected cells after 60 min of chase at either temperature.

Thus, F protein was detected in greater amounts on the cell surface at 39 °C than at 35 °C, probably due to more efficient cell surface expression of F protein at 39 °C.
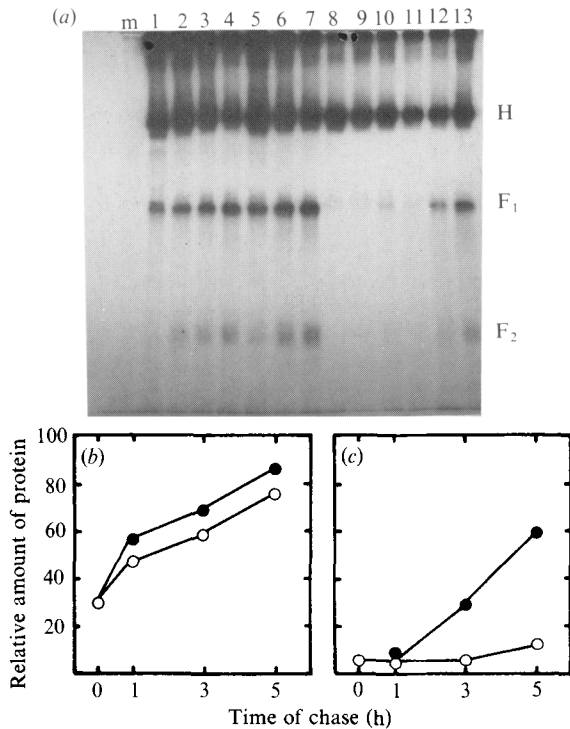
Fig. 3. Appearance of F protein at the surface of infected cells. At 24 h p.i., MV-infected HeLa cells were pulse-labelled with [^{35}S]methionine for 1 h at 35 °C, chased with unlabelled methionine (2 mM) at 35 °C or 39 °C for various times and then subjected to total cell (lanes m and 1 to 7) and cell surface (lanes 8 to 13) immunoprecipitation using a mixture of anti-F and anti-H monoclonal antibodies. The immunoprecipitates were analysed by SDS-PAGE. (a) Fluorograph of the gel; lane m, mock infection; lane 1, 35 °C, 0 h; lanes 2 and 8, 35 °C, 1 h; lanes 3 and 9, 35 °C, 3 h; lanes 4 and 10, 35 °C, 5 h; lanes 5 and 11, 39 °C, 1 h; lanes 6 and 12, 39 °C, 3 h; lanes 7 and 13, 39 °C, 5 h. Scanning of the fluorograph of (b) total cell immunoprecipitation and (c) cell surface immunoprecipitation showed the amount of F$_1$ protein recovered. Cells were chased with unlabelled methionine at 35 °C (O) or 39 °C (●).

The detection of enhanced syncytium formation in MV-infected cells due to temperature elevation may support the idea that F protein appears on the cell surface more efficiently at 39 °C than at 35 °C.

There is a possibility that unmasking of F protein at the cell membrane or faster transport of F protein to the cell membrane occurs at 39 °C. Defective expression of M protein was reported to be involved in the masking of viral glycoproteins expressed on the cell surface by prompting antibody-induced redistribution of the viral glycoproteins on the surface of infected cells (Yoshida *et al.*, 1979). In MV-infected cells, M protein was stable at either temperature (Ogura *et al.*, 1987) and antibody treatment in cell surface immunoprecipitation was performed at 4 °C for a very short time to avoid this redistribution of glycoproteins. Gupta *et al.* (1977) showed the distinction between cell surface antigens and cell membrane antigens that are not accessible to antibodies by cell surface iodination followed by immunoprecipitation. Therefore, it is possible that F protein becomes more accessible to antibody at the cell surface at 39 °C because of increased membrane fluidity. Analysis by cell surface iodination followed by immunoprecipitation is now in progress.

Glycosylation of the F protein, which is thought to occur at two sites on the F$_2$ subunit (Richardson *et al.*, 1986), is essential for its integration into the cell membrane (Sato *et al.*, 1988). No alteration in F$_2$ mobility, however, was observed at either temperature.

Slower turnover of F protein or decreased shedding at 39 °C is unlikely. As demonstrated previously (Ogura *et al.*, 1988) and in this study, there was no significant difference between 35 °C and 39 °C in total cell immunoprecipitation of F protein after chase periods, eliminating the possibility that F protein degrades or turns over faster at 35 °C than 39 °C. MV was produced more efficiently at 35 °C than at 39 °C but cell-associated infectivity was about 10 times greater than cell-free infectivity at 35 °C (Ogura & Nakamura, 1989), suggesting that shedding could not account for the reduced cell surface expression of F protein at 35 °C.

H protein plays an essential role in the functional expression of F protein which leads to the attachment of adjacent cells to each other, as indicated by our observation that anti-H monoclonal antibody completely inhibited MV-induced syncytium formation (data not shown). However, elevated temperature did not affect cell surface expression of H protein.

As Mottet *et al.* (1986) reported the different effects of reduced temperature in restricting Sendai virus HN and F$_0$ transport to the plasma membrane, we observed the different effects of elevated temperatures on cell surface expression of MV F and H glycoproteins. It remains to be clarified how the different sensitivities to elevated temperatures of the two glycoproteins arise in MV-infected cells.

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