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Synthesis of M Protein of HVJ (Sendai Virus) in Rat Glial Cells is Selectively Restricted at a Non-permissive Temperature

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SUMMARY

Production of HVJ (Sendai virus) wild-type in rat glial cells was characteristically restricted at high temperature. The synthesis of the M protein was selectively decreased in infected cells at 39 °C. This temperature-sensitive (*ts*) character of HVJ was not observed in infection of LLCMK2 cells or chick embryo fibroblast cells. Newcastle disease virus, mumps virus and vesicular stomatitis virus did not show *ts* growth in glial cells.

It is well known that paramyxoviruses cause persistent infection in susceptible animals and man. Measles virus, for example, can induce a chronic neurological disease in the central nervous system (CNS), known as subacute sclerosing panencephalitis (SSPE), in which the virus seems to persist in a cell-associated, non-budding state with lack of synthesis of M (matrix) protein (Hall & Choppin, 1979; Stephenson & ter Meulen, 1979; Wechsler *et al.*, 1979). Isolations of parainfluenza virus type 1 from the brain of a patient with multiple sclerosis (ter Meulen *et al.*, 1972) and simian virus 5-like virus from peripheral blood lymphocytes of a patient with SSPE (Robbins *et al.*, 1981) have also been reported. In addition, Sendai virus HVJ, a type 1 parainfluenza virus, has recently been shown to be pathogenic for the CNS of mice after intracerebral inoculation of the virus. Viral antigens were detectable in ependymal cells and choroid plexus epithelial cells during acute infection (Shimokata *et al.*, 1976; Kristensson *et al.*, 1983). However, its growth characteristics in neural cells of brain are poorly understood. In view of the possible causative role of paramyxoviruses in several CNS diseases as described above, the present study was undertaken to examine HVJ replication in rat glial cells in culture at different temperatures.

A rat glial cell line (C6) (Benda *et al.*, 1969), which was kindly supplied through Dr H. Higashida of the Cancer Research Institute, Kanazawa University, Japan, was grown in Dulbecco's modified Eagle's medium (DMEM) with 10% foetal bovine serum and 100 µg/ml kanamycin. Primary chick embryo fibroblast (CEF) cells and LLCMK2 (from rhesus monkey kidney) cells were cultured with Eagle's minimum essential medium containing 10% calf serum and 10% tryptose phosphate broth. Monolayer cultures were infected with HVJ wild-type (three strains; Nagoya 1-60, Z and RL), Newcastle disease virus (NDV) (Miyadera strain), mumps virus (R79-310 strain) or vesicular stomatitis virus (VSV) (New Jersey serotype) at a multiplicity of infection of 5 p.f.u./cell. Infectivities of HVJ, NDV, mumps virus and VSV in the culture media were assayed by plaque formation on LLCMK2, CEF, Vero and L929 cells, respectively. Estimation of viral genome replication and transcription were performed as described by Roux & Waldvogel (1981). To examine intracellular viral protein synthesis, infected cell monolayers were labelled with 50 µCi/ml [³⁵S]methionine (1490 Ci/mmol, Amersham) for 1 h in methionine-free DMEM and then solubilized in lysing buffer (0.15 M-NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0.1% SDS, 0.01 M-Tris-HCl pH 7.4) containing 100 µg/ml phenylmethylsulphonyl fluoride (PMSF). The cell lysates were subjected to immunoprecipitation as described previously (Wechsler *et al.*, 1979; Sato *et al.*, 1981) and then analysed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970). For chases, the

Table 1. Yield efficiency at 32 °C and 39 °C of HVJ, NDV, mumps virus and VSV on C6, LLCMK2 and CEF cells

Virus	Cell type	Virus yields* (p.f.u./ml)		Yield efficiency (39 °C/32 °C)
		32 °C	39 °C	
HVJ Nagoya 1-60	C6	4.3×10^6	5.0×10^1	1.2×10^{-5}
	LLCMK2	2.5×10^8	5.2×10^7	2.1×10^{-1}
	CEF	2.8×10^6	5.3×10^5	1.9×10^{-1}
HVJ Z	C6	6.5×10^6	1.5×10^2	2.3×10^{-5}
	LLCMK2	6.8×10^8	7.1×10^6	1.0×10^{-2}
	CEF	6.9×10^6	1.0×10^5	1.4×10^{-2}
HVJ RL	C6	5.1×10^7	1.3×10^2	2.5×10^{-6}
	LLCMK2	2.3×10^8	2.9×10^5	1.3×10^{-3}
	CEF	8.0×10^5	2.9×10^4	3.6×10^{-2}
NDV	C6	6.8×10^6	2.3×10^6	3.4×10^{-1}
	LLCMK2	3.0×10^7	1.8×10^6	6.0×10^{-2}
	CEF	4.4×10^6	7.3×10^5	1.7×10^{-1}
Mumps virus	C6	1.6×10^6	2.1×10^6	1.31
	LLCMK2	9.3×10^5	1.3×10^6	1.40
VSV	C6	5.3×10^7	3.2×10^6	6.0×10^{-2}
	LLCMK2	2.5×10^8	2.4×10^8	9.6×10^{-1}
	CEF	9.0×10^7	9.5×10^6	1.1×10^{-1}

* Culture media of cells infected with HVJ, NDV, mumps virus and VSV were harvested for plaque titration 48 h, 48 h, 72 h and 24 h after infection, respectively.

radioactive medium was removed and the cells were washed twice with DMEM and incubated in DMEM containing a tenfold excess of methionine.

First, HVJ yields in C6 cells at various temperatures were assayed. As shown in Table 1, HVJ Nagoya 1-60 strain grew well in C6 cells at 32 °C, but not at 39 °C, showing a yield efficiency between these two temperatures of 1.2×10^{-5} . The infection of C6 cells at 39 °C exhibited weaker cytopathic effects than those at 32 °C. Similar results were obtained with the other strains of HVJ, Z and RL. Thus, HVJ appeared to show temperature-sensitive (*ts*) growth in C6 cells at 39 °C or higher when less than 10^{-3} yield efficiency was defined as *ts* growth. On the other hand, the above *ts* character of HVJ infection could not be observed in either LLCMK2 or CEF cells. In addition, NDV, mumps virus and VSV could multiply well at either temperature in C6, LLCMK2 and CEF cells (Table 1).

In the culture media of cells infected at the non-permissive temperature (39 °C), no significant amounts of non-infectious incomplete virions could be detected by radioisotopic uridine and methionine labelling followed by ultracentrifugation analysis (data not shown).

In order to measure viral RNA synthetic activity, the infected C6 cells were labelled with 20 $\mu\text{Ci/ml}$ [^3H]uridine in the presence of 5 $\mu\text{g/ml}$ actinomycin D and analysed by sedimenting on CsCl gradients. Viral genome replication rates (ct/min in the nucleocapsids) were the same at either temperature, but viral transcription rates (ct/min in the CsCl pellets) at 39 °C were found to be doubled when compared to those of the cells at 32 °C (data not shown).

Next, intracellular viral protein synthesis was examined by SDS-PAGE analysis of [^{35}S]methionine-labelled cells. Initially, labelled cell lysates were directly subjected to SDS-PAGE and fluorography, but the M protein band could not be clearly resolved because of the overlapping of M with host cell proteins (data not shown); for this reason, radioimmunoprecipitation was performed using an anti-HVJ serum containing polyclonal antibody to M protein. In C6 cells infected with HVJ Nagoya 1-60 strain at 39 °C, the rate of synthesis of M protein selectively decreased, while synthesis of the other structural proteins was unaffected by the temperature of incubation (Fig. 1*a*). No new protein bands with different mobilities appeared at 39 °C. A similar specific reduction of M protein synthesis was observed in C6 cells infected at 39 °C with the other two strains of HVJ (Z and RL), but was not observed in the case of LLCMK2 cells (Fig. 1*b*).

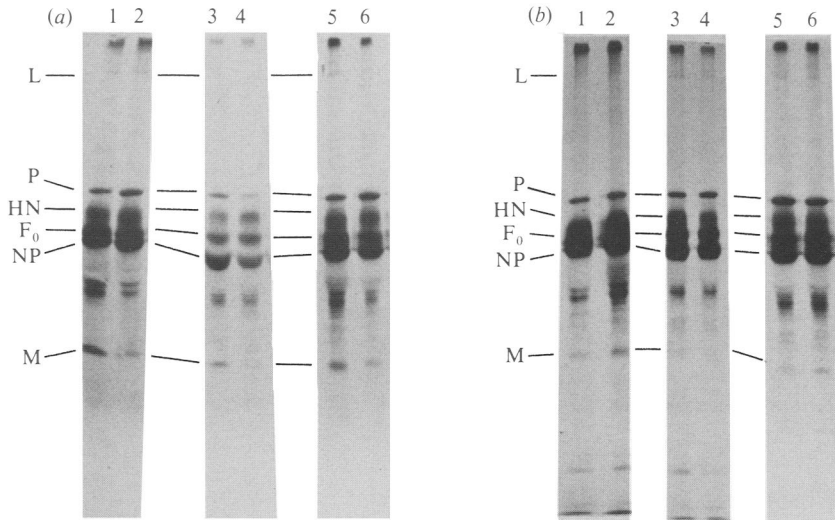


Fig. 1. Immunoprecipitation and SDS-PAGE of ^{35}S -labelled HVJ proteins in C6 (a) and LLCMK2 (b) cells. Infected cells were incubated at 32 °C or 39 °C for 24 h and then labelled with [^{35}S]methionine (50 $\mu\text{Ci}/\text{ml}$) for 1 h. Cell lysates were immunoprecipitated and subjected to SDS-PAGE and fluorography. Lanes 1, 3 and 5, incubated and labelled at 32 °C; lanes 2, 4 and 6, incubated and labelled at 39 °C; lanes 1 and 2, infected with HVJ Nagoya 1-60 strain; lanes 3 and 4, infected with HVJ Z strain; lanes 5 and 6, infected with HVJ RL strain.

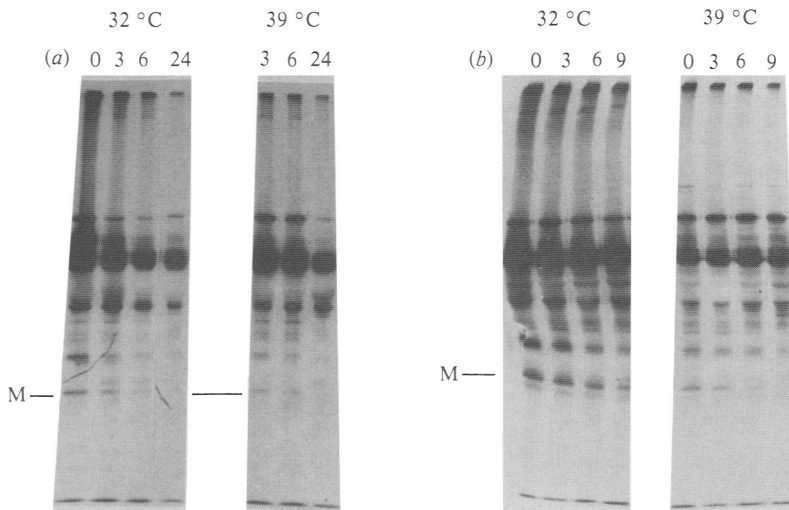


Fig. 2. Degradation of synthesized M protein of HVJ in infected C6 cells. (a) Infected cells were incubated at 32 °C for 24 h, labelled with [^{35}S]methionine for 1 h, and then chased at 32 °C or 39 °C. (b) Infected cells were incubated at 32 °C or 39 °C for 12 h, labelled with [^{35}S]methionine for 1 h, and then chased at the respective temperatures. The cells were harvested at the various times (h) shown after the chase and were subjected to immunoprecipitation and SDS-PAGE.

Rapid degradation or extreme heat lability of the M protein of HVJ in infected C6 cells might also result in an apparent reduction in its synthesis at 39 °C. Pulse-chase experiments (Fig. 2) showed that M protein synthesized at 32 °C did not appear to be heat-labile at 39 °C (Fig. 2a). In addition, M protein, barely detectable 12 h after infection at 39 °C, was not rapidly degraded in comparison with that synthesized at 32 °C (Fig. 2b). Although our antibody against M protein is polyclonal, the possibility is not completely ruled out that M protein might be present in a form

that is not accessible or immunoreactive with the specific antibody as mentioned by Miller & Carrigan (1982).

A selective inhibition of M protein synthesis has been observed in abortive infections with influenza A (Lohmeyer *et al.*, 1977) and B (Nakamura & Homma, 1981) viruses. In influenza B virus-infected MDCK cells, a similar phenomenon to our findings in C6 cells has been demonstrated; the synthesis of M protein was selectively inhibited at high temperatures accompanied by reduced production of virus particles (Tobita *et al.*, 1982).

The reduction of M protein synthesis in abortive infection with influenza A virus, which contains eight distinct single-stranded RNA segments of negative polarity and is transcribed into monocistronic messengers (Scholtissek *et al.*, 1976; Inglis *et al.*, 1977), is known to be due to a reduced concentration of M-specific mRNA (Bosch *et al.*, 1978, Valcavi *et al.*, 1978). It has also been demonstrated by Stephenson *et al.* (1981) that a block of M protein expression in persistent infection with measles (SSPE) virus, which contains non-segmented negative-stranded RNA, is at the level of translation of its polypeptide. The mechanisms underlying the specific reduction of M protein synthesis in our C6 cells at the non-permissive temperature are still unclear at present. Analysis of mRNA isolated from infected C6 cells in a cell-free translation system is now in progress.

Growth of HVJ wild-type in C6 cells exhibited host-dependent temperature sensitivity, suggesting some contribution of host cell function(s) to HVJ replication in the rat glial cell. This factor does not seem to be involved in the functions essential for cell growth because C6 cells can grow normally even at 39 °C. Recently, Miller & Carrigan (1982) have demonstrated that addition of papaverine, an inhibitor of cyclic AMP (cAMP) phosphodiesterase, to the culture medium causes selective disappearance of intracellular M protein in measles virus-infected neural cells, accompanied by inhibition of virus production. Similar results have been reported for non-neural human amnion (AV3) cells treated with exogenous cAMP (Robbins & Rapp, 1980). In contrast, it has been shown recently that dibutyryl cAMP activates rubella virus replication restricted in normal rat glial cells (Van Alstyne & Paty, 1983). Since C6 cells have been reported to differentiate morphologically into astrocytes by increasing intracellular cAMP (Hamprecht *et al.*, 1973), cyclic nucleotide concentrations might be responsible for our present observations.

The host-dependent *ts* system presented here may yield information not only on host factor(s) in HVJ replication but also on mechanisms of paramyxovirus persistence in the CNS.

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