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Restriction of Measles Virus Gene Expression in Acute and Subacute Encephalitis of Lewis Rats

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Measles virus (MV) replication in brain tissue of Lewis rats with acute (AE) and subacute (SAME) encephalitis was characterized by biochemical techniques. Messenger RNAs specific for measles virus nucleocapsid (N), phospho (P)-, matrix (M), fusion (F), and haemagglutinin (H) protein were detected in all brain extracts examined. The quantity of the individual MV mRNA species was quite different in comparison to lytically infected Vero cells. A steep gradient of MV transcripts was found in brain tissue which is most likely due to strongly attenuated transcription of mRNAs along the viral genome, representing particularly low transcription of the glycoprotein genes. In addition, in vitro translation assays only revealed synthesis of N and P protein in consistent fashion. The mRNAs for the glycoproteins did not direct the synthesis of detectable viral proteins whereas the M mRNA revealed some activity in animals with AE. The data indicate a strong restriction of the MV envelope gene expression in infected brain tissue, which is independent of the incubation time and type of the central nervous system (CNS) disease. This phenomenon which is similar to the findings observed in measles inclusion body encephalitis and subacute sclerosing panencephalitis suggest that host factors may initially be responsible for the initiation of transcriptional and translational alterations.

INTRODUCTION

Measles virus (MV) is responsible for measles inclusion body encephalitis (MIBE) and subacute sclerosing panencephalitis (SSPE) in man (ter Meulen and Carter, 1982). Both diseases develop after long incubation periods on the basis of a persistent viral infection. To date the pathogenetic mechanisms leading to these CNS infections are not completely understood but studies of the viral gene expression have recently led to a better understanding of the state of measles virus persistence in brain cells of these two human CNS diseases. It has been found that MV persistence in brain tissue is characterized by a restriction of envelope viral gene expression implicating MV transcriptional and translational defects (Baczko et al., 1986, 1988; Cattaneo et al., 1986, 1987a, 1988; Liebert et al., 1988). However, these observations do not help to explain which factors are involved in the establishment of persistence since the studies have been carried out on autopsy material. Therefore, it is not possible to determine which of the defects observed in MV replication in brain tissue of patients with MIBE or SSPE is a consequence of long-lasting virus host–cell interaction and which are the initial events determining persistence once MV has entered the brain.

To study these parameters we analyzed MV gene expression in a recently described animal model system (Liebert and ter Meulen, 1987). Following intracerebral inoculation of a neurotropic rat brain adapted CAM-RBH strain of MV, Lewis rats develop different types of CNS diseases. Newborn animals invariably succumb to an acute encephalitis (AE) within a few days following infection. Weanling animals develop either an AE or a subacute encephalomyelitis (SAME) weeks or months after infection. Previous analyses of MV structural proteins in infected brain tissue by immunohistochemical staining procedures using monoclonal antibodies have suggested a restriction of MV envelope gene expression in all animals. Already in animals with AE, only one third of the infected brain cells expressed MV envelope proteins, and this was observed to decrease further to almost undetectable levels in brains of animals with SAME (Liebert and ter Meulen, 1987). We show in this study that the restriction of MV gene expression in infected brain tissue of Lewis rats results from transcriptional and translational alterations. The data obtained indicate that the defects observed in measles virus replication in acute or subacute mosaics encephalomyelitis in Lewis rats parallel many findings described in MIBE and SSPE.

MATERIALS AND METHODS

Infection of animals and tissue culture

Inoculation of the rat brain adapted CAM/R 40 strain of measles virus was performed as described pre-
viously (Liebert and ter Meulen, 1987). Rats were infected in the left cerebral hemisphere with 25 μl of CAM/RBH (4 × 10⁵ TCID₅₀/ml) using a dispenser syringe. The animals were killed under ether narcosis. Brain material was obtained immediately after death and RNA extracted. Vero cells were infected with CAM/RBH virus at a m.o.i. of 0.1.

**Immunohistology**

Immunohistological studies on brain sections were carried out with a library of monoclonal antibodies directed against MV structural proteins as described previously (Liebert and ter Meulen, 1987).

**Extraction of RNA**

Total RNA from MV-infected Vero cells and from brain tissue was extracted as described previously (Chirgwin et al., 1979; Baczko et al., 1984). Cell homogenate from infected Vero cells was obtained by lysis in guanidinium isothiocyanate buffer when CPE was almost complete. Organ material was homogenized in guanidinium isothiocyanate buffer by using a Potter homogenizer. The homogenate was overlaid on a CsCl cushion (1.7 g/cm³) and centrifuged in an SW 28 rotor (Beckman) at 20³ and 24,000 rpm overnight. The RNA pellet was dissolved in SEH buffer (100 mM NaCl, 10 mM Hapes, pH 7.0, 1 mM EDTA) containing 0.1% SDS and ethanol precipitated. The average yield was 0.05 μg of total RNA/g tissue. Polyadenylated RNA was selected from total RNA by one cycle of oligo(dT)-cellulose chromatography (Sigma).

**S1-Nuclease protection assay**

Total RNA extracted from different rat organs (see Results) was screened for the presence of N-specific sequences by S1-analysis as described previously (Palavolo et al., 1980; Billeter et al., 1984). Briefly, single-stranded M13 hybridization probes covering the 3' end of the MV genome (PstI/BamHI fragment, nucleotides U-230) (Billeter et al., 1984) were labeled with [³²P]dATP by primer extension according to the conditions for sequence determination by chain termination (Davis et al., 1986) and hybridized to 10 μg of total RNA for 16 hr at 42³ in a buffer containing 80% formamide. Nuclease S1 digestion was performed for 60 min at 30³ with 700 units S1-nuclease/ml in the appropriate buffer. Following phenol extraction and ethanol precipitation, protected fragments were separated on 0% polyacrylamide sequencing gel.

**Northern blots**

MV-specific RNAs were analyzed as described previously (Cattaneo et al., 1987b). Briefly, 1 μg of +pA-RNA isolated from infected Vero cells or rat brain tissue was loaded on a 3-cm broad slot of a 1.5% formaldehyde-containing agarose gel together with a mixture of standard RNAs described below (usually 2 fmol each). After electrophoresis, the gel was blotted onto nitrocellulose which was cut in strips for hybridization. The strips were hybridized with [³²P]CTP-labeled RNA probes transcribed from MV-specific cDNA templates specific for all MV structural genes except L cloned in the pGem-1 vector. RNA probes used for all hybridization experiments were the precise complements of the external standard RNAs mentioned above and were transcribed from the following cDNA fragments: pGem N, 851-bp EcoRV/XbaI fragment; pGem P, 531-bp BalI/HindIII fragment; pGem M, 520-bp PstI/Smal fragment; pGem F, 320-bp TaqI/Ddel fragment; pGem H, 787-bp BglI/Aval fragment (Cattaneo et al., 1987b). Hybridization was performed at 54³ in buffer containing 50% formamide. Following autoradiography, bands corresponding to the monocistronic MV mRNAs and the external standard RNAs were excised and the amount of radioactivity retained was determined by scintillation counting.

**In situ hybridization**

MV-specific RNA was localized in brain sections with ³⁵S-labeled DNA and RNA probes using modifications of the procedures described by Haase et al. (1984) and Cox et al. (1986). Paraffin sections (5–6 μm thick, deparaffinized through xylene) or cryostat sections were rehydrated through graded ethanol, air-dried, immersed in 0.2 M HCl (20 min, room temperature), 2X SSC (30 min, 70³), 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin (Sigma), and finally dehydrated through graded ethanol and air-dried. ³⁵S-labeled probes were obtained either by nick-translation of the pGem N clone in the presence of [³⁵S]dATP (Amersham) or by in vitro transcription of the MV-specific pGem-1 clones in the presence of [³⁵S]UTP (Amersham) in anti-mRNA sense orientation using SP6- or T7-RNA polymerase, respectively (Boehminger). Specific activity for the strand-specific RNA probes was in the order of 5 × 10⁶ and 5 × 10⁷ dpm/μg for the nick-translated probe.

Labeled probes were applied to the sections at a concentration of 0.2 μg/ml in a mixture containing 50% deionized formamide, 10% dextran sulfate, 0.3 M NaCl, 10 mM Tris, pH 7.2, 1 mM EDTA, 0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, 100 μg/ml +pA-RNA, 100 μg/ml yeast tRNA, and 50 mM DTT. Siliconized coverslips were applied to the
sections and sealed with rubber cement. Hybridization was performed at 48°C overnight for RNA probes and at room temperature for 48 hr for the DNA probe.

Following hybridization, slides were immersed in 2x SSC for 10 min at room temperature and afterward in hybridization wash mixture (HWM: 50% formamide, 0.6 M NaCl, 10 mM Tris–HCl, pH 7.4, 2 mM EDTA) (for RNA probes, 30 min at 45°C, for the DNA probe, 30 min at 30°C). Following two rinses in 0.1 x SSC for 10 min, sections were dehydrated in graded ethanol containing 0.3 M ammonium acetate. Slides were air-dried and dipped in Kodak NTB2 emulsion diluted 1:1 with 0.6 M ammonium acetate at 4°. They were kept at 4° for 3 days, developed with Kodak D 19 developer, fixed with 30% sodium thiosulfate, and counterstained with hematoxylin. Controls included hybridization of MV-specific probes with brain sections of noninfected Lewis rats and labeled pGem-1 vector transcripts or a murine coronavirus JHM-specific transcript to brain sections from MV-infected Lewis rats. The murine coronavirus JHM probe was kindly provided by Dr. S. Siddell, Würzburg.

In vitro translation and immunoprecipitation

The ability of the different MV mRNAs obtained from the rat brains to direct the synthesis of the respective proteins was investigated using an in vitro translation system derived from rabbit reticulocyte lysate (Amer sham) following a procedure described previously (Baczko et al., 1984). Proteins made in vitro were immunoprecipitated using a polyclonal rabbit-anti-MV serum and monoclonal antibodies against MV structural proteins. Total translates and immunoprecipitated proteins were analyzed on a discontinuous 12% polyacrylamide gel as described (Carter et al., 1982; Baczko et al., 1984).

RESULTS

Detection of measles virus (MV) transcripts in different organs of infected Lewis rats

To determine if MV replication is restricted to brain cells following intracerebral inoculation of MV into weanling Lewis rats, RNA from different organs was extracted at various time intervals following infection (3–12 days postinfection). Total RNA obtained from tissue of two different brain regions and spinal cord as well as lung, liver, kidney, bone marrow, thymus, spleen, and peripheral blood mononuclear cells was screened for MV-specific transcripts by nuclease S1 analysis using 32P-labeled M 13 probes derived of the 3' end of the N gene in both orientations. As summarized in Table 1, MV-specific transcripts were always detectable in brain specimens as early as 4–5 days following infection but not in other tissues. In only one animal was a weak signal seen in total RNA derived from thymus and bone marrow.

Expression of MV-specific transcripts in brain material from CAM/RBH-Infected Lewis rats

MV-specific transcription in brain tissue was analyzed by Northern blot using +pA-RNA fractions isolated from brain material of CAM-infected Lewis rats with different types of CNS diseases. For comparison, +pA-RNA from Vero cells infected with CAM/RBH virus was included in this study. Figure 1 shows the pattern of MV transcription in Vero cells (Fig. 1A), brain tissue of a newborn and a weanling Lewis rat with AE (Figs. 1B and C), and an animal with SAME (Fig. 1D). All MV structural genes investigated were expressed as monocistronic and bi- or polycistronic polyadenylated transcripts in all RNA specimens. However, the absolute amount and transcriptional efficiency of the individual MV-specific mRNAs in Vero cells were clearly different from those found in brain material. Similar to the results obtained for HeLa cells lytically infected with the Edmonston strain of MV (Cattaneo et al., 1987b), we detected a polar expression gradient for CAM-infected Vero cells reflecting an almost linearly decreasing transcriptional attenuation of the structural genes along the gene order. In contrast, the gradient of mRNAs derived from brain was steeper and did not differ significantly between AE in newborn or weanling rats and in SAME (Figs. 1B–D). As in the lytic infection, the mRNAs for the first three genes, N, P, and M genes,
FIG. 1. Northern blot analysis of +pA-RNA derived from infected Vero cells (Fig. 1A), and from brain tissue of CAM/RBH-infected Lewis rats: a newborn animal 5 days postinfection (Fig. 1B), a weanling animal at Day 10 postinfection (Fig. 1C) both with AE, and an animal with SAME (Fig. 1D) at Day 30 postinfection. Isolated +pA samples were mixed with 2 fmol standard RNA transcripts (except Fig. 1C where there is only 1 fmol of the H standard that is hardly visible) and separated on a 1.5% formaldehyde-containing agarose gel. Filter strips were hybridized to 32P-labeled strand-specific RNA probes and following hybridization were ordered according to the MV gene order. A, signals for the monocistronic; Δ, signals for the poly-cistronic RNAs; and ○, signals for the standard RNA transcripts migrating corresponding to their individual lengths.

were efficiently transcribed as visualized by strong hybridization signals. However, transcription of the glycoprotein-specific mRNAs for the F and the H genes was significantly reduced in brain-derived RNAs. This was already evident in the acute form of infection (Figs. 1B and C) and was further enhanced in the subacute type of disease (Fig. 1D).

This typical expression pattern was observed in the large number of the infected animals we studied and was independent of the incubation period and type of CNS disease. In the case of AE, 20 newborn and 28 weanling Lewis rats were examined while for SAME 30 animals were studied.

Quantitation of the signals obtained for the monocistronic mRNAs revealed that the amount of N gene transcription proved to be about 5 to 7-fold lower in brain material as compared to lytically infected tissue culture (Table 2). The expression gradient, generally steeper for the brain-derived MV-specific mRNAs, showed the most obvious restriction occurring at the M-F gene boundary. The values for the absolute expression/10 pg RNA varied slightly for the individual mRNAs (Table 2). With increasing age and incubation period the expression gradient of MV transcription became steeper, affecting in particular the envelope protein genes (Figs. 1C and D, Table 2). For negative control, MV-specific probes were hybridized to +pA-RNA from the brain of noninfected Lewis rats. No signal except the hybridization of the standard RNAs was observed (data not shown).

Analysis of the biological activity of the isolated +pA-RNAs from brain material

The finding of a transcriptional restriction of MV-genes, together with the observation of a reduced expression of envelope proteins in brain tissue of Lewis rats with AE and SAME (Liebert and ter Meulen, 1987), indicated that further characterization of the brain-derived mRNAs might be instructive. mRNAs from brain material of Lewis rats with AE and SAME were isolated, and their ability to direct the synthesis of the corresponding proteins was analyzed by in vitro translation (Fig. 2). Immunoprecipitation of MV-specific proteins synthesized in vitro from +pA-RNA derived from CAM-infected Vero cells revealed signals for the P, H, N, and M proteins (Figs. 2A and 2B, lane 2). +pA-RNAs derived brain material from either newborn Lewis rats with AC (Fig. 2A, lanes 3 and 4) or weanling animals with AC (Fig. 2A, lane 5 and 6) which directed the synthesis of the N and P protein. However, the signals obtained for the P proteins were significantly reduced compared to the lytic control (Fig. 2A, lane 2). In lanes 3-6 of Fig. 2A, a band can be seen at the position of the M protein. +pA RNAs derived from the brains of Lewis rats with SAME revealed only N and small amounts of P-specific translation products (Fig. 2B, lanes 4-7). In lanes 1 and 2 of Figs. 2A and 2B a faint band with an approximate molecular weight of 70 kDa which represents MV specific H protein can be seen. This band was not detectable in the other lanes. In addition, in lanes 1 and 2 of Figs. 2A and 2B a protein of approximately 46 kDa is visible which could not be identified by the application of our monoclonal antibodies directed against MV structural proteins. F protein expression could not be examined since our monoclonal and monospecific polyclonal anti-F antibodies did not immunoprecipitate this protein from the in vitro synthesized products.

Sensitivity of the in Vitro translation assay

In order to exclude the possibility that the failure to detect M protein synthesis in the in vitro translation experiments using polyadenylated RNAs from brain of animals with SAME results from low sensitivity of the in...
TABLE 2

QUANTITATIVE ANALYSIS OF MV-SPECIFIC GENE EXPRESSION IN BRAINS OF CAM-INFECTED LEWIS RATS

| MV-specific mRNA | Vero/CAM | AE newborn rat | AE weanling rat | SAME weanling rat |
|------------------|----------|----------------|-----------------|------------------|
|                  | Copies   | %              | Copies          | %                |
| N                | 21,400   | 100            | 5500            | 100              |
| P                | 17,800   | 82             | 3300            | 59               |
| M                | 14,200   | 67             | 2800            | 51               |
| F                | 10,600   | 49             | 850             | 16               |
| H                | 8,400    | 39             | 450             | 8                |

"Copy numbers of the individual mRNA were determined as described (Cattaneo et al., 1987b) and refer to 10 μg of RNA, the amount estimated per cell. Radioactivity bound to the nitrocellulose filters from Figs. 1A–D at the positions of the standard RNA transcripts and the corresponding monocistronic mRNA was measured by scintillation counting.

Relative expression of the MV structural genes was determined in relation to the individual N-gene expression.

vitro translation system employed, +pA from lytically infected Vero cells was translated at different concentrations. The amount of RNA of infected cells was adjusted to the individual copy number calculated for the N-, M-, and H-specific mRNAs in the brain from animals with SAME. Figure 3 shows the results of the in vitro translation experiment using serially diluted +pA-RNA from infected cells (lanes 1–7) and from the brain of an animal with SAME (lane 8). The positions of the L-, P-, H-, N-, and M-specific translation products are indicated as well as the position of the 46-kDa protein (designated X) also seen in Figs. 2A and 2B. As an internal control for equal amounts of +pA-RNA translated in vitro (1 μg each) a cellular protein band marked 1 is recognizable in each lane (Fig. 3A) with approximately the same intensity. In lane 3 of Fig. 3 the concentration of +pA-RNA represents about 5000 copies of the N-mRNA, in lane 4, about 1400 copies of the M-mRNA, and in lane 6, about 200 copies of the H-mRNA. In the total translate (Fig. 3A) all three viral proteins are detectable at the adjusted concentrations in infected Vero cell lysate as well as in the immune precipitate (Fig. 3B), except for the haemagglutinin which was not clearly precipitable as a distinct band. The intensity of the N-specific bands in lanes 3 and 8 was comparable. The M-specific protein, additionally identified by using a monospecific antiserum (data not shown), could be immunoprecipitated at the appropriate concentration (lane 4) but not visualized in the brain-derived RNA (lane 8).

Expression of MV transcripts at the single cell level

The results of the Northern blot analysis and the in vitro translation studies indicated the development of transcriptional and translational alterations in the course of MV infection in brain tissue. In order to determine whether this phenomenon could also be demonstrated at the single cell level in situ hybridization on brain sections was carried out. For this purpose, we first determined the distribution and localization of MV-infected cells in the brain of an animal with SAME using a double-stranded N-gene-specific probe labeled by nick-translation with 35S (Fig. 4). MV-specific signals occurred mainly in the gray matter of the brain, but were also found in the white matter.

To analyze the expression of the individual MV-specific mRNAs a brain area of an animal with SAME was chosen in which only N and P proteins but no M, F, and
Fig. 3. In vitro translation and immunoprecipitation of isolated +pA-RNA from CAM-infected and uninfected Vero cell culture and brain material from an animal with SAME. +pA-RNA from CAM-infected Vero cells was diluted to decreasing concentrations with +pA-RNA from uninfected Vero cells to a total amount of 1 µg +pRNA and translated using a rabbit reticulon system. Total translation products were immunoprecipitated employing a polyclonal rabbit-anti-MV hyperimmune serum. (A) Total translation products of 1 µg (lane 1), 0.5 µg (lane 2), 250 ng (lane 3, about 5000 copies N-mRNA/10 µg RNA), 100 ng (lane 4 about 1400 copies M-mRNA/10 µg RNA), 50 ng (lane 5, about 200 copies H-mRNA/10 µg RNA), and 10 ng (lane 6) +pA-RNA from infected cells, and 1 µg of +pA from an animal with SAME (lane 8). Lane 9, ¹⁴C-labeled protein marker. (B) Immunoprecipitation of the total translation products from Fig. 3A, lanes 1–8 using a polyclonal rabbit-anti-MV hyperimmune serum. Lane 9, ¹⁴C-labeled protein marker.

H proteins were detectable by immunohistology. From this area, serial frozen sections were alternately stained for immunohistology or hybridized to the same strand-specific RNA probes used for the Northern blot analyses showing the expression of the N- (Fig. 5A), P- (not shown), M- (Fig. 5B), F- (Fig. 5C), and H- (Fig. 5D)

Fig. 4. In situ hybridization of a paraffin-embedded 6-µm-thick brain section of an animal with SAME using an N-gene specific, double-stranded probe labeled by nick-translation with [³²P]dATP. Tissue was counterstained with hematoxylin/eosin, magnification is 200X.
mRNAs. About the same number of cells showed hybridization signals for the N-, P-, and M-mRNA with a gradual reduction in intensity from N to M. In contrast, F- and H-specific signals were detectable in a smaller number of cells. The presence of M-, F-, and H-specific mRNA in detectable amounts in the same brain area where no expression of the corresponding proteins were seen could either reflect the higher sensitivity of the RNA detection method or suggest the existence of translational defects for the F- and H-specific mRNAs. A similar hybridization pattern was obtained with material from newborn and weanling Lewis rats with AE (data not shown), in which, in contrast to SAME, 20–30% of infected brain cells expressed MV envelope proteins (Liebert and ter Meulen, 1987).

DISCUSSION

In the present study we analyzed MV gene expression at the transcriptional and translational level in brain material derived from infected Lewis rats showing different types of CNS diseases. The transcription of the glycoprotein-specific mRNAs was generally restricted, the degree of transcriptional restriction being related to the age of the animals and the type of disease. In addition, the synthesis of the viral M proteins and probably also the H proteins was shown to be inhibited, due to a failure in translation of the corresponding mRNA in Lewis rats with SAME. Thus, data obtained at the molecular level support the in vivo observation of a restricted expression of the M, F, and H proteins in the brains of infected rats (Liebert and ter Meulen, 1987).

Transcriptional restriction of the glycoprotein-specific mRNAs could be seen as soon as MV-specific mRNA was detectable in infected brains. This phenomenon was independent both of the incubation time after infection and the humoral immune response of the animals, since measles antibodies in serum and CSF could only be detected in diseased weanling rats (Dörries et al., 1988). The fact that a restriction of MV transcription has already occurred in newborn rats a few days after infection suggests that brain-specific host cell factors may primarily interfere with MV gene expression. At present we cannot determine whether brain cells lack factors necessary for efficient MV transcription or contain factors actively interfering with MV gene expression leading to a premature termination of MV transcripts.
Interestingly, restriction occurred predominantly at the M–F gene boundary located within the only long noncoding region of the MV genome spanning about 1000 nucleotides. This genomic region is indeed peculiar, since three open reading frames have been defined, but no translation products of these reading frames have been detected in vivo or in vitro (Bellini et al., 1986; Wong et al., 1987; Greer et al., 1987). Moreover, no preferential accumulation of mutations has occurred in this genomic region compared to the sequences of lytic MV strains (Cattaneo et al., 1988), in spite of the high variability of viral RNA genomes (Steinhauer and Holland, 1987; Domingo and Holland, 1987). Furthermore, noncoding sequences at the 3′ end of the M mRNA have also been defined for other paramyxoviruses (Bellini et al., 1986; Blumberg et al., 1984; Hidaka et al., 1984). Although there is only chance homology between those noncoding regions within the different viruses, the analogy in location and the conservation of these particular sequences could argue for a regulatory function in the expression of viral genes. Interestingly, the 5′ untranslated region of the MV F gene has been shown to contain an unusually high content of cytosine residues (44%) (Buckland et al., 1987) with an overall GC content of 64%. This may not only influence translational efficiency of the corresponding mRNA as has been shown for cellular genes (Takeishi et al., 1985; Ordahl et al., 1984; Sap et al., 1986) but also transcriptional regulation. The viral polymerase complex could slowdown or stall along this region which could possibly favor termination. Alternatively, the restriction of MV transcription could possibly result from a selective instability of the glycoprotein-specific mRNAs in brain tissue. This has been described in other viral and cellular systems in which instability of viral and cellular transcripts occur, probably due to the activity of endogenous RNases (for review, see Brawerman, 1987; Pontecorvi et al., 1988).

The finding that during AE in newborn Lewis rats the restriction observed is less pronounced than in weanling animals with AE may support the hypothesis that cellular differentiation of the developing brain could influence the restriction of viral gene expression. It is known from tissue culture experiments that viral replication is down-regulated with increased differentiation of the infected cell (Robbins and Rapp, 1980; Miller and Carrigan, 1982; Yoshikawa and Yamanouchi, 1984). The level of endogenous signal transmitters (cAMP and cGMP) and the mitotic activity (Joseph et al., 1975; Lucas et al., 1978; McChesney et al., 1987) have been shown to influence MV replication in different tissue culture systems especially of neuronal and lymphoid origin. Similar phenomena may occur in our animal model and may interfere with the replication of the mouse virus.

Besides the transcriptional alterations, translation of the envelope proteins has been shown to be restricted. This could be documented for the M-specific mRNA by dilution experiments with RNA from infected cells (Fig. 3) and at the single cell level for the F and H protein. The mechanism underlying these restrictions probably resides in the mRNAs themselves because they occur not only in vivo but also in vitro translation experiments. Cap-dependent translation alterations have been shown for VSV methylation mutants (Horikami and Moyer, 1982), but mutational events should also be considered. Although the time course for mutations seems to be very short one should keep in mind that RNA-editing mechanisms have been shown recently for the P genes of negative strand RNA viruses (Thomas et al., 1988; Cattaneo et al., 1989).

The observed alterations of MV transcription and translation in infected brain tissue of rats are very similar to the changes seen in MV persistence in human CNS diseases (Baczko et al., 1986; Cattaneo et al., 1987a). In MIBE as well as in SSPE a restriction at a transcriptional level is found, affecting mainly MV M, F, and H genes. This leads to a rather steep gradient of mRNA transcripts according to the location of the individual genes in the MV genome (Cattaneo et al., 1987b). In vitro translation experiments using isolated +pA RNA from human brain tissue only MV N and P proteins were always detectable whereas the viral envelope proteins were either absent or only occasionally immunoprecipitated (Baczko et al., 1986). These in vitro findings were supported by immunohistological investigations (Liebert et al., 1986). Alterations in translation of MV genes caused by mutations have been observed in some cases of SSPE and in MIBE (Cattaneo et al., 1988). In one case of MIBE a mutation caused elimination of the initiation codon of the M gene (Cattaneo et al., 1988), and in one SSPE case a mutation introduced a stop codon in the M gene sequence (Cattaneo et al., 1986, 1988). However, mechanisms other than mutations could be implicated in alteration of translation of MV protein (Ogura et al., 1987, 1988).

The observation of restricted MV transcription and translation in infected rat brain a few days after infection demonstrates that this phenomenon is independent of long incubation periods. One can assume that similar events may have taken place in SSPE or MIBE a long time before the onset of disease. By this mechanism, which appears to be host-cell dependent, infected brain cells may not be destroyed by virus replication. In the absence of F protein, no cell fusion occurs and the defect of M protein synthesis blocks the maturation of complete virus particles thus keeping the infection strictly cell-associated. Moreover, the overall restriction of the expression of MV surface glycoproteins saves the infected cells from being recognized
and destroyed by antiviral antibodies. These events probably prevent a lytic infection of MV and allow the establishment of persistence. Since the ribonucleocapsid-complex is infectious (Rozenblatt et al., 1979), the infection is probably maintained by spread via cell processes. The pathogenetic role played by the observed mutations in the two human diseases is not understood. It is conceivable that mutations amplify the restriction of gene expression and support persistence rather than initiate MV gene restriction.

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