yielded estimates of processing time with a standard error of ~5 msec (that is, by taking into account response variability and the slope of the psychometric curve). Experiments were performed with highly trained observers who were familiar with psychophysical experiments in which displays are rapid and, in particular, with these experiments (through thousands of trials); except for one of them (D.S.), subjects were not familiar with the purpose of the experiments.

The mean slope of the detection curves is 1.9 msec per target with a standard deviation of 6.3 msec per target (Fig. 2). Thus, there was no significant increase in processing time with increasing number of targets. However, processing time for discrimination increased at a constant rate with the number of targets (Fig. 2). The average slope is 16.6 msec per target with standard deviation of 3.2 msec per target. Detection and discrimination of orientation clearly differ qualitatively. During a first stage, local gradients of orientation can be detected in parallel, but the direction and the magnitude of these gradients are unknown. In order to know *what* the orientation is in the neighborhood of the gradients, a second stage of serial inspection is required. The inspection time is ~17 msec per target, a time that seems to be independent of the detection time, which varies among observers. Interestingly, for each observer the discrimination time for two targets is the same as the detection time (Fig. 2).

Thus, knowing *what* the target is already requires serial inspection by focal attention. We next asked what process underlies knowing *where* a target is. We devised another experiment to show that at the detection stage observers are accurate in positional judgment. The observers were presented with either two or three targets; the three targets were arranged in two different patterns that can be described as triangles, one having a right angle and the other not (Fig. 3). The observers reported the number of targets, and when there were three, which spatial arrangement was presented. The psychometric curves for detection and localization overlap (Fig. 4). Localization reached its maximum at the same SOA as detection. Observer D.S. could perform the localization task with no better than 90 percent accuracy; that level was reached at the same time the targets could be detected—longer SOA's did not improve localization performance. Localization could be equated to detection by increasing the difference between the two triangles. The positional accuracy required to tell the two kinds of triangles apart was one or two line spacings between line elements; thus positional judgment is quite accurate at the detection stage.

Thus, detecting feature (orientation) gradients and locating them can be done in parallel, but identifying the features (orientation) and knowing what they are requires serial inspection with focal attention. What is surprising is that we find such scrutiny to be necessary for as simple a feature as the orientation of a line element that is regarded as a basic dimension in vision (*4–6*). This finding is inconsistent with the proposal that initial parallel feature processing in nontopographical feature spaces is followed by serial processing (with focal attention) to localize different features and to combine them according to their location (*1, 7*). In our experiments, the location of an object can be found by the parallel preattentive system; that parallel system is probably limited by its ability to detect feature gradients (over short spatial range) but not what occurs at these gradients. The role of focal attention might be suggested now as a necessary condition for knowing what actual features an object has. Whether our interpretation of detection versus discrimination as two different processes—parallel versus serial—will hold beyond orientation, that is, for other single features, such as color, direction of movement, and line segment width, remains to be seen.

Note added in proof: Recently we briefly presented a mixture of a few red and green disks (targets) embedded in an aggregate of yellow disks (each having the same size and luminance) followed by a mask of an aggregate of bipartite red-green disks. We found again that detecting and locating the colored targets could be done in parallel, whereas discriminating between their red and green colors required serial search by focal attention.

DOV SAGI
BELA JULESZ
AT&T Bell Laboratories,
Murray Hill, New Jersey 07974

References and Notes

1. A. Treisman and G. Gelade, *Cognit. Psychol.* **12**, 97 (1980).
2. B. Julesz, *Nature (London)* **290**, 91 (1981).
3. J. R. Bergen and B. Julesz, *ibid.* **303**, 696 (1983).
4. D. H. Hubel and T. N. Wiesel, *J. Physiol. London* **148**, 106 (1959).
5. H. B. Barlow, *Proc. R. Soc. London Ser. B* **212**, 1 (1981).
6. D. Marr, *Vision* (Freeman, San Francisco, 1982).
7. D. H. Ballard, G. F. Hinton, T. J. Sejnowsky, *Nature (London)* **306**, 21 (1983).

6 August 1984; accepted 8 February 1985

Measles Virus Matrix Protein Synthesized in a Subacute Sclerosing Panencephalitis Cell Line

Abstract. *Measles virus generally produces acute illness. Rarely, however, persistent infection of brain cells occurs, resulting in a chronic and fatal neurological disease, subacute sclerosing panencephalitis (SSPE). Evidence indicates that expression of the measles virus matrix protein is selectively restricted in this persistent infection, but the mechanism underlying this restriction has not been identified. Defective translation of matrix messenger RNA has been described in one SSPE cell line. This report presents evidence that in a different SSPE tissue culture cell line IP-3-Ca, the matrix protein is synthesized but fails to accumulate. A general scheme is proposed to reconcile the different levels at which restriction of matrix protein has been observed.*

Subacute sclerosing panencephalitis (SSPE) is a rare, chronic, and invariably fatal disease resulting from persistent measles virus (MV) infection of the human central nervous system (*1*). Distinguishing SSPE from the far more common acute MV infection is the failure of virion progeny to be produced by the persistently infected brain cells (*1*). The inability to consummate viral reproduction has been ascribed to a defect in the expression of a single MV gene product, the matrix protein (*2, 3*). This conclusion is supported by studies showing matrix protein to be the only structural protein of MV undetected in brain cells from patients with SSPE or in derivative SSPE cell lines (*2*) and by the presumably related observation that patients with this disease have a selective deficiency of antibodies to matrix protein (*3*). To investigate the mechanism by which matrix protein is restricted, we examined the MV matrix gene products of the SSPE cell line IP-3-Ca. In contrast to the results of prior studies of SSPE, IP-3-Ca was found to direct the synthesis of all MV proteins, including matrix protein. Once synthesized, however, matrix protein did not accumulate because it was unstable.

The IP-3-Ca cell line was originally

obtained by subculturing the IP-3 cell line—the product of the cocultivation of SSPE brain cells and BSc-1 African green monkey kidney cells (*4*). IP-3-Ca replicates as a stable carrier culture, develops syncytia that "heal" spontaneously, and does not produce viral progeny (*5*). It is further characterized by the presence of MV-related antigen in all cells, as determined by ultrastructural and immunocytochemical analyses; the ability to adsorb rhesus monkey red blood cells; and the capacity to transfer viral macromolecules to susceptible cells by membrane fusion (*5*). The demonstrated cell-to-cell spreading of viral material in the absence of assembly, budding, or release of virions appears to mimic SSPE in vivo.

The MV-related proteins synthesized by and accumulated within the IP-3-Ca cell were characterized by electrophoresis of viral proteins immunoprecipitated from radioactively labeled infected cell lysates. Edmonston MV proteins produced during lytic infection of BSc-1 cells were examined in parallel. Selection of the BSc-1 line for this comparative analysis was based on its parental relation to the IP-3-Ca virus-carrier cell line. The serological probe used was a polyvalent hyperimmune rabbit serum generated by immunization with highly purified, detergent-disrupted Edmonston MV (*6*). Its capacity to recognize the MV-related antigens of the SSPE virus carrier cell was confirmed by immunofluorescence microscopy. Subsequent immunoprecipitations performed with this hyperimmune serum revealed that 8 to 10 percent of the labeled BSc-1 intracellular proteins accumulated late in acute infection were virus-specified. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of such immunoprecipitates demonstrated that each of the major Edmonston MV structural proteins (*6, 7*) was synthesized and accumulated (Fig. 1a). Polypeptides other than the major viral structural proteins described above were usually immunoprecipitated as well. These included discrete polypeptide fragments of the MV nucleocapsid (NP) protein and a major protein of ~90K. The latter species also appears to be related to the NP protein (*8*), although its origin and its role in MV replication are unknown.

Immunoprecipitation of lysates derived from IP-3-Ca cells exposed to [^{35}S]methionine for 48 hours (two cell doublings) revealed that 2 to 3 percent of the labeled protein that accumulated in these persistently infected cells was MV-specified. The electrophoretic profile of the immunoprecipitated MV proteins did not show a labeled polypeptide corresponding to the MV matrix protein (lane 2 in Fig. 1b). Otherwise the MV structur-

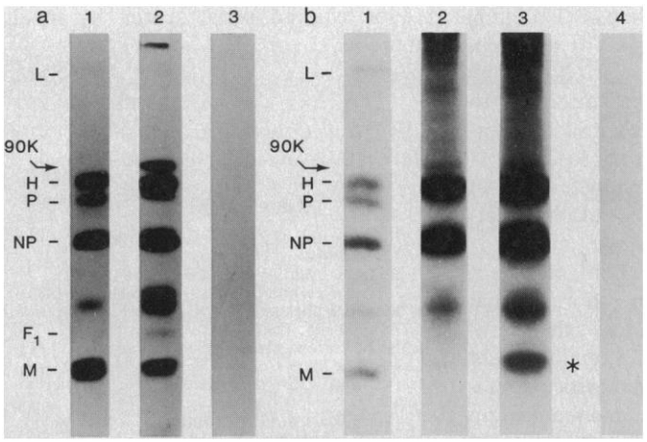

Fig. 1. Identification of a putative matrix protein synthesized but not accumulated in IP-3-Ca cells. (a) Confluent BSc-1 cell monolayers were infected with Edmonston MV (multiplicity of infection, 5 plaque-forming units per cell). One culture was labeled with [^{35}S]methionine (400 μCi/ml) in complete nutrient medium for the duration of acute infection, while another was labeled (800 μCi/ml) for 2 hours in medium lacking methionine after significant viral cytopathology developed. Cell lysates were prepared and immunoprecipitated and the MV proteins were analyzed by SDS-PAGE (*12, 14*). Fluorograms of 10 percent polyacrylamide gels are shown. Lane 1 shows structural proteins from purified measles virions; lane 2, MV proteins immunoprecipitated from infected BSc-1 cells (since the distribution of label among the MV proteins was unaffected by the duration of isotope incorporation, only the immunoprecipitate obtained from cells labeled throughout the infection is shown); and lane 3, immunoprecipitate from labeled uninfected BSc-1 cells. (b) The MV proteins of the IP-3-Ca cells were similarly analyzed after monolayer cultures were labeled with [^{35}S]methionine for 48 or 2 hours. Lane 1 shows measles virion proteins; lane 2, immunoprecipitated MV proteins accumulated after 48 hours of labeling; lane 3, immunoprecipitated MV proteins after 2 hours; and lane 4, control immunoprecipitate of labeled IP-3-Ca cell lysate treated with preimmunization serum. The nonspecific background radioactivity in lanes 2 and 3 was exaggerated by the prolonged (14-day) fluorographic exposure used to detect the presence of any labeled polypeptide corresponding to the MV matrix protein (compare Fig. 3). The asterisk denotes the ~38K putative matrix protein found in IP-3-Ca cells labeled for 2 hours. Abbreviations: L and P, nucleocapsid-associated proteins; H, hemagglutinin protein; F_1, fusion protein; M, matrix protein; and NP, nucleocapsid protein.

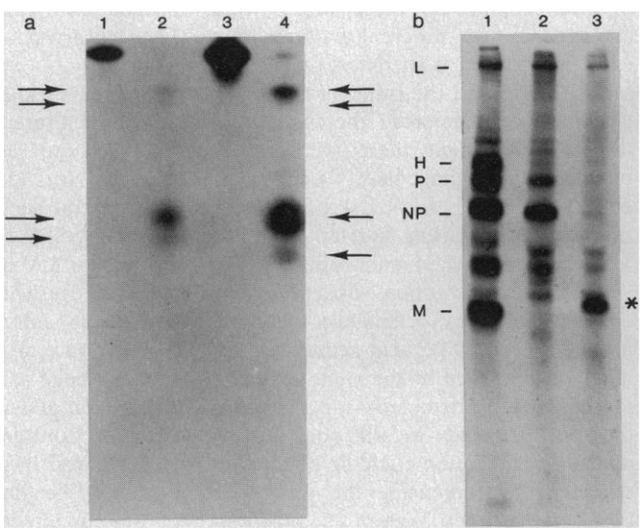

Fig. 2. Confirmation of the assignment of the ~38K IP-3-Ca polypeptide as matrix protein. (a) Peptide maps of the ~38K IP-3-Ca protein and the matrix protein of Edmonston MV. These two proteins were identified and excised from gels like those shown in Fig. 1a, lane 2, and Fig. 1b, lane 3. They were then subjected to limited proteolysis with *Staphylococcus aureus* V8 protease and the peptides generated were analyzed by electrophoresis in 17 percent polyacrylamide gel (*15*). Lanes 1 and 2 show the IP-3-Ca protein untreated and protease-digested, respectively; lanes 3 and 4, the corresponding undigested and digested Edmonston matrix protein. Arrows denote the proteolytic cleavage products. (b) Immunoprecipitation of the IP-3-Ca protein by monospecific antibodies to the Edmonston matrix protein. Lysates of labeled IP-3-Ca cells were prepared and subjected to preliminary immunoprecipitation with monospecific antiserum to NP. This procedure was used to immunoselect NP, the nucleocapsid-associated proteins (L and P), potential NP–matrix protein complexes, and the fragments of NP that might comigrate with matrix protein (lane 2). The portion of the IP-3-Ca cell lysate that was not removed by the antiserum was then exposed to monospecific antiserum to the Edmonston MV matrix protein (lane 3). The asterisk denotes the single major labeled protein of ~38K immunoprecipitated by this procedure. For comparison, the proteins of purified MV were analyzed in parallel (lane 1).

al proteins accumulated in IP-3-Ca cells and in acutely infected BSc-1 cells were similar. Although undetected by the above analysis, synthesis of a putative matrix protein was evident in immunoprecipitates of briefly labeled IP-3-Ca cells (lane 3 in Fig. 1b). The electrophoretic mobility of this labeled protein (~38K) was similar to that of the Edmonston MV matrix protein (~36K) and was well correlated with the estimated mass of matrix proteins specified by several other MV strains (*9*). The tentative assignment of the ~38K protein as matrix protein was confirmed by demonstrating both primary structure similarity (Fig. 2a) and antigenic relatedness (Fig. 2b) to the matrix protein of Edmonston MV.

Restriction of matrix protein expression in SSPE has been observed by several investigators (*2*), who proposed that the restriction may occur at transcription or translation. Recently, Carter *et al.* (*10*), studying the SSPE cell line N-1, showed that transcription of the matrix messenger RNA (mRNA) occurred, but that this transcript was unable to direct synthesis of matrix protein. The failure to program matrix protein translation occurred in vivo and in vitro, indicating that the restriction was unlikely to be host cell–specified. In our study the IP-3-Ca line also manifested restricted expression of this protein, but the restriction was not due to a defect in transcription or translation of the matrix gene product but rather to failure of the synthesized protein to accumulate normally. Additional evidence for this was obtained by comparing the rates at which matrix protein and the other MV proteins accumulated in IP-3-Ca cells (Fig. 3). A prominent band of newly synthesized matrix protein was readily apparent after short periods of labeling (0.5 to 2 hours). Thereafter, however, the contribution of matrix protein to the total accumulated labeled viral proteins diminished progressively, becoming barely detectable within 12 hours of continuous labeling.

These experiments and more recent studies (*11*) showing both adequate transcription and efficient translation of the IP-3-Ca matrix mRNA lead us to conclude that the matrix protein undergoes rapid posttranslational degradation. To our knowledge this is the first indication that an unstable matrix protein may be synthesized in SSPE (assuming, of course, that the IP-3-Ca cell adequately models SSPE in vivo). While host cell factors may be responsible for restricted expression of matrix protein, the identification of at least two disparate mechanisms by which the restriction may occur, defective translation of matrix mRNA (*10*) and posttranslational degradation of matrix protein, suggests other explanations. Furthermore, the recent demonstration of posttranslational degradation of matrix protein in BHK cells persistently infected with Sendai virus (*12*) indicates that defects in the products of the gene coding for matrix protein are not unique to SSPE, but rather may be common in persistent paramyxovirus infections.

How can the restriction of matrix protein expression in persistent infections of different host cells by different viruses be regulated by different molecular mechanisms? The answer may lie in the propensity of replicating RNA virus genomes to mutate frequently and in the capacity of these mutant genomes to survive during persistent infection (*13*). Certainly SSPE can be viewed as a persistent infection by an RNA virus. Since the matrix gene products are required neither for genome replication and transcription nor for cell-to-cell propagation of this infection, there should be no constraint on the survival of genomes containing mutations in the nucleotide sequence of the MV matrix gene. The nature and position of each mutation will then determine the level at which matrix protein restriction occurs. Missense mutations may result in the synthesis of matrix proteins that are biologically inactive, inherently unstable, or selectively degradable by host cell proteases. Nucleotide substitutions in noncoding regions may ablate or diminish binding of matrix mRNA to ribosomes or may alter translational efficiency. Other mutations may produce new nonsense codons, resulting in premature termination of matrix protein chain elongation. The nature and the role of these mutations in SSPE remain to be elucidated.

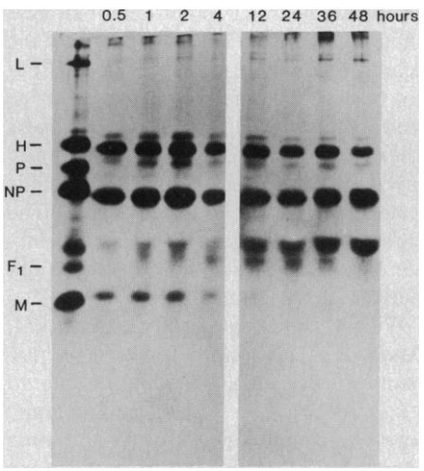

Fig. 3. Evaluation of the rate at which the matrix protein contribution to the total MV proteins accumulated in IP-3-Ca cells becomes negligible. Identical monolayers of IP-3-Ca cells were labeled for increasing periods of time, as described in the legend to Fig. 1. Immunoprecipitates prepared from each cell lysate were solubilized and a portion was assessed for radioactivity. The volume of solubilized immunoprecipitate subjected to SDS-PAGE was adjusted so that each sample contained identical amounts of radioactivity. The track at the far left shows the Edmonston MV structural proteins; this is followed in temporal sequence by the immunoprecipitates of IP-3-Ca cells.

RACHEL D. SHEPPARD*
CEDRIC S. RAINE
Department of Pathology, Albert Einstein College of Medicine, Bronx, New York 10461
MURRAY B. BORNSTEIN
Department of Neurology, Albert Einstein College of Medicine
STEPHEN A. UDEM†
Departments of Cell Biology, Medicine, and Microbiology and Immunology, Albert Einstein College of Medicine

References and Notes

1. G. Agnarsdottir, in *Recent Advances in Clinical Virology*, A. P. Waterson, Ed. (Churchill Livingstone, London, 1977), pp. 21–49; S. L. Wechsler and H. C. Meissner, *Prog. Med. Virol.* **28**, 65 (1982); V. ter Meulen, J. R. Stephenson, H. W. Kreth, in *Comprehensive Virology*, vol. 18, *Virus-Host Interactions: Receptors, Persistence, and Neurological Diseases*, H. Fraenkel-Conrat and R. R. Wagner, Eds. (Plenum, New York, 1983), pp. 105–159.
2. W. W. Hall and P. W. Choppin, *Virology* **99**, 443 (1979); F. H. Lin and H. Thormar, *Nature (London)* **285**, 490 (1980); W. W. Hall and P. W. Choppin, *N. Engl. J. Med.* **304**, 1152 (1981); C. E. Machamer, E. C. Hayes, H. J. Zweerink, *Virology* **108**, 515 (1981).
3. W. W. Hall, R. A. Lamb, P. W. Choppin, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 2047 (1979); S. L. Wechsler, H. L. Weiner, B. N. Fields, *J. Immunol.* **123**, 884 (1979).
4. T. Burnstein *et al.*, *Infect. Immun.* **10**, 1378 (1974).
5. R. D. Sheppard *et al.*, *ibid.* **12**, 891 (1975); R. D. Sheppard *et al.*, *J. Neuropathol. Exp. Neurol.* **34**, 87 (1975).
6. S. A. Udem, *J. Virol. Methods* **8**, 123 (1984).
7. W. E. Mountcastle and P. W. Choppin, *Virology* **78**, 463 (1977); D. L. J. Tyrrell and E. Norrby, *J. Gen. Virol.* **39**, 219 (1978); S. L. Wechsler and B. N. Fields, *J. Virol.* **25**, 285 (1978).
8. T. F. Wild and P. Giraudon, *J. Gen. Virol.* **63**, 247 (1982); unpublished observations.
9. W. W. Hall, W. Kiessling, V. ter Meulen, *Nature (London)* **272**, 460 (1978); S. L. Wechsler and B. N. Fields, *ibid.*, p. 458; B. K. Rima, S. J. Martin, E. A. Gould, *J. Gen. Virol.* **42**, 603 (1979).
10. M. J. Carter, M. M. Willcocks, V. ter Meulen, *Nature (London)* **305**, 153 (1983).
11. R. D. Sheppard and S. A. Udem, in preparation.
12. L. Roux and F. A. Waldvogel, *Cell* **28**, 293 (1982).
13. J. Holland *et al.*, *Science* **215**, 1577 (1982).
14. M. J. Birrer, B. R. Bloom, S. Udem, *Virology* **108**, 381 (1981).
15. D. W. Cleveland *et al.*, *J. Biol. Chem.* **252**, 1102 (1977).

16. Supported by National Multiple Sclerosis Society grants 1001 D-4 and 1241 C-3; National Institutes of Health grants NS 08952, NS 11920, and CA13330-12; and American Cancer Society grant MV-26. S.A.U. is a recipient of a Rita Allen Foundation scholarship and an Irma T. Hirschl Trust research career award.

* Present address: Department of Cellular, Viral, and Molecular Biology, University of Utah School of Medicine, Salt Lake City 84132.
† To whom correspondence should be addressed.

7 December 1984; accepted 6 March 1985