PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 1014–1017 (1974)

The Pattern of Virus-Specific Peptide Synthesis in Rabbit Cells Infected with Eastern Equine Encephalitis Virus and Its Response to Interferon (37944)

ANTHONY S. LUBINIECKI, JOHN A. ARMSTRONG, AND MONTO HO

Department of Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania 15261

Eastern equine encephalitis (EEE) virus is a group A arbovirus (togavirus) indigenous to North America. The virion consists of a core of single-stranded RNA encapsidated by multiple copies of a nucleocapsid protein, which is surrounded by a lipoprotein envelope (1, 2). Previous studies of protein and RNA synthesis of group A arboviruses have generally concentrated on later stages of infection, at or after the time of maximal viral RNA synthesis. This study shows that qualitative and quantitative changes in viral protein synthesis occur earlier during the infection cycle, and point to the existence of a control mechanism.

Materials and Methods. The preparation and storage of EEE stocks in chick embryo cell cultures have been previously described (2). Rabbit kidney (RK) cells were cultured in 5.0-cm Falcon (Oxnard, CA) plastic petri dishes, as reported earlier (3). Interferon pretreatment as accomplished by exposing cultures to partially purified rabbit interferon 16 hr before infection (2). RK cultures were infected at time zero with an input multiplicity of about 80 plaque-forming units (pfu)/cell of EEE virus. After 45 min at 37°C, cultures were refed with 2.0 ml minimal medium (2 mg/ml bovine serum albumin, 0.4 × BME amino acids, 2 mg/ml $NaHCO_3$ in Hanks' balanced salt solution, HBSS), and at times indicated labeled with 100 μCi ^{3}H-reconstituted protein hydrolysate (RPH, Schwarz Mixture, Schwarz-Mann, Orangeburg, NY) for 30 min or 3 hr at 37°C in 1.0 ml minimal medium without amino acids. After labeling, cultures were washed three times with ice-cold HBSS, and dissolved in 1.5 ml 0.1 *M* sodium phosphate (pH 7.2) containing 10 mg/ml sodium dodecyl sulfate and 10 mg/ml dithioerythritol.

Extracts were coelectrophoresed with small amount of purified ^{14}C-EEE virions in 10 cm 5% polyacrylamide gels for 7.5 hr at 4 V/cm constant voltage, as previously described by Strauss *et al.* (5). Gels were frozen, and cut into 1.0-mm transverse sections. Gel slices were treated with 0.25 ml in NaOH at 56°C overnight, neutralized, and counted in the presence of 10 ml Triton X-100: toluene (1 part: 4 parts) containing 4 g/liter PPO and 0.25 g/liter POPOP. Viral proteins were identified by aligning electropherograms of infected and uninfected cells using ^{14}C-EEE virion proteins as internal markers. Correction for virus-induced shutoff of normal host cell protein synthesis was accomplished by the method of Zweerink and Joklik (4). Subtraction of corrected cpm/fraction in uninfected cell electropherograms from the corresponding fraction of the infected cell electropherograms yielded viral proteins (4). When this procedure was employed on two uninfected gels, stable base lines reproducibly resulted.

Myoglobin, ovalbumin, bovine serum albumin, and rabbit IgG (all obtained from Sigma Chemical Co., St. Louis, MO) were employed as molecular weight standards and electrophoresed in parallel with labeled samples. After electrophoresis, standard gels

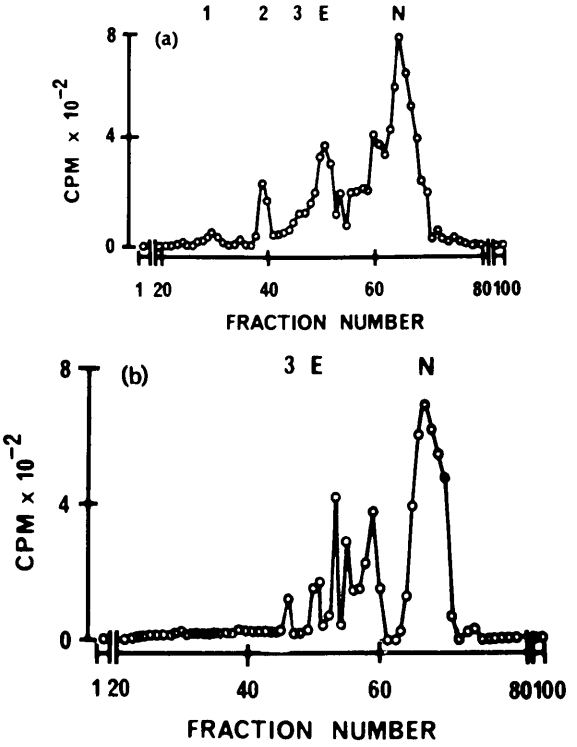

FIG. 1. Viral peptides found in RK cultures labeled 4–4.5 hr (a) or 5–5.5 hr (b) after infection.

were fixed in 20% sulfosalicylic acid, stained in 0.025% Coomassie blue, and destained in 10% trichloroacetic acid.

Results and Discussion. RK cultures infected with EEE virus synthesized viral proteins in two distinct patterns. Cultures labeled for 30 min at 1, 2, 3, or 4 hr postinfection demonstrated the same qualitative findings as those shown in Fig. 1a. Peptides labeled E and N coelectrophoresed with ^{14}C-EEE envelope and nucleocapsid proteins, respectively. Nonstructural proteins labeled 1, 2, and 3 were also reproducibly observed at all four times tested. Five similar peptides have also been described by other workers studying related group A arboviruses (5–9). Their average molecular weights, in decreasing order, were 105,000, 85,000, 65,000, 53,000, and 30,000, based on 5–12 determinations. Their sum (338,000) is within the theoretical coding capacity of the EEE RNA genome whose size is estimated to be 3.5×10^6 (10). Several additional peaks were often observed of molecular size intermediate between the E and N peptide. Analogous proteins have occasionally been reported in other related systems (5, 11). Viral proteins smaller than 30,000 were never observed. In general, the amounts of the various viral proteins increased with time after infection during the first 4 hr.

When cultures were labeled at 5, 6, 7, or 8 hr after infection, the peptide pattern was different from that observed earlier. Figure 1b shows that peptides 1 and 2 did not incorporate ^{3}H-amino acids at 5 hr. Identical results were obtained in cultures labeled at later times. To test whether failure to observe peptides 1 and 2 was due to increased turnover or decreased synthesis, cultures were labeled from 3–6 hr with 10 μCi ^{3}H-RPH. Figure 2 shows that peptides 1 and 2 labeled before 5 hr were still present at 6 hr after infection. Therefore, the synthesis of peptides 1 and 2 is probably terminated

between 4.5 and 5 hr after infection. Increased rate of turnover is not considered a likely mechanism to explain the absence of these two proteins from cultures labeled after 5 hr.

The above experiments were repeated with several modifications. Actinomycin D, added before or after virus infection to a final concentration of 2 μg/ml, had no effect on either final virus yield at 8–9 hr or the cessation of synthesis of peptides 1 and 2 at above 5 hr. In addition, cultures pretreated with 3000 units of interferon were labeled at 4 or 7 hr after infection. Interferon-treated cultures labeled at 4 or 7 hr contained peptides 1 and 2, while mock-treated cultures contained these two peptides only at 4 hr. Figure 3 shows the presence of peptides 1 and 2 at 7 hr. Cultures receiving less interferon (300, 30, or 3 units) synthesized peptides 1 and 2 at 4 hr, but not at 7 hr. This effect was also observed in the presence or absence of actinomycin D. These experiments show that the reproducible termination of peptide 1 and 2 synthesis is independent of host RNA synthesis. Since interferon has been shown to reduce EEE protein and RNA synthesis by amounts related to the dose of interferon employed (2), termination may be governed by the concentration or rate of synthesis of EEE RNA or protein. None of the doses of interferon employed produced any observable effect on the electrophoretic pattern of labeled uninfected cell proteins, or on acid-precipitable radioactivity after pulses of ^{3}H-RPH or ^{3}H-uridine in treated uninfected cells (2).

A control mechanism governing the synthesis of peptides 1 and 2 or their equivalents in related systems has not been previously described. Most other workers examined viral protein synthesis at relatively late times in the infection cycle, at or after viral RNA synthesis was proceeding at its maximum rate. One study of the entire infection cycle failed to detect qualitative changes in Semliki Forest virus (SFV) protein synthesis, but did find quantitative ones (7). No solid explanation is currently apparent to explain the discrepancy between the EEE and SFV data, although differences in cell type and virus strain are potential explanations. It is interesting that the rate of EEE RNA synthesis in RK cells steadily increased until about 4.5 hr, and declined thereafter (2). Since this decline in RNA synthesis is temporally coincident with cessation of synthesis of peptides 1 and 2, it is possible that these peptides are the two components of the viral RNA polymerase complex predicted from genetic studies of the closely related Sindbis virus (12).

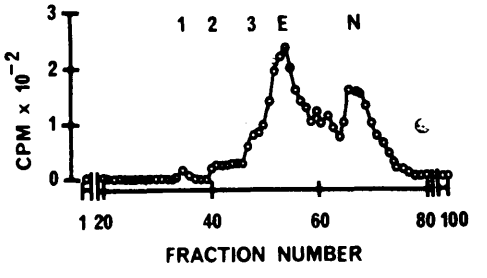

FIG. 2. Viral peptides found in RK cultures labeled 3–6 hr after infection.

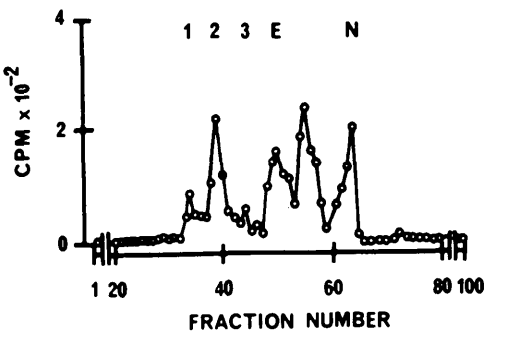

FIG. 3. Viral peptides found in RK cultures pretreated with 3000 units of interferon labeled 7–7.5 hr after infection.

Summary. Eastern equine encephalitis virus replicated extensively in rabbit kidney cells. Initially, five peptides were detected which are characteristic of cells infected by group A arboviruses. By 5 hr after infection, the two largest nonstructural peptides were no longer detected. Infected cultures labeled from 3–6 hr after infection demonstrated all five peptides. Failure to detect the two largest nonstructural proteins after 5 hr probably resulted from termination of their syntheses rather than increased rates of

degradation. This phenomenon may represent a regulatory mechanism governed by viral RNA and/or protein concentrations, since termination was not apparent in cultures pretreated with large doses of interferon.

This work was supported in part by NIH Grant 5ROI-AI-02953, 5R22-AI-02686, and NIH Predoctoral Fellowship 5 GM 41, 441-03. The technical assistance of Alma Tillman, Annie McCoy, Carlie White, and Mary White is gratefully acknowledged.

1. Armstrong, J. A., Freeberg, L., and Ho, M., Proc. Soc. Exp. Biol. Med. 137, 13 (1971).

2. Lubiniecki, A. S., "Effects of Interferon on the Replication of Eastern Equine Encephalitis Virus in Rabbit Kidney Cell Cultures." Doctoral Thesis, University of Pittsburgh, Pittsburgh, PA (1972).

3. Ke, Y. H., and Ho, M., J. Virol. 1, 883 (1967).

4. Zweerink, H. J., and Joklik, W. K., Virology 41, 501 (1971).

5. Strauss, J. H., Burge, B. W., and Darnell, J. E., Virology 37, 367 (1969).

6. Scheele, C. M., and Pfefferkorn, E. R., J. Virol. 3, 369 (1969).

7. Hay, A. J., Skehel, J. J., and Burke, D. C., J. Gen. Virol. 3, 175 (1968).

8. Friedman, R. M., J. Virol. 2, 1081 (1968).

9. Burrell, C. J., Martin, E. M., and Cooper, P. D., J. Gen. Virol. 6, 319 (1970).

10. Armstrong, J. A., Edmonds, M., Nakazoto, H., Phillips, B. A., and Vaughan, M. H., Science 176, 526 (1972).

11. Igarashi, A., Biken J. 13, 289 (1970).

12. Burge, B. W., and Pfefferkorn, E. R., Virology 30, 214 (1966).

Received Oct. 26, 1973. P.S.E.B.M., 1974, Vol. 145.