Shutoff of Host Translation by Encephalomyocarditis Virus Infection Does Not Involve Cleavage of the Eucaryotic Initiation Factor 4F Polypeptide That Accompanies Poliovirus Infection

JUDITH MOSENKIS,¹ SUSAN DANIELS-McQUEEN,¹ STEVEN JANOVEC,¹ ROGER DUNCAN,² JOHN W. B. HERSHEY,² JAMIE A. GRIFO,³ WILLIAM C. MERRICK,³ AND ROBERT E. THACH^{1*}

Department of Biology, Washington University, St. Louis, Missouri 63130¹; Department of Biological Chemistry, University of California School of Medicine, Davis, California 95616²; and Department of Biochemistry, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106³

Received 3 December 1984/Accepted 8 January 1985

Studies were conducted to determine whether encephalomyocarditis virus infection causes proteolytic cleavage of any of the polypeptides which comprise eucaryotic initiation factor 4F. Since no such alterations in the components of the initiation factor were detected, these observations confirmed that the mechanisms whereby encephalomyocarditis virus and poliovirus shut off host translation are different.

The mechanism by which poliovirus infection shuts off host cellular translation has been widely studied (2, 4, 5, 7, 8, 13, 17, 19, 20). Most investigators agree that the primary event responsible for the shutoff phenomenon is likely to be the proteolytic cleavage of the 220,000 M_r component (p220) of eucaryotic initiation factor 4F (eIF-4F). This cleavage appears to inactivate eIF-4F, thereby preventing the translation of capped mRNAs. Since virtually all host mRNAs are capped, whereas poliovirus mRNA is not (9, 16), this single event could easily account for the specificity of the shutoff phenomenon.

In view of the simplicity and elegance of this mechanism, it was disturbing to find that encephalomyocarditis virus (EMC) infection did not appear to produce the same result (10, 11, 14, 18). Specifically, no evidence for the inactivation of the ability to translate capped mRNAs could be detected in EMC-infected HeLa cells. In contrast, virtually complete inactivation was observed in the poliovirus-infected control (10). Although these experiments demonstrate a difference in the effects of EMC and poliovirus infections on the ability to translate capped mRNAs, it seemed important to determine whether structural changes in eIF-4F components might still be induced by EMC infection. To this end, we assayed for the proteolytic cleavage of eIF-4F p220 in EMC-infected L cells by two different techniques.

To determine whether endogenous p220 is cleaved in EMC-infected L cells, the immunoblotting procedure previously described was employed (5). Briefly, L cells were infected with EMC at a multiplicity of 20 PFU per cell as described before (11). After 4 h, when both the shutoff of host translation and the rate of viral translation were maximal, infected and mock-infected cells were lysed, and proteins were analyzed by sodium dodecylsulfate-polyacrylamide gel electrophoresis. Proteins in these electrophoretograms were then electroblotted onto a nitrocellulose sheet. The presence of p220 or its cleavage products was then detected in these Western blots with an affinity-purified antibody to p220 (15), followed by exposure to ¹²⁵I-labeled rabbit anti-goat immunoglobulin G (IgG) and autoradiography. A typical experimental result is shown in Fig. 1. The

single dark band in lane 1 (EMC infected) and lane 2 (mock infected) is p220. Little, if any, evidence of proteolytic cleavage was detected. For the sake of comparison, results from a parallel experiment with rhinovirus type 14-infected HeLa cells are shown in lane 3 (rhinovirus infected) and lane

FIG. 1. Lack of cleavage of eIF-4F component p220 in EMC-infected L cells. Cells were infected at a multiplicity of infection of 20 with EMC (or mock infected), and lysates were prepared 4 h postinfection as previously described (11). These lysates were assayed for p220 by the Western blotting technique with an affinity-purified antibody against rabbit p220 as previously described (5, 15). This antibody cross-reacts strongly with both mouse and human p220 (data not shown). The location of the antigen reacting with this antibody was detected by a second ¹²⁵I-labeled antibody (rabbit anti-goat IgG) and autoradiography. In a control experiment, HeLa cells were infected with human rhinovirus type 14 (or mock infected), and lysates were prepared by standard procedures (4a). Lanes contained the following lysates: 1, EMC-infected L cell; 2, mock-infected L cell; 3, rhinovirus-infected HeLa cell; 4, mock-infected HeLa cell.

* Corresponding author.

FIG. 2. Absence of proteolytic activity against eIF-4F components. Purified eIF-4F was labeled by reductive methylation with $H_2^{14}CO$ (12), then incubated with lysates prepared from infected and mock-infected cells. The fate of labeled peptides in these reactions was then determined by sodium dodecylsulfate-polyacrylamide gel electrophoresis and fluorography as previously described (11). Lanes contained the following lysates: 1, poliovirus-infected HeLa cell; 2, mock-infected HeLa cell; 3, EMC-infected L cell; 4, mock-infected L cell; 5, equal portions of poliovirus-infected HeLa cell and EMC-infected L cell. The p220, p48, and p24 components of eIF-4F are indicated. The only identifiable cleavage product (c.p.) generated in poliovirus-infected lysates is also indicated.

4 (mock infected). Thus, whereas minimal (less than 1%) cleavage of p220 occurred in EMC-infected cells, in rhinovirus (as in poliovirus)-infected cells virtually 100% of the p220 was cleaved. The cleavage of p220 in rhinovirus type 14 cells was observed previously by Etchison and Fout (4a).

Whereas these experiments indicated that p220 was not cleaved as a result of EMC infection, they did not rule out the possibility that other components of eIF-4F are altered. To test for this possibility, highly purified eIF-4F (6) was radiolabeled with $H_2^{14}CO$ and reduced with $NaCNBH_3$ (12). Samples of this factor were then incubated with lysates prepared from infected or mock-infected cells. Approximately 1 μg of labeled factor was added to a 25- μl reaction mixture containing 10 μl of infected or mock-infected cell lysate, 18 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (pH 7.5), 2.5 mM $Mg(OAc)_2$, 100 mM KOAc, 0.05 mg of spermidine per ml, 0.16 mg of creatine phosphokinase per ml, 10 mM creatine phosphate, 1 mM ATP, 0.6 mM CTP, 0.1 mM GTP, and 0.4 mM dithiothreitol; reaction mixtures were incubated for 2 h at 30°C. The products of these reactions were analyzed by sodium dodecylsulfate-polyacrylamide gel electrophoresis and fluorography. A typical result is shown in Fig. 2. The results of a control experiment with poliovirus-infected HeLa cell lysates prepared as previously described (10) are shown in lane 1 (poliovirus infected) and lane 2 (mock infected). Under the conditions of the assay, most of the p220 band was cleaved by the poliovirus-infected lysate. The only obvious product of this cleavage was the band labeled c.p. in Fig. 2. In contrast, lysates from EMC-infected L cells had no detectable effect on any of the labeled eIF-4F bands, as is evident in lane 3 (EMC infected) and lane 4 (mock infected). The lack of protease activity in the EMC-infected lysate was not due to the presence of an inhibitor, since an equipartite mixture of EMC- and poliovirus-infected lysates still resulted in the cleavage of p220 (lane 5). In separate experiments, all lysates were active in translating both endogenous and exogenous messages and in processing viral

polypeptide precursors (data not shown). These results confirmed and extended those shown in Fig. 1; in neither case was any evidence for the proteolysis of eIF-4F components detected in EMC-infected cells.

It may be concluded on the basis of these experiments that the mechanisms by which poliovirus and EMC shut off host cell translation are distinctly different. This finding confirmed those of previous studies that employed activity assays (10). Whereas the mechanism employed by poliovirus may be simply the inactivation of eIF-4F via proteolysis, that induced by EMC is more complex and actually may involve more than one effect (1, 3, 10, 11). We cannot at present rule out the possibility that EMC infection results in some other type of modification of eIF-4F. For example, phosphorylation of eIF-4F seemed an attractive possibility.

It will be of interest to see which other types of viruses have adopted the poliovirus-type shutoff mechanism. Thus far, only rhinovirus type 14 appears to fall into this category (4a). Other viruses, such as Mengovirus, reovirus types I and III, vaccinia virus, and frog virus III, are quite dissimilar in that they lack mechanisms for cleaving p220 (R. Duncan and J. W. B. Hershey, unpublished data).

This work was supported by United States Public Health Service grants AI 20484, GM 26796, GM 07250, and GM 22135 from the National Institutes of Health.

Rhinovirus-infected HeLa cell extracts were kindly provided by Shay Fout.

LITERATURE CITED

1. Alonso, M. A., and L. Carrasco. 1981. Reversion by hypotonic medium of the shutoff of protein synthesis induced by encephalomyocarditis virus. *J. Virol.* 37:535-540.
2. Baltimore, D. 1969. The replication of picornaviruses, p. 101-176. In H. B. Levy (ed.), *The biochemistry of viruses*. Marcel Dekker, Inc., New York.
3. Carrasco, L., and A. E. Smith. 1976. Sodium ions and the shutoff of host cell protein synthesis by picornaviruses. *Nature*

- (London) 264:807-809.
4. **Ehrenfeld, E.** 1982. Poliovirus-induced inhibition of host-cell protein synthesis. *Cell* 28:435-436.
 - 4a. **Etchison, D., and S. Fout.** 1985. Human rhinovirus 14 infection of HeLa cells results in the proteolytic cleavage of the p220 cap-binding complex subunit and inactivates globin mRNA translation in vitro. *J. Virol.* 54:634-638.
 5. **Etchison, D., S. C. Milburn, I. Edery, N. Sonenberg, and J. W. B. Hershey.** 1982. Inhibition of HeLa cell protein synthesis following poliovirus infection correlates with the proteolysis of a 220,000 dalton polypeptide associated with initiation factor eIF-3 and a cap binding protein complex. *J. Biol. Chem.* 257:14806-14810.
 6. **Grifo, J. A., S. M. Tahara, M. A. Morgan, A. J. Shatkin, and W. C. Merrick.** 1983. New initiation factor required for globin mRNA translation. *J. Biol. Chem.* 258:5804-5810.
 7. **Helentjaris, T., and E. Ehrenfeld.** 1978. Control of protein synthesis in extracts from poliovirus-infected cells. I. mRNA discrimination by crude initiation factors. *J. Virol.* 26:510-521.
 8. **Helentjaris, T., E. Ehrenfeld, M. L. Brown-Leudi, and J. W. B. Hershey.** 1979. Alteration in initiation factor activity from poliovirus-infected HeLa cells. *J. Biol. Chem.* 254:10973-10978.
 9. **Hewlett, M. J., J. K. Rose, and D. Baltimore.** 1976. The 5' terminal structure of poliovirus polyribosomal RNA is pUp. *Proc. Natl. Acad. Sci. U.S.A.* 73:327-330.
 10. **Jen, G., B. M. Detjen, and R. E. Thach.** 1980. Shutoff of HeLa cell protein synthesis by encephalomyocarditis virus and poliovirus: a comparative study. *J. Virol.* 35:150-156.
 11. **Jen, G., and R. E. Thach.** 1982. Inhibition of host translation in encephalomyocarditis virus-infected L cells: a novel mechanism. *J. Virol.* 43:250-261.
 12. **Jentoft, N., and D. G. Dearborn.** 1979. Labelling of proteins by reductive methylation using sodium cyanoborohydride. *J. Biol. Chem.* 254:4359-4365.
 13. **Kaufmann, Y., E. Goldstein, and S. Penman.** 1976. Poliovirus-induced inhibition of polypeptide initiation in vitro on native polyribosomes. *Proc. Natl. Acad. Sci. U.S.A.* 73:1834-1838.
 14. **Lawrence, C., and R. E. Thach.** 1974. Encephalomyocarditis virus infection of mouse plasmacytoma cells. I. Inhibition of cellular protein synthesis. *J. Virol.* 14:598-610.
 15. **Meyer, L. J., S. C. Milburn, and J. W. B. Hershey.** 1982. Immunochemical characterization of mammalian protein synthesis initiation factors. *Biochemistry* 21:4206-4212.
 16. **Nomoto, A., N. Kitamura, F. Golini, and E. Wimmer.** 1977. The 5' terminal structures of poliovirion RNA and poliovirus mRNA differ only in the genome-linked protein VPg. *Proc. Natl. Acad. Sci. U.S.A.* 74:5345-5349.
 17. **Rose, J. K., H. Trachsel, K. Leong, and D. Baltimore.** 1978. Inhibition of translation by poliovirus: inactivation of a specific initiation factor. *Proc. Natl. Acad. Sci. U.S.A.* 75:2732-2736.
 18. **Svitkin, Y. V., V. A. Ginevskaya, T. Y. Ugarova, and V. I. Agol.** 1978. A cell-free model of the encephalomyocarditis virus-induced inhibition of host cell protein synthesis. *Virology* 87:199-203.
 19. **Tahara, S. M., M. A. Morgan, J. A. Grifo, W. C. Merrick, and A. J. Shatkin.** 1982. Interactions of cap binding proteins with eukaryotic mRNAs. *Der. Biochem.* 21:359-372.
 20. **Trachsel, H., N. Sonenberg, A. J. Shatkin, J. K. Rose, K. Leong, J. E. Bergmann, J. Gordon, and D. Baltimore.** 1980. Purification of a factor that restores translation of vesicular stomatitis virus mRNA in extracts from poliovirus-infected HeLa cells. *Proc. Natl. Acad. Sci. U.S.A.* 77:770-774.