JOURNAL OF VIROLOGY, July 1972, p. 162-165
Vol. 10, No. 1

Host-Virus Interactions in *Escherichia coli*: Effect of Stationary Phase on Viral Release from MS2-Infected Bacteria

C. PROPST RICCIUTI

Department of Microbiology, Yale University Medical School, New Haven, Connecticut 06510, and Department of Engineering and Applied Science, Yale University, New Haven, Connecticut 06520

Received for publication 15 March 1972

Infection of stationary-phase *Escherichia coli* with MS2 bacteriophage results in the production, but not the release, of progeny virus by the host. Substantial protein synthesis in stationary-phase cells indicates that general protein synthesis is not sufficient to assure cell lysis or viral release.

The production of progeny ribonucleic acid (RNA) bacteriophage usually terminates with the release of these viruses from the host bacterium. Very little is known about the process of viral release. Under normal growing conditions (37 C, log phase), this release is accompanied by cellular lysis. It has been suggested that bacteriophage coat protein is involved in the lysis process (12). Under conditions of reduced temperature (31 C), however, phage release appears to occur without cell lysis (2, 6). Recently Engelberg and Soudry have suggested that a short-lived, host-controlled protein(s) is essential for bacteriophage release at both temperatures (2). This conclusion is based on the finding that chloramphenicol- and rifampin-treated cells, which do not synthesize proteins, do not release virus, as well.

In studying host-virus interactions between MS2 and stationary-phase *Escherichia coli*, it was found that cell lysis did not occur in stationary-phase infections. Figure 1 shows a typical experiment in which *E. coli* 3000 (Hfr Thi$^-$ from R. L. Sinsheimer) was grown into stationary phase in a minimal medium of the following composition: 0.1 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffered at *p*H 7.6, 0.085 M NaCl, 0.1 M KCl, 0.02 M NH_4Cl, 0.025 mM Na_2SO_4, 0.34 mM KH_2PO_4, 2.5 mM $CaCl_2$, and 2.5 mM $MgCl_2$. The medium was also supplemented with 0.1 mg of thiamine and 5 mg of glucose per ml. Growth was followed by optical density at 450 nm. At different times in growth, samples were removed and infected with MS2 virus. Before use, the virus was banded in CsCl by centrifugation for 24 hr at 40,000 rev/min using a type 65 rotor and a model L2-65B ultracentrifuge. The density of the virus-CsCl solution was 1.385. CsCl fractions having high titers of virus were further purified through Sephadex G-75 and collected in 0.1 M NaCl-0.01 M Tris (*p*H7.6) buffer. The multiplicity of infection (MOI) was between three and five. After infection, the optical density of both infected and uninfected cultures was followed. From Fig. 1 it is evident that cell lysis occurred in log-phase but not stationary-phase infections.

Although growth of virus in stationary-phase cells is not as productive as growth in log-phase cells (partially due to the failure of phage to adsorb as well to stationary-phase cells), previous experiments in this laboratory had shown substantial growth of MS2 in stationary-phase *E. coli*. It was therefore of interest to determine whether the absence of cell lysis in stationary phase represented a failure of infected cells to release phage or whether the viruses were being released via a mechanism not involving cell lysis. To answer this question *E. coli* 3000 was grown into stationary phase and infected with purified MS2 at a MOI of five. The arrow in Fig. 1 marks the approximate time in stationary phase at which this experiment was run. At different times after infection, duplicate samples were removed and assayed for (i) intracellular plus extracellular (produced) virus, and (ii) extracellular (released) virus. For determination of intracellular plus extracellular virus, the infected cells were artificially lysed using a lysozyme-ethylenediaminetetraacetic acid procedure (5). For determination of extracellular virus, the infected cells remained unlysed and were removed prior to the assay by centrifugation. Unadsorbed phage contribute approximately 3×10^8 plaque-forming units/ml to both extracellular and intracellular plus extracellular virus assays. The results of this experiment (Fig. 2) show that few if any

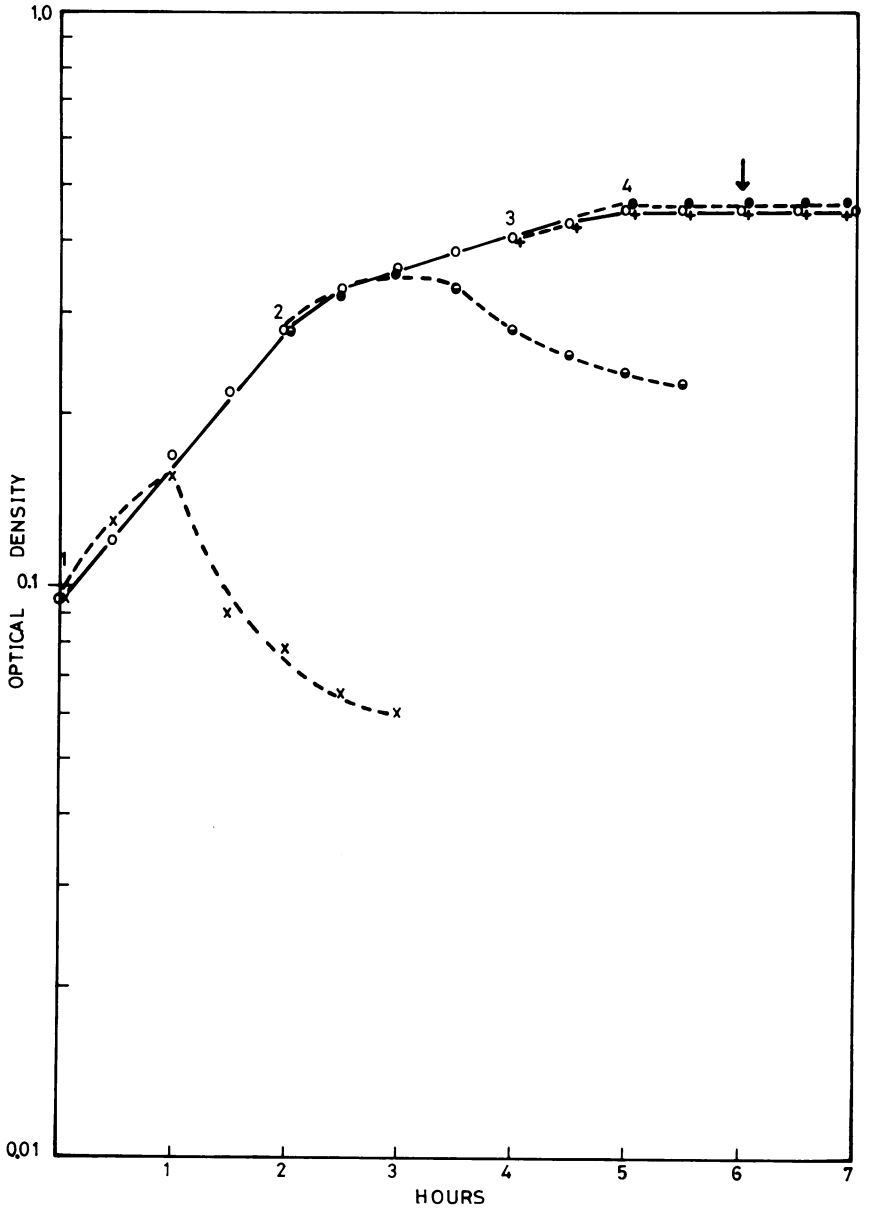

FIG. 1. *Effect of culture age on lysis of MS2-infected E. coli. At different times in culture growth, indicated by the numbers 1 to 4 along the optical density curve, samples were removed and infected with MS2 virus. The optical density (OD) at 450 nm of the uninfected culture and each infected sample was followed. Symbols: ○, OD of the uninfected culture; ×, OD of the first infected sample; ◒, OD of the second infected sample; +, OD of the third infected sample; ●, OD of the fourth infected sample.*

viruses are being released by stationary-phase cells, despite the fact that progeny viruses are being synthesized by these cells. This phenomenon appears to be common to nongrowing cells since similar results have been found under stationary-phase conditions using Robert's C medium plus 0.1% Casamino Acids (11) and starvation conditions using GSO medium without amino acids (3), which was made limiting for either glucose or nitrogen.

Engelberg and Soudry have suggested that host-controlled protein synthesis is required for MS2

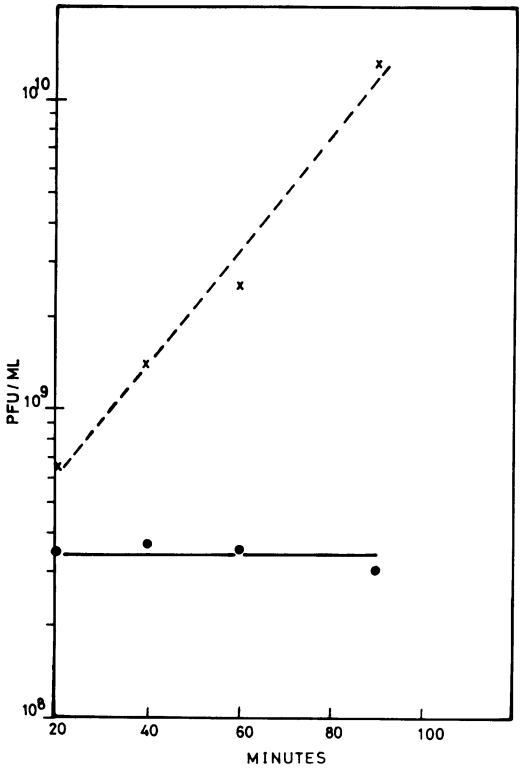

FIG. 2. *Effect of stationary phase on the production and release of MS2 by infected E. coli. Symbols:* ●, *extracellular (released) virus;* ×, *intracellular plus extracellular (produced) virus. "Minutes" are minutes postinfection.*

release and that the inability of cells treated with chloramphenicol or rifampin to release progeny phage is due to a failure of these cells to synthesize proteins (2). To determine whether the inability of stationary-phase cells to release phage was correlated with an inability to synthesize proteins, the protein synthesis of log- and stationary-phase cells was measured. *E. coli* 3000 was grown into stationary phase, and, at different times in growth, protein synthesis was measured by labeling cells with ^{3}H-leucine (6 Ci/mmole, 2 μCi/ml). The incorporation of label was stopped after 2 min by adding sodium azide to 0.2 M and quick chilling the sample in a dry ice-ethanol bath. Samples were put onto 3M filters, hot trichloroacetic acid-precipitated, and counted in Liquifluor-toluene scintillation fluid. The results of such an experiment (Fig. 3) indicate that there is substantial protein synthesis occurring in stationary-phase cells, which in three experiments averaged 44% of the maximal, log-phase protein synthesis. The ability of stationary-phase cells to continue protein synthesis is not totally unexpected. It is well documented in vitro that protein synthesis activity is increased with culture age and remains high in stationary-phase cell extracts, indicating that these cells retain their potential for extensive protein synthesis (9). The materials necessary for stationary-phase synthesis are believed to come from two major sources. Stationary-phase cells, under the conditions used here, appear capable of synthesizing some new materials since these cells continue to increase in mass at a slow rate, approximately one-seventh of that found in log-phase growth (note Fig. 3). The cells may also reuse amino acids freed by degradation of preexisting proteins. The contribution of turnover is difficult to ascertain, however, since rates for nongrowing *E. coli* vary from 4 to 20% per hr depending on the technique used (1, 7, 8). Furthermore, since the determination of turnover rates often involves the transfer of cells from growth medium to buffer, results obtained under these conditions may not adequately represent what occurs in stationary phase.

The fact that significant protein synthesis does occur in stationary-phase cells indicates that general protein synthesis is not sufficient to assure virus release. If a short-lived, host-controlled protein is essential for RNA bacteriophage release, it is possible that this protein is not made or activated by infected, stationary-phase cells. There is ample precedent for changes in specific proteins under stationary-phase conditions. Polynucleotide phosphorylase shows decreased activity (9), and

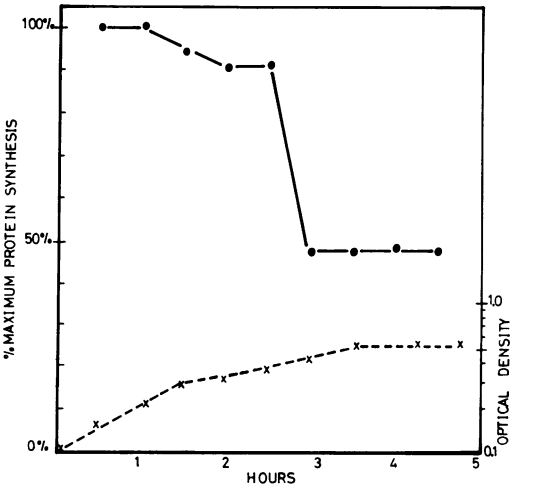

FIG. 3. *Protein synthesis in uninfected, stationary- and log-phase E. coli. Symbols:* ●, *percent maximum protein synthesis;* ×, *optical density at 450 nm of growing culture. Protein synthesis data have been corrected for cell numbers.*

catalase (4) and thymidine kinase (10) increased production in stationary-phase cells. Without eliminating other alternatives, it is also possible, since cell membranes are changed in stationary-phase cells (9), that the host-controlled protein is synthesized or activated properly, or both, but is incapable of reacting with the modified cell membrane to allow phage release.

This investigation was carried out in the laboratory of Anne M. Haywood and supported jointly by Public Health Service grant AI 07788 from the National Institute of Allergy and Infectious Diseases and Public Health Service research fellowship 5-f01-GM 36985-04 from the National Institute of General Medical Sciences.

I thank Bennett Kaufman for performing the preliminary experiment which suggested that lysis of infected stationary-phase cells did not occur.

LITERATURE CITED

1. Borek, E., L. Ponticorvo, and D. Rittenberg. 1958. Protein turnover in microorganisms. Proc. Nat. Acad. Sci. U.S.A. 44:369–374
2. Engelberg, H., and E. Soudry. 1971. Ribonucleic acid bacteriophage release: requirement for host-controlled protein synthesis. J. Virol. 8:257–264.
3. Garwes, D., A. Sillero, and S. Ochoa. 1969. Virus-specific proteins in *Escherichia coli* infected with phage Qβ. Biochim. Biophys. Acta 186:166–172.
4. Gray, E., A. Haywood, and E. Chargaff. 1964. Rapidly labeled ribonucleic acids of *Rhodopseudomonas spheroides* under varying conditions of catalase synthesis. Biochim. Biophys. Acta 87:397–415.
5. Haywood, A., and J. Harris. 1966. Actinomycin inhibition of MS2 replication. J. Mol. Biol. 18:448–463.
6. Hoffmann-Berling, H., and R. Maze. 1964. Release of male specific bacteriophage from surviving host bacteria. Virology 22:305–313.
7. Mandelstam, J. 1957. Turnover of protein in starved bacteria and its relationship to the induced synthesis of enzyme. Nature (London) 179:1179–1181.
8. Mandelstam, J. 1958. Turnover of protein in growing and nongrowing populations of *E. coli*. Biochem. J. 69:110–119.
9. Moore, L. D., F. J. Kocan, and W. W. Umbreit. 1966. Cell-free protein synthesis: effects of age and state of ribosomal aggregation. Science 154:1350–1353.
10. Okazaki, R., and A. Kornberg. 1964. Deoxythymidine kinase of *E. coli*: I. Purification and some properties of the enzyme. J. Biol. Chem. 239:269.
11. Roberts, R., D. Cowie, P. Abelson, E. Bolton, and R. Britten. 1957. Studies of biosynthesis in *E. coli*, p. 5. Carnegie Inst. Wash. Publ. no. 607.
12. Zinder, N. D., and I. B. Lyons. 1968. Cell lysis: another function of coat protein of the bacteriophage f2. Science 159:84–85.