CELL-FREE PROTEIN SYNTHESIS DIRECTED BY COLIPHAGE MS2 RNA: SEQUENTIAL SYNTHESIS OF SPECIFIC PHAGE PROTEINS

Kathleen Eggen, Max P. Oeschger, and Daniel Nathans
Department of Microbiology, The Johns Hopkins University
School of Medicine, Baltimore, Maryland 21205

Received July 17, 1967

In E. coli infected with an RNA phage, three phage-specific proteins have been detected which correspond with the three known cistrons in the phage RNA (Nathans, Oeschger, Eggen and Shimura, 1966; Vinuela, Algranati, and Ochoa, 1967). One of these proteins, the phage coat protein, is synthesized at a greater rate and for a longer time than the other two (Oeschger and Nathans, 1966; Nathans et al., 1966; Vinuela et al., 1967). In E. coli extracts the phage RNA also directs the synthesis predominantly of phage coat protein and lesser amounts of non-coat proteins (Nathans, Notani, Schwartz and Zinder, 1962; Ohtaka and Spiegelman, 1963; Nathans, 1965). In contrast to the situation in infected cells, in extracts coat protein appears prior to the appearance of non-coat proteins, as measured by the incorporation of histidine, which is lacking in the phage coat (Ohtaka and Spiegelman, 1963). We are exploring these possible manifestations of regulation at the translational level by measuring the synthesis of phage-specific proteins under various conditions, both in infected cells and in cell extracts. As an extension of our previous findings (Nathans et al., 1966), we report here that cell-free extracts of E. coli incubated with MS2 RNA synthesize phage proteins which are similar in electrophoretic mobility to the three

phage proteins found in MS2-infected cells, and that these proteins appear sequentially during incubation of the extract.

MATERIALS AND METHODS

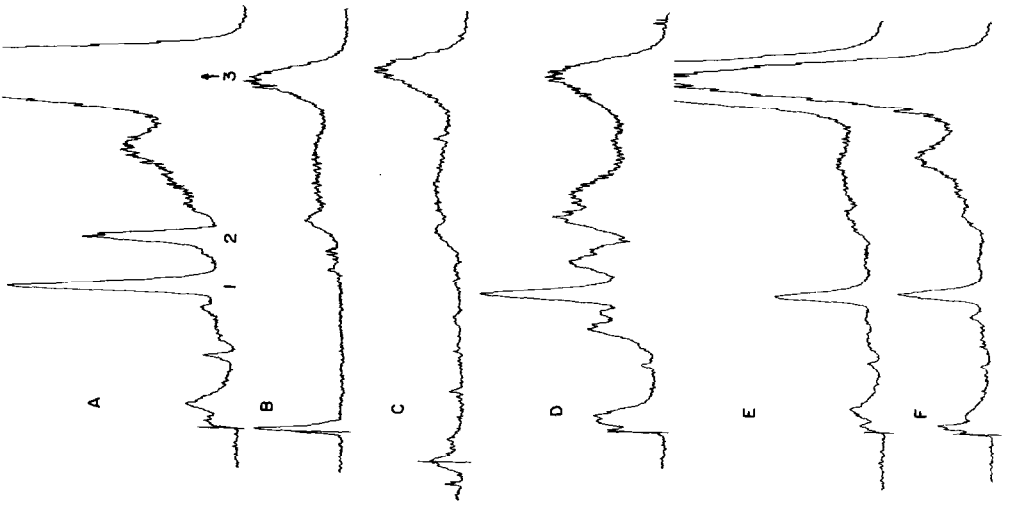
The source and growth of coliphage MS2 and its host E. coli C3000 and the method of preparing MS2 RNA have been given previously (Shimura et al., 1965). Amber mutants of MS2 were isolated after nitrous acid treatment of the phage as described by Zinder and Cooper (1964) and by Gussin (1966), employing E. coli K37 and K38 as indicator bacteria (Zinder and Cooper, 1964). Phage proteins were labelled in actinomycin-treated E. coli C3000-42 ($lys^-ileu^-val^-arg^-$) infected with MS2 (Oeschger and Nathans, 1966) and analysed by acrylamide gel electrophoresis and radioautography (Nathans et al., 1966). Cell-free synthesis of proteins was carried out as previously described (Nathans, 1965) except that E. coli Q13 (kindly provided by W. Gilbert) was used to prepare cell extracts. The protein products were solubilized by treating the incubation mixture with sodium dodecyl sulfate (final concentration 1%), ethylenediaminetetraacetate (0.05 M) and mercaptoethanol (0.05 M). After 1 hour at 37°, 1/20 volume of 0.1 M C^{12}-amino acids was added, and the samples were dialysed on a rapid, semi-micro dialyzer against 0.1% sodium dodecyl sulfate—0.01 M mercaptoethanol. At the end of dialysis, 1/4 volume of 60% sucrose and 1/100 volume of 1 M potassium phosphate, pH 7.2, were added and the samples electrophoresed in 10% acrylamide gels (Summers, Maizel and Darnell, 1965). After electrophoresis, the gel columns were removed to 5% trichloracetic acid and left overnight in the cold. They were then extracted with 5% trichloracetic acid at 80° for 15 minutes and washed five times with 7 1/2% acetic acid prior to being sliced and dried for radioautography (Fairbanks, Levinthal and Reeder, 1965). The trichloracetic acid washing procedure eliminates small peptides attached to sRNA and results in better defined protein bands in the

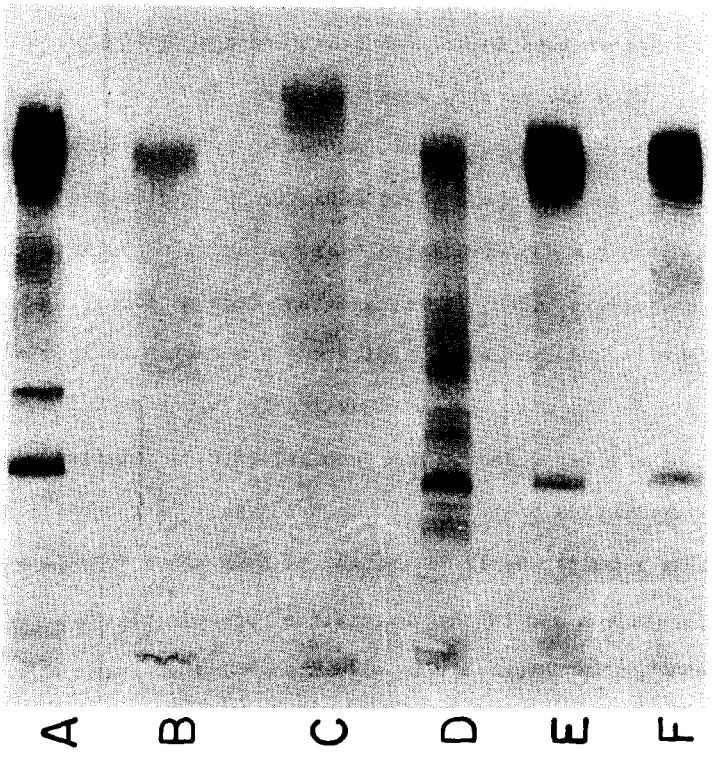
radioautograms. In instances where proteins were quantitated, this was done by planimeter measurements of microdensitometer tracings of the radioautograms. In each case the exposure time was such that film blackening was proportional to radioactivity, as shown by simultaneous exposure of a series of standard gel segments.

RESULTS AND DISCUSSION

Identification of phage proteins in infected cells

As shown previously (Nathans et al., 1966), non-permissive cells infected with amber mutants of the RNA phage lack specific phage proteins, thus allowing identification of these proteins. We have extended our earlier results by examining several newly isolated amber mutants of MS2 whose properties will be reported in more detail elsewhere. Figure 1 illustrates the electrophoretic analysis of phage proteins synthesized in actinomycin-treated E. coli C3000-42 after infection with MS2 or with amber mutants. Am 5, am 8, and am 13 appear to have single amber mutations, as judged by reversion frequencies of 0.5 to 3%. Am 5 and am 8 yield defective particles and therefore have mutations in the "RNA-protecting" or "maturation" protein (Lodish et al., 1965; Heisenberg, 1966; Argetsinger and Gussin, 1966). Am 13 is defective in RNA synthesis, indicating a mutation in the RNA synthetase cistron. Am 12, which has a reversion frequency of 0.03%, appears to be a double amber mutant. Non-permissive cells infected with this mutant synthesize phage RNA but do not lyse. On the basis of these and other results, am 12 is thought to have amber mutations in the coat protein cistron and in the "maturation" protein cistron. As shown in figure 1, cells infected with wild type MS2 show three phage-specific protein peaks (labelled 1, 2, 3) and a variable fourth component (see below). Cells infected with am 5 or am 8 lack the peak 2 protein, which is therefore the "maturation" protein, confirming our previous





See Legend on following page

Figure 1. Radioautograms (left) and microdensitometer tracings (right) of C^{14}-proteins made in actinomycin-treated E. coli C3000-42 infected with MS2 or amber mutants of MS2. Log phase cells were treated with ethylenediaminetetraacetate and actinomycin as previously described (Oeschger and Nathans, 1966), infected with phage at an input multiplicity of 4, and C^{14}-lysine, isoleucine, valine and arginine added at 8 minutes after infection. At 50 minutes after infection, the cells were chilled and washed and the proteins analysed. Cells were infected with the following phages: A. MS2 B. None C. Am 13 D. Am 12 E. Am 5 F. Am 8.

---

finding with the f2 mutant sus 11 (Lodish and Zinder, 1965). Cells infected with am 12 lack both peak 2 and peak 3 but have peak 1, again confirming that peak 3 is the coat protein. From this result we have also inferred that peak 1 protein is the RNA synthetase; the only mutants (e.g., am 13) which fail to yield peak 1 protein in infected cells show no detectable phage protein or RNA synthesis. Since the identification of peak 1 is indirect, we refer to it as (?) RNA synthetase.

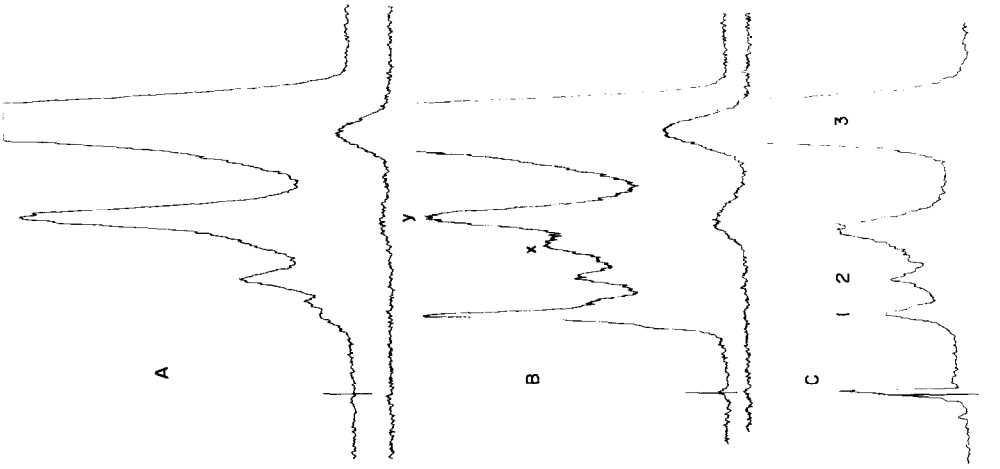
In the radioautogram of phage proteins from cells infected with wild type MS2 (figure 1-A), it was noted above that a fourth phage peak is evident. As previously reported (Nathans et al., 1966), this peak is variable in amount and position and was considered a breakdown product, probably of the "maturation" protein. This conclusion is strengthened by the observation that certain mutants which lack the "maturation" protein (e.g., am 8, figure 1) also lack the variable protein. Presumably such mutations are near the beginning of the cistron.

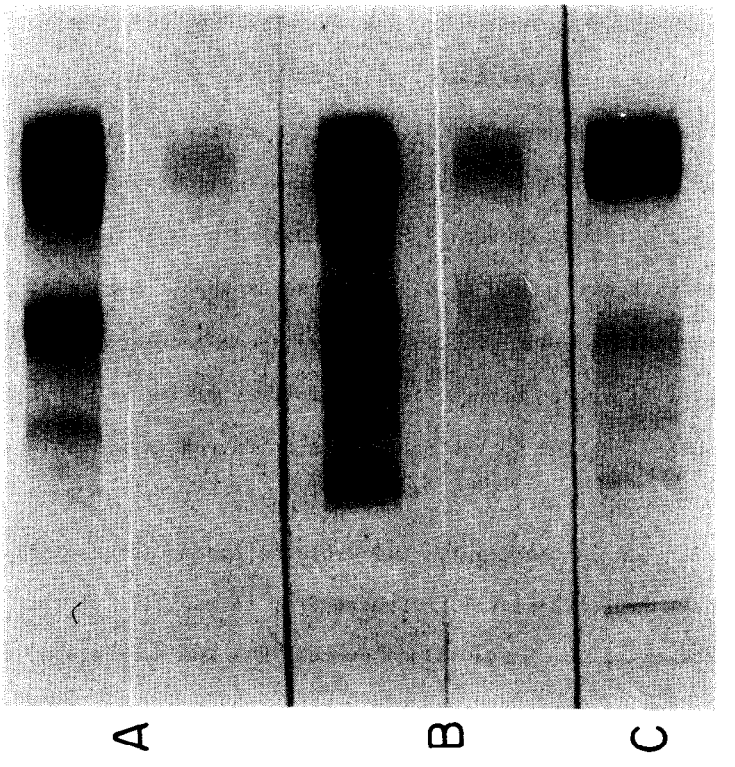
Gel electrophoresis of phage proteins made in cell extracts

Among the proteins synthesized in E. coli extracts in the presence

of coliphage RNA, phage coat and RNA synthetase had already been identified (Nathans et al., 1962; Capecchi, 1966). When the cell-free protein products were analysed by gel electrophoresis as described above, proteins corresponding to peak 3 (phage coat) and to peak 1 ((?) RNA synthetase) were detected, but little or no peak 2 ("RNA-protecting" or "maturation" protein) was observed (Nathans et al., 1966; Vinuela et al., 1967). In these experiments, the products analysed were taken after relatively long incubation times. With samples taken at earlier times, however, a more distinct protein peak was evident which corresponded in mobility to peak 2 protein seen in infected cells (figure 2). Therefore, as judged by similarity in electrophoretic mobility (and in the case of peak 3, by analysis of tryptic peptides), the three phage proteins synthesized in MS2-infected cells were also synthesized in E. coli extracts programmed with MS2 RNA (figure 2). Also, in both infected cells and in cell-free extracts, the peak 2 or "maturation" protein appeared to be unstable. Although these data strongly suggest that some of the proteins formed in extracts correspond to those found in infected cells, the identification of the non-coat protein peaks is based essentially on one criterion, electrophoretic mobility. We are now trying to identify these proteins by use of template RNA from amber mutants and by comparison of primary structures with those of phage proteins isolated from infected cells.

It is evident from the radioautograms and tracings shown in figure 2 that two other phage-specific protein peaks are present (labelled X and Y). These proteins have not been identified. Peak X is not present at early times, as shown in figure 2, and may be a breakdown product of the "maturation" protein. Peak Y, however, appears early and is therefore not likely to consist only of breakdown products. Whether it consists of precursor polypeptides or is an entirely different phage protein remains to be determined. In addition to these proteins,





See Legend on following page

Figure 2. Radioautograms (left) and microdensitometer tracings (right) of the proteins made in E. coli extracts in the presence or absence of MS2 RNA. Protein synthesis occurred in the presence of C^{14}-isoleucine, threonine, arginine, valine and lysine. A. 15 minute incubation. Top: with MS2 RNA; Bottom: no MS2 RNA. B. 30 minute incubation. Top: with MS2 RNA; Bottom: no MS2 RNA. C. Phage proteins from cells infected with MS2. The phage-specific peaks are labelled as in figure 1.

other bands have been observed in the radioautogram after long exposure of the dried gels. These minor protein bands vary in position and quantity and have not been identified.

Sequential synthesis of phage proteins in cell extracts

The time course of synthesis in cell-free extracts of proteins 1, 2 and 3 is shown in figure 3. The proteins were quantitated by radioautography as described above. The rate of synthesis of peak 3 protein (coat protein) far exceeds that of the other two proteins, as previously reported. (Note the two different ordinates of figure 3.) On a weight basis (i.e., incorporation of five common amino acids), the rate of coat protein synthesis is about 10 times greater than the rate of synthesis of either of the other proteins. Since the molecular weight of the coat protein is likely to be much lower than that of the other proteins, the preferential reading of the coat protein cistron is probably considerably greater than 10 fold.

As seen in the figure, the proteins appear sequentially in the order peak 3-peak 2-peak 1, corresponding in mobility to coat protein-"RNA-protecting" or "maturation" protein-(?) RNA synthetase, respectively. This finding confirms and extends earlier observations of Ohtaka and Spiegelman (1963). There are several possible explanations of this finding. For example, each cistron may be read successively, beginning

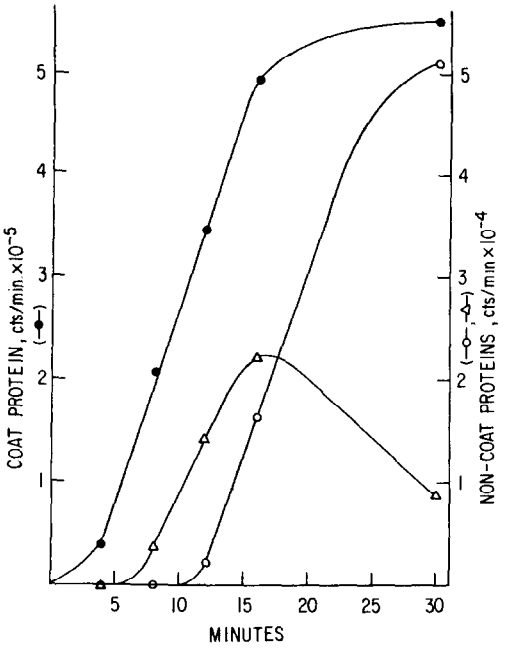


Figure 3. Time course of synthesis of three phage proteins in E. coli extracts programmed with MS2 RNA. Aliquots of an incubation mixture which contained C^{14}-lysine, arginine, isoleucine, valine and threonine were removed at the times indicated in the figure and analysed by gel electrophoresis and radioautography as described in the text. The radioactivity present in each protein band was determined from the densitometer tracings. ●, peak 3; △ , peak 2; ○, peak 1. Note that peak 3 protein is represented on the left-hand ordinate and peaks 1 and 2 on the right-hand ordinate, which is 10-fold lower.

---

at one end of the RNA, as suggested by the results of Zinder (1966) with polar coat mutants of f2. If this were the case, the data suggest that the order of cistrons may be, from the 5' end: coat protein-"maturation" protein-(?) RNA synthetase. Another possibility is that the differential lag may be related to the size of each protein: the larger the protein,

the longer the time needed for synthesis of a complete molecule. Whatever the explanation, the differential lag is likely to be peculiar to or exaggerated in the cell-free synthesis of phage proteins since in infected cells the coat protein does not appear prior to the other proteins (Vinuela et al., 1967; Nathans and Oeschger, unpublished results).

This research has been supported by a grant from the U. S. Public Health Service. K. E. is a pre-doctoral trainee, and M. P. O. is a post-doctoral fellow of the U. S. Public Health Service.

References

Argetsinger, J. E. and Gussin, G. N. (1966). J. Mol. Biol. 21, 421.

Capecchi, M. R. (1966). J. Mol. Biol. 21, 173.

Fairbanks, G., Levinthal, C. and Reeder, R. H. (1965). Biochem. Biophys. Res. Com. 20, 393.

Gussin, G. N. (1966). J. Mol. Biol. 21, 435.

Heisenberg, M. (1966). J. Mol. Biol. 17, 136.

Lodish, H. F., Horiuchi, E. and Zinder, N. D. (1965). Virology 27, 139.

Loeb, T. and Zinder, N. D. (1961). Proc. Nat. Acad. Sci., Wash. 47, 282.

Nathans, D., Notani, G., Schwartz, J. H. and Zinder, N. D. (1962). Proc. Nat. Acad. Sci., Wash. 48, 1424.

Nathans, D. (1965). J. Mol. Biol. 13, 521.

Nathans, D., Oeschger, M. P., Eggen, K. and Shimura, Y. (1966). Proc. Nat. Acad. Sci., Wash. 56, 1844.

Oeschger, M. P. and Nathans, D. (1966). J. Mol. Biol. 22, 235.

Ohtaka, Y. and Spiegelman, S. (1963). Science 142, 493.

Shimura, Y., Moses, R. E. and Nathans, D. (1965). J. Mol. Biol. 12, 266.

Summers, D. F., Maizel, J. V. and Darnell, J. E. (1965). Proc. Nat. Acad. Sci., Wash. 54, 505.

Vinuela, E., Algranati, I. and Ochoa, S. (1967). Europ. J. Biochem. 1, 1.

Vinuela, E., Salas, M. and Ochoa, S. (1967). Proc. Nat. Acad. Sci., Wash. 57, 729.

Zinder, N. D. and Cooper, S. (1964). Virology 23, 152.

Zinder, N. D., Engelhardt, D. L. and Webster, R. E. (1966). Cold Spring Harbor Sym. Quan. Biol. 31, 251.