The Identification of Prereplicative Bacteriophage T4 Proteins*

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SUMMARY

Amber and deletion mutants of bacteriophage T4 were used to identify prereplicative T4 proteins on sodium dodecyl sulfate acrylamide gels. The molecular weights of 25 identified T4 proteins are given.

The succeeding two papers describe the transcriptional and translational patterns which occur following bacteriophage T4 infection of Escherichia coli (1, 2). Most of the analyses described are dependent on the assignment of prereplicative gene products to specific proteins detected by electrophoresis in sodium dodecyl sulfate-acrylamide gels. We present here the assignments we have made thus far by analyzing phage-specified proteins in cells infected with nonsense mutants or deletion mutants of T4.

MATERIALS AND METHODS

Preparation of Radioactive Proteins in Phage-infected Cells—Mutant stocks of coliphage T4 were prepared from single plaques by infection of E. coli B or CR63 in M9 media (3) plus tryptophan (10 µg per ml) at 30°C. All phage mutants, which are noted in Table I, were originally from the collections of Drs. W. Wood, C. Yegian, R. Epstein, S. Champe, L. Black, D. Parma, J. Corbett, and J. Weil. We thank each of them for their generous aid.

All infections were performed at a multiplicity of eight in E. coli B (cell density 3 X 10⁸ cells per ml) in M9 media plus tryptophan (10 µg per ml) at 30°C. All phage mutants, which are noted in Table I, were originally from the collections of Drs. W. Wood, C. Yegian, R. Epstein, S. Champe, L. Black, D. Parma, J. Corbett, and J. Weil. We thank each of them for their generous aid.

All infections were performed at a multiplicity of eight in E. coli B (cell density 3 X 10⁸ cells per ml) in M9 media plus tryptophan (10 µg per ml) at 30°C. Bacteria were irradiated with ultraviolet light prior to infection in order to diminish host transcription and translation (which normally masks T4 proteins synthesized at early times after infection (14)). The cells were irradiated for 13 min with an ultraviolet lamp (UVSL 25, Ultraviolet Products Incorporated); the lamp was placed 6 inches above the cells, which were gently stirred in a 150-mm diameter Petri dish. The cells were transferred to a flask and aerated for 10 min before infection. After irradiation in this manner, T4-infected cells synthesize proteins at four times the rate of uninfected cells. This rate of protein synthesis is 20 to 40% of the rate measured in T4-infected unirradiated cells.

Phage and mixed ¹⁴C-amino acids (Schwarz-Mann No. 3122-09) were added simultaneously to the cells. The final concentration of radioactive amino acids was 0.375 µCi per ml. Cells labeled by this method incorporated about 25% of the input amino acids into protein. The cells were lysed and prepared for electrophoresis by resuspension in 1 volume of water plus 4 volumes of SDS-sample buffer (10). Complete lysis was ensured by heating the samples at 90°C for 1 min.

Gel Electrophoresis and Autoradiography—Radioactive protein samples were run on the vertical slab apparatus described by F. W. Studier and J. Maizel. The SDS gel and buffer system used is that of Laemmli and Maizel, described by Laemmli (10). A 12.5% separating gel was poured and always allowed to sit overnight before electrophoresis. A 4.5% stacking gel was poured the following morning and, following polymerization, the gel was used immediately for electrophoresis. Gels were run for 6 hours at constant current (10 ma per gel for a gel thickness of 1/4 inch). After electrophoresis, the proteins were fixed in the gel with 50% trichloroacetic acid for 30 min, and then stained with 0.1% Coomassie brilliant blue solution in 50% trichloroacetic acid for 30 min. The gels were destained overnight by repeated washing in 7% acetic acid. The gels were dried (10) and placed under film (Kodak No-Screen medical x-ray film) for various lengths of time. The gel patterns shown here were obtained in Boulder. At Stanford ³⁵S (as H₂S⁵O₄) was used as the radioactive precursor (15); identical assignments were made for many of these genes.

RESULTS AND DISCUSSION

The autoradiograms of SDS-gels in Fig. 1 show some of the T4 prereplicative proteins which are synthesized during the first 12 min of infection of E. coli B. Each column represents a separate infection using either a nonsense or deletion mutant of T4. The arrows indicate the gene product which is absent in each infection, when compared with that of a wild type T4-infected culture.

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In Table I we have listed the genes whose protein products we have identified. We have listed the molecular weights of these proteins and the mutants which were used in the assignments. In addition, we list several unmapped deletion mutants which yield missing (presumably nonessential) prereplicative protein bands.

The sum of the molecular weights of the prereplicative proteins (encoded by both mapped and unmapped genes) identified on gels is about 1,120,000. This total accounts for about 17% of the coding capacity of T4 DNA. By the criterion of RNA-DNA saturation hybridization, prereplicative T4 RNAs are derived from about 50% of the total DNA (16). Thus, a com-
Molecular weights of T4 prereplicative gene products

The molecular weights were derived by comparing the electrophoretic migration of T4-specific proteins in SDS-polyacrylamide gels with the migration of the following standards: bovine serum albumin, β-lactoglobulin, catalase, carbonic anhydrase, pepsin, E. coli RNA polymerase, E. coli α factor, hemoglobin, myoglobin, ovalbumin, creatine phosphokinase, and trypsin. The references for published molecular weights are given in the table.

| Molecular weight | Gene | Published molecular weight | Mutants |
|------------------|------|---------------------------|---------|
| 112,000          | 43   | 112,000 (4)               |         |
| 103,000          | 556  | 103,000                   |         |
| 95,000           | rIIA | 95,000 (7)                |         |
| 71,000           | 46   | 71,000 (5)                |         |
| 70,000           | deletion 9A  | 70,000 (6) |         |
| 68,000           | 30   | 68,000 (10)               |         |
| 66,000           | 116  | 66,000 (11)               |         |
| 64,000           | 39   | 64,000 (12)               |         |
| 51,000           | 52   | 51,000 (15)               |         |
| 50,000           | deletion (39-56)  | 50,000 (16) |         |
| 47,000           | 63   | 47,000 (19)               |         |
| 40,000           | 32   | 35,000 (7)                |         |
| 37,000           | 44   | 37,000 (8)                |         |
| 35,000           | rII B| 35,000 (9)                |         |
| 33,500           | 21   | 33,500 (10)               |         |
| 33,000           | deletion 20A, 107B | 33,000 (11) |         |
| 29,000           | 42   | 29,000 (12)               |         |
| 28,500           | 44   | 28,500 (13)               |         |
| 24,000           | E10  | 24,000 (14)               |         |
| 23,500           | 47   | 23,500 (15)               |         |
| 22,000           | 1    | 22,000 (16)               |         |
| 20,000           | Internal protein III  | 20,000 (17) |         |
| 18,000           | 62   | 18,000 (18)               |         |
| 17,000           | 55   | 17,000 (19)               |         |
| 15,000           | 56   | 15,000 (20)               |         |
| 12,000           | deletion (39-56)  | 12,000 (21) |         |

Total 1,116,500

TABLE I—Continued

a Mutant 293 is an unmapped mutation which was once thought to be in gene 57.

b These deletions were provided by Dr. D. Parma; they are unmapped.

c An additional band disappears.

d These mutants of gene 44 appear to be polar on gene 62.

e Deletion (39-56)1 has been described previously (13).

The complete catalogue of prereplicative T4 proteins on gels is by no means complete. There still remain 27 genetically defined prereplicative cistrons whose gene products cannot be identified by our present technique. As more nonsense or deletion mutants become available to us this catalogue will be expanded.

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