THE PROTEINS OF BACTERIOPHAGE M13

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Abstract.—Particles of the small filamentous coliphage M13 contain not only the major coat protein, which is the product of phage gene 8, but also a minor coat protein, the A protein, which is the product of gene 3. The A protein has a molecular weight of approximately 70,000 daltons, is present in one copy per virion, and is responsible for phage attachment to host cells. Also associated with purified M13 particles is a minor quantity of very small proteinaceous material, but its origin as a phage-coded product has not been demonstrated.

At least five phage-specific proteins, including the two coat proteins, are present in appreciable quantities in M13-infected cells. The principal phage protein synthesized is the product of gene 5, which is responsible for phage single-stranded DNA synthesis. This protein has a molecular weight of about 8,000 daltons. Its precise function in DNA synthesis is not yet known.

Phage proteins are synthesized at nearly normal rates in cells in which replication of phage double-stranded DNA is blocked by gene 2 mutations. This result suggests that the initial double-stranded DNA molecule serves as the principal template, perhaps the only template, for phage messenger RNA synthesis.

Introduction.—M13 is a male-specific bacteriophage containing 2×10^6 daltons of single-stranded DNA.[1] We have been interested in determining both the number of proteins in the filamentous M13 virion and the total number of phage-specific proteins formed within infected *Escherichia coli* cells. Based on genetic evidence,[2, 3] it appears that the virions should contain not only the major coat protein[4, 5] but also a minor protein for phage attachment to the F pili of the host cells. Based on the genome size, the *total* number of proteins encoded by M13 should be about ten. This is in fair agreement with the presently known number of phage genes (eight) as defined by conditional lethal mutants.[2, 6]

In this communication, we report on polyacrylamide gel studies of virion and intracellular M13 proteins, with particular emphasis on establishing which proteins are coded by known phage genes.

Materials and Methods.—The bacterial strains, bacteriophages, cultural techniques, and media routinely used for M13 have been described previously.[6-8] Radioactive amino acids were purchased from the New England Nuclear Corp. M13 virions, labeled either singly with H^3-leucine or doubly with H^3-valine plus C^{14}-arginine, were prepared as described in the figure legends. The labeled virions were purified by three cycles of differential centrifugation and banding in a CsCl density gradient. Virion proteins were extracted and prepared for gel electrophoresis using the procedures described by Nathans *et al.*[9] for proteins from sonicated cells.

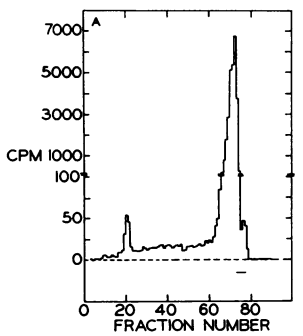
Labeled intracellular phage proteins were prepared according to the general procedure developed by Ptashne,[10] involving ultraviolet (UV)* irradiation of the host cells before infection to suppress cellular protein synthesis. The UV-sensitive *su-* bacterial strain $159F^+$ was grown to a cell density of 4×10^8/ml in M9 medium containing 0.1% casamino acids and 0.2% glucose. The culture was irradiated in a Petri dish cover at a depth of

1.6 mm with a UV dose of 3900 ergs/mm² (75 sec at 14.2 cm from a 15-watt germicidal lamp). This dose reduces protein synthesis to a few per cent of that in unirradiated cells: subsequent infection with phage M13 results in a twofold stimulation of protein synthesis. The irradiated cells were sedimented and resuspended in one half the original volume of medium, using M9 supplemented with glucose, 2×10^{-2} *M* $MgSO_4$, 19 nonlabeled amino acids at 5 μg/ml, and nonlabeled leucine at 0.1 μg/ml. The resuspended culture was split into two 15-ml fractions. To one were added H^3-leucine, to give a specific activity of approximately 10 μc/mμmole, and M13 phage to a ratio of 100 phage/cell. To the other fraction of the culture was added C^{14}-leucine, to give a specific activity of 2 μc/mμmole. Both the cultures were incubated for 35 min at 37°C with gentle shaking, then chilled in ice water. The cells were washed three times in the cold with 0.1 *M* phosphate buffer, pH 7.0, containing 0.05% casamino acids. The two cultures were then combined, resuspended in approximately 0.5 ml of water, and sonicated for 3 min at 10,000 cps. The proteins were extracted and prepared for electrophoresis as described by Nathans *et al.*[9]

Polyacrylamide gels, 7 mm diameter × 10 cm long, containing 0.1% sodium dodecyl sulfate were prepared and run essentially as described by Summers *et al.*,[11] but with the addition of 8 *M* urea to the gels. Electrophoresis was carried out at 10 v/cm of gel, 5 ma/tube, for approximately 4 hr, by which time the bromphenol blue dye, used as a marker, had migrated 8–9 cm. After electrophoresis, the gel was removed from the tube, frozen on a block of dry ice, and sliced with a razor blade device which gives 100 slices from 8.5 cm of gel. The radioactive proteins were eluted from gel slices as described by Eggen *et al.*[12] The vials were counted for H^3 and C^{14} in a Packard 3375 Tri-Carb scintillation counter, using a dioxane-base scintillation fluid. Most samples were counted for sufficient time to give a 90% certainty that the ratio of H^3/C^{14} was within 2% of the measured value, but slices near the origin had low counting rates and 90% confidence envelopes on the order of 15%.

Results.—Proteins present in wild-type M13 virions: Protein was extracted from labeled M13 phage and subjected to electrophoresis in sodium dodecyl sulfate–containing polyacrylamide gels. Two electropherograms are shown in Figure 1. It is apparent that the phage particles contain at least one and possibly two minor proteins in addition to the major coat protein. Figure 1*A* shows the results for protein from M13 virions labeled with H^3-leucine. One minor peak is clearly present at fraction 20. A second minor peak appears as a shoulder on the front side of the major protein peak.

The minor peaks are more evident in the electropherogram of protein which was extracted from phage doubly labeled with C^{14}-arginine and H^3-valine (Fig. 1*B*). The major coat protein of M13 lacks arginine[15]; consequently the major



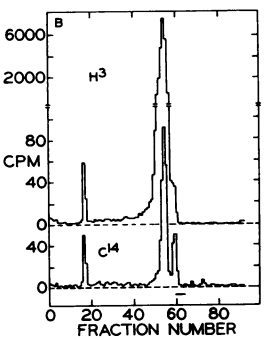


FIG. 1.— Gel electrophoresis of labeled proteins from wild-type M13 virions. M13 phage were grown with (*A*) H^3-leucine at 2 μc/ml in half-strength H broth, host cell *E. coli* HB 11[13]; (*B*) H^3-valine plus C^{14}-arginine at concentrations of 10 and 5 μg/ml and specific activities of 1 and 4 μc/mμmole, respectively, in M9 medium supplemented with 18 nonlabeled amino acids at 20 μg/ml, host cell *E. coli* C 3000 *arg-val-*.[14] Phage purification, protein extraction, and gel electrophoresis were carried out as described in *Materials and Methods*. In these and subsequent graphs, the anode is to the right and the horizontal bar represents the position of the bromphenol blue dye marker.

peak in the Figure 1*B* gel had very few C^{14} counts relative to H^3. (The quantity of C^{14} found in the major protein in such experiments was often less than that shown in this figure, and varied with the particular batch of arginine used for labeling, thus may have been due to a radioactive impurity.) Each minor peak had approximately equal numbers of C^{14} and H^3 counts, showing that its protein contained both arginine and valine. Therefore, these peaks cannot represent aggregation or fragmentation products of the major coat protein subunit. The protein in each minor peak contained about 0.5 per cent of the total leucine or valine present in the virion.

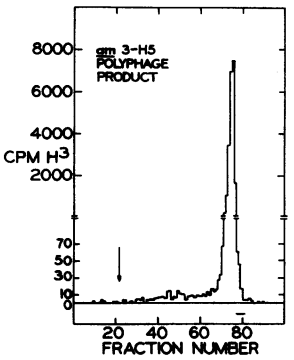
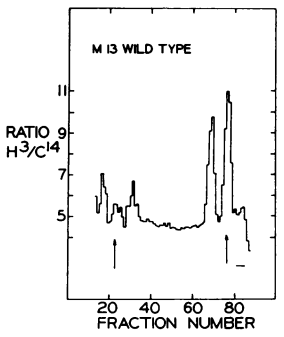


FIG. 2.—Gel electrophoresis of H^3-leucine-labeled proteins from M13 polyphage. Polyphage were prepared by infecting *su-* cells, *E. coli* K38, with M13 *amber* mutant 3-H5, in H-broth plus 1.7 μc/ml H^3-leucine. The infected culture was incubated for 6 hr at 30° and the resultant defective product was purified by differential centrifugation and banding in CsCl. The arrow indicates the expected position of the slower-migrating minor protein peak.

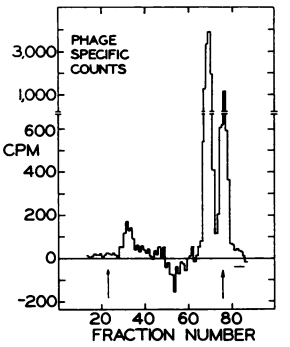
Protein present in M13 polyphage: Polyphage are defective phagelike particles produced by gene 3 *amber* mutants infecting *su-* host cells.[2, 3] They contain infective DNA, are capable of reacting with anti-M13 antibodies, and have the same buoyant density in CsCl as wild-type phage, but they fail to adsorb to host cells. When examined with the electron microscope, they have the usual filamentous appearance of phage M13 particles, but are many times longer.

The proteins were extracted from H^3-leucine-labeled polyphage particles and run on a polyacrylamide gel; the resulting electropherogram is shown in Figure 2. The major coat protein peak is present, as with wild-type virions, but the slower-migrating minor peak is clearly absent (the expected position is marked by the arrow). It is uncertain from this experiment whether the faster-migrating minor peak is present or absent, and the appropriate arginine-label experiments have not yet been carried out to answer the question.

Phage-specific proteins synthesized in cells infected with wild-type M13: A culture of UV-sensitive *su-* cells was irradiated with ultraviolet light to suppress host cell protein synthesis. The irradiated culture was split; one half was infected with wild-type phage and labeled with H^3-leucine; the other half was left uninfected and was labeled with C^{14}-leucine. After incubation, the cultures were combined and washed, the proteins were extracted, and gel electrophoresis was carried out. The gel was sliced and each slice was counted for H^3 and C^{14}. The detailed procedures are described under *Materials and Methods*. The results are shown in Figure 3, as ratio of H^3/C^{14} *versus* fraction (slice) number. Each peak represents a section of the gel which received more labeled protein from the infected cell extract than from the uninfected, and is presumably due to the presence of one or more phage-specific proteins. At least five such peaks are evident. Their electrophoretic mobilities, relative to the bromphenol blue dye band (horizontal bar), are: 0.22, 0.28, 0.35, 0.82, and 0.91. Among the group of five phage protein peaks, two have the same electrophoretic mobilities as the major coat



(*Left*) FIG. 3—Gel electrophoresis of leucine-labeled intracellular proteins: M13 wild-type infection. The radioactive protein extracted from infected H^3-leucine-labeled cells plus uninfected C^{14}-leucine-labeled cells was prepared, electrophoresed, and counted for H^3 and C^{14} as described in *Materials and Methods*. The arrows in this and subsequent figures indicate the positions of the major coat protein and the slower-moving minor protein present in M13 virions.



(*Right*) FIG. 4.—Phage-specific protein counts from the M13 wild-type experiment shown in Fig. 3. The ratios shown in Fig. 3 were converted to phage-specific counts as described in the text.

protein and the slower-moving minor coat protein, 0.91 and 0.28. These are marked by upward-pointing arrows in Figure 3 and subsequent figures. An additional peak is often found at approximately the position of the dye. This might represent the rapidly moving minor virion protein (see *Discussion*).

The ratio peaks in Figure 3 were converted to phage-specific counts by first estimating a base-line ratio, 4.5, below which the counts were considered as due to host proteins, then allocating the remainder of the counts in each slice to phage-specific proteins (Fig. 4). It is apparent from this figure that the principal phage product is the peak with mobility 0.82, just slower than the major coat protein. This product plus the major coat protein account for over 90 per cent of the phage-specific proteins. The slowest-moving peaks, which are quite evident when plotted as ratios, actually contain relatively few counts and are barely discernible when plotted as phage-specific counts.

To confirm the identity of the presumed major coat protein peak, wild-type infected and uninfected cells were labeled with H^3- and C^{14}-histidine and the labeled proteins were examined by gel electrophoresis. Histidine is known to be absent from the major coat protein subunit of phage M13.[15] The results for the histidine-labeled extract were similar to those for the leucine-labeled extract shown in Figure 3, except that the large peak of mobility 0.91, the major coat protein peak, was missing. The other phage-specific peaks were still present, showing that they are not aggregation products of the major coat protein.

Phage-specific proteins synthesized in su- cells infected with M13 amber mutants: *Amber* mutants infecting nonpermissive host cells produce, for the mutated gene, only a fragment of the protein normally encoded.[16] Upon electrophoresis of labeled proteins extracted from such cells, the normal protein peak should be missing. A new faster-migrating peak might appear, depending on the size, quantity, and labeling of the fragment produced. The disappearance, or shifting, of a peak as a result of infection with an *amber* mutant is therefore a way to correlate protein products with viral genes.

UV-irradiated cells were infected with the gene 3 *amber* mutant 3-H4. Labeling, protein extraction, and gel electrophoresis were carried out as already described for infections with wild-type phage. The results are shown in Figure

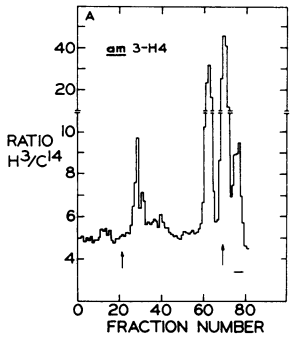
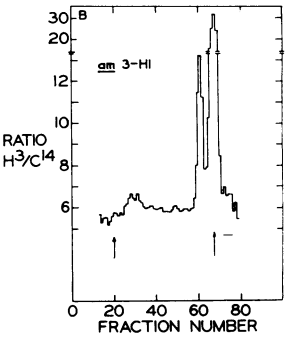


FIG. 5.—Infections of *su-* cells with gene 3 *amber* mutants: gel electrophoresis of leucine-labeled intracellular proteins. The procedures were as in Fig. 3 legend except that the infections were with (*A*) *amber* mutant 3-H4; (*B*) *amber* mutant 3-Hl.



5*A*, and are similar to those for the wild-type extract except that one peak is missing from this gel, the peak corresponding to the slower migrating minor coat protein. The same type of experiment was carried out with another gene 3 mutant, *am* 3-H1, and gave the electropherogram shown in Figure 5*B*. Although the results are not as clear-cut as those for *am* 3-H4, the *am* 3-H1 extract also appears to be missing the slower-migrating minor coat protein.

The results of infections with two different gene 5 mutants are shown in Figure 6. With *amber* mutant 5-H3 (Fig. 6*A*) the principal phage-specific peak is clearly absent (downward-pointing arrow). With *amber* mutant 5-H27 (Fig. 6*B*) this peak is also missing, and apparently is replaced by a new, slightly faster-moving peak. To confirm that the new peak migrates more rapidly than the principal protein peak, the *am* 5-H27 extract was coelectrophoresed with a small amount of an *am* 3-H5 extract, containing the principal protein. The result is shown in the inset to Figure 6*B*. As expected, there are two peaks just to the left of the major coat protein peak, one at the position expected for the principal protein (marked by the arrow), and one with slightly greater mobility.

Electrophoresis of the intracellular proteins extracted after infection with the gene 8 *amber* mutant 8-H1 gave rise to the electropherogram shown in Figure 7. In this extract, the major coat protein peak is missing. The inset to Figure 7 shows the results of a control experiment to confirm the identity of the missing peak. H^3-labeled coat protein purified from wild-type M13 virions was added

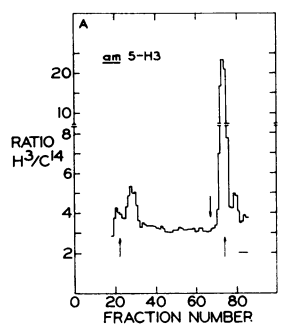
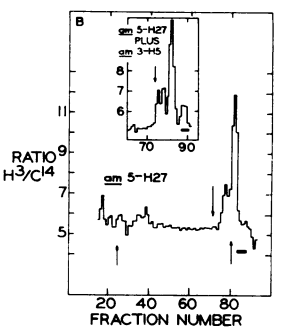


FIG. 6.—Infections of *su-* cells with gene 5 *amber* mutants: gel electrophoresis of leucine-labeled intracellular proteins. The procedures were as in the Fig. 3 legend except that the infections were with (*A*) *amber* mutant 5-H3; (*B*) *amber* mutant 5-H27. The downward-pointing arrows indicate the expected position for the principal phage-specific protein. The inset in (*B*) is a portion of the electropherogram from coelectrophoresis of the *am* 5-H27 extract with a small amount of an *am* 3-H5 extract, which contains the principal protein.



to the *am* 8-H1 extract and the mixture was electrophoresed. The H^3 label from the coat protein moved, as expected, to the position of the missing peak.

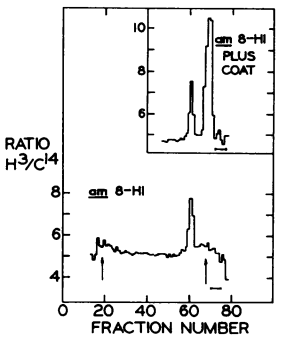
Proteins extracted from cells infected with the gene 2 *amber* mutant 2-H2 gave rise to the electropherogram shown in Figure 8. All of the usual peaks are present and a new peak is found, at fraction 48. This presumably represents a large fragment of the normal gene 2 protein, although it is not clear where this protein itself migrates in gels. With another gene 2 mutant, *am* 2-H7, the usual peaks were again present and there appeared also to be a still larger fragment.

Extracts prepared from cells infected with *amber* mutants in genes 1, 4, 6, and 7 gave electrophoretic patterns in which it was not possible to identify either missing peaks or new peptide fragments.

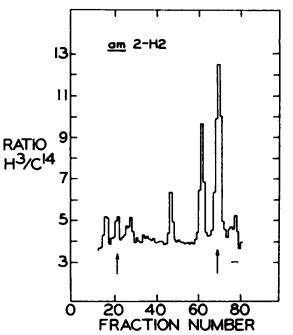
Discussion.—M13 virion proteins: The major coat protein: The major protein subunit of the filamentous phage virions has been well characterized previously.[4,5] It has only 49 amino acids and occurs in approximately 2,000 copies per virion. Results in this paper and in press[2] show that this protein is the product of M13 gene 8.

The A protein: In gel electrophoresis of proteins from wild-type M13 virions, a slow-migrating minor protein is found. This protein is missing from the long, defective, "polyphage" particles produced by *su-* cells infected with M13 gene 3 *amber* mutants. A minor phage-specific protein of the same electrophoretic mobility is found in cells infected with wild-type phage, but is missing from *su-* cells infected with gene 3 *amber* mutants. Thus, this minor coat protein appears to be the product of gene 3. The product of this gene has previously been implicated in phage attachment to host cells, as many gene 3 mutants, and no others, have abnormal attachment properties.[2, 3] The gene 3 protein will be called the A protein, by analogy with the minor capsid component of the small RNA phage R17,[17] and in agreement with the usage of Rossomando and Zinder,[18] who have recently demonstrated a minor coat protein in the filamentous phage f1.

The A protein contains approximately 0.5 per cent of the leucine and valine present in the entire M13 virion. Assuming that it also contains this proportion of the total coat protein, there would be about 50,000 daltons of A protein per virion. It is possible to estimate protein subunit molecular weights from electrophoretic mobilities on sodium dodecyl sulfate–polyacrylamide gels of the type



(*Left*) FIG. 7.—Infection of *su-* cells with the gene 8 *amber* mutant 8-H1: gel electrophoresis of leucine-labeled intracellular proteins. The procedures were as in the Fig. 3 legend except for the infecting phage used. The inset shows a portion of the electropherogram resulting from coelectrophoresis of the *am* 8-H1 extract with added H^3-leucine-labeled protein purified from M13 virions.



(*Right*) FIG. 8.—Infection of *su-* cells with the gene 2 *amber* mutant 2-H2. The procedures were as in the Fig. 3 legend except for the infecting phage used.

used in this study.[19] By this means, the estimated molecular weight for the A protein is 70,000 daltons. Thus, there appears to be only one A protein subunit per virion. Since the A protein is involved in phage attachment to the F pili of host cells, and since the phage-pilus attachment is end to end,[20] the A protein must be located at one end of each virion. This is in agreement with the data of Fareed *et al.*,[21] who showed that sonicating bacteriophage f1 into fragments did not increase the number of ends capable of competing with intact phage for attachment to host cells. It is also in agreement with the observation made by Caro and Schnös[20] that in mixtures of F pili fragments and filamentous phages, the pili attached at one but not both ends of the phage particles.

The formation of polyphage particles when the A protein is missing from infected cells (gene 3 *amber* mutants in *su-* cells) suggests that this protein also functions in preventing end-to-end fusion of normal-length particles. The occasional double-length particles found in stocks of filamentous phages[20, 22, 23] might be due to a limited supply of A protein within infected cells.

Is there a second minor protein in M13 virions? On gel electrophoresis of proteins from highly purified virions, one minor peak migrates very rapidly, at almost the rate of bromphenol blue dye. This suggests a molecular weight of 2,000 daltons or less, exceedingly low for a protein molecule. On the basis of leucine and valine content, it can be estimated that there are on the order of 50,000 daltons, at least 25 molecules, of this material per virion. A protein peak of similar electrophoretic mobility was found in extracts of *su-* cells infected with either wild-type phage or *amber* mutants in any of the eight known genes of M13. With the cell extracts, this peak would probably include a variety of incomplete phage protein fragments, as well as any small structural protein. Thus, its presence in each *amber* mutant extract is inconclusive for determining whether there is any such rapidly migrating phage-coded structural protein. In sum, there is not sufficient evidence at present to say whether the rapidly migrating protein found associated with M13 virions is actually a structural protein of the virus or only contaminating short polypeptide fragments.

The gene 5 protein: The product of M13 gene 5 is known to control the synthesis of phage single-stranded DNA molecules, although its specific role in that process is not known.[7] From the electrophoretic results presented here, the molecular weight of the gene 5 polypeptide is about 8,000 daltons. Surprisingly, it is the principal phage-specific protein synthesized in M13-infected cells, at least under the conditions of these experiments. With the wild-type phage infection, there were on the order of 1,000–10,000 molecules/cell at 35 minutes after infection. It should be possible to purify sufficient quantities of this protein for further study.

Protein synthesis by gene 2 amber mutants: The gene 2 product is known to control replication of the double-stranded, replicative form of M13 DNA.[7] In the absence of the gene 2 product, the "parental" RF molecule is formed, but no pool of "progeny" RF molecules is synthesized. It is apparent from results shown here that protein synthesis by gene 2 *amber* mutants in *su-* host cells proceeds at approximately the same rate as in infections with wild-type phage, despite the lack of progeny RF molecules. Thus, the parental RF molecule must

be the principal template, perhaps the only template, for transcription of phage M13 messenger RNA. This is also true with phage ϕX174.[24]

Remaining genes and proteins: Genes have not yet been identified for the intracellular phage-specific proteins having electrophoretic mobilities of 0.22 and 0.35, nor have proteins been identified for genes 1, 4, 6, and 7. The establishment of further gene-protein correlations may require, first, the testing of additional *amber* mutations in these genes and, second, improving the procedures for detecting minor phage-specific proteins in infected cells.

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* *Abbreviations:* UV, ultraviolet light; *su-*, nonsuppressing strain for *amber* mutants; *arg-*, arginine-requiring; *val-*, valine-requiring.

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