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Identification of the Gene Controlling the Synthesis of the Major Bacteriophage T5 Membrane Protein

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After infection of *Escherichia coli* B by bacteriophage T5, a major new protein species, as indicated by polyacrylamide gel electrophoresis, appears in the cells' membranes. Phage mutants with amber mutations in the first-step-transfer portion of their DNA have been tested for their ability to induce membrane protein synthesis after they infect *E. coli* B. We have found that phage with mutations in the A1 gene of T5 do not induce the synthesis of the T5-specific major membrane protein, whereas phage that are mutant in the A2 gene do induce its synthesis. We conclude that gene A1 must function normally for T5-specific membrane protein biosynthesis to occur and that only the first 8% (first-step-transfer piece) of the DNA need be present in the cell for synthesis to occur.

We recently demonstrated that when T5 bacteriophage infects *Escherichia coli* a major new protein of an approximate molecular weight of 57,000 appears in the cell membrane (3a). This protein, found in both the inner and outer membrane, accounts for as much as 10% of the membrane proteins synthesized early after infection of *E. coli* B by T5. The membrane protein is not seen if the phage are UV irradiated prior to infection. The kinetics of synthesis of the T5 membrane protein are consistent with it being a pre-early protein, i.e., a protein coded for by the initial 8% piece of T5 DNA (first-step-transfer [FST] DNA) that is transferred spontaneously to the cell after adsorption of the phage. Transcription and translation of this 8% of the DNA are necessary for the transfer of the remaining 92% of the DNA to the cell, which is followed by early and late protein synthesis (8; D. J. McCorquodate, Crit. Rev. Microbiol., in press). Proteins coded for by the initial 8% segment of DNA are synthesized most rapidly between 3 and 9 min postinfection (10, 11), as is the T5-specific membrane protein. It is also synthesized in cells that have been infected in the presence of chloramphenicol and then blended prior to the removal of the chloramphenicol (and hence contain only the FST DNA), and in T5-infected cells containing the colicinogenic factor Col Ib. Infection of these latter cells results in little or no synthesis of early or late proteins (14).

A variety of mutants that map in the FST region have been isolated, and their properties have been studied (2, 11). Mutants in gene A1 do not transfer the whole phage DNA molecule, do not break down host DNA after infection, and do not shut off the synthesis of host and pre-early phage proteins. Some A1$^-$ mutants are also unable to grow in cells containing the λ prophage (7). Mutants in gene A2 are defective only in the transfer of the whole phage DNA molecule. This paper presents evidence that mutants in gene A2 induce normal synthesis of the T5-specific membrane protein, whereas mutants in gene A1 do not. We conclude that only the first 8% of the DNA is necessary for synthesis of the membrane protein and that gene A1 may be the structural gene for it.

MATERIALS AND METHODS

Organisms and media. *E. coli* B was obtained from the laboratory of M. J. Bessman, and *E. coli* CR63 was from M. L. Dirksen. M9 medium was made as described by Herriot and Barlow (5). Wild-type T5 was originally obtained from S. E. Luria. T5 *am*H16d, carrying an amber mutation in pre-early gene A1, and T5 *am*H231, carrying an amber mutation in pre-early gene A2, were isolated by Hendrickson and McCorquodale (4) after in vivo mutagenesis with 5-bromodeoxyuridine. The amber mutants were grown on *E. coli* CR63 by the confluent lysis method (1).

Isolation of bacterial membranes. The experiments empolyed a double-labeling procedure in which proteins made in uninfected cells were labeled with [^{14}C]amino acids, and proteins made in infected cells were labeled with [^{3}H]amino acids. Cells were grown at 37 C in M9 medium containing 0.5% yeast extract, 5×10^{-4} M $CaCl_2$, and 10 μg each of leucine and tyrosine per ml from a 5% overnight inoculum in the same medium. Cultures of 100 ml were routinely used. Infection was carried out when the cells reached a concentration of about 10^9 cells/

ml, using a multiplicity of 10 T5 phage particles per cell. [^{3}H]leucine and [^{3}H]tyrosine were added 30 s after phage addition. Uninfected cells that had been labeled with [^{14}C]leucine and [^{14}C]tyrosine were chilled on ice, and 50 μg of chloramphenicol was added per ml of culture. Amino acid incorporation in the infected cells was stopped by mixing them with a chilled, uninfected culture containing chloramphenicol. The cells were removed by centrifugation, washed once with 30 to 40 ml of 0.01 M HEPES buffer (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pH 7.4), resuspended in 15 ml of HEPES, and finally broken by one or two passages through a French pressure cell. $MgCl_2$ was added to give a final concentration of 0.01 M and about 1 mg each of DNase and RNase was added. The unbroken cells were removed by low-speed centrifugation, and the cell envelopes were isolated by centrifugation for 60 min at 50,000 rpm in a no. 60 (Spinco) rotor at 4 C and washed with 7 to 8 ml of HEPES.

Preparation of samples for gels. Samples were prepared for electrophoresis by the method of Schnaitman (16). The whole membrane pellet was suspended in 0.1 M phosphate buffer (pH 7.2), containing 1.6% sodium dodecyl sulfate (SDS), 4×10^{-3} M EDTA, and 0.08% mercaptoethanol, to yield a suspension containing about 7 mg of protein per ml (as assayed by the method of Lowry et al. [9]). After the air in the tubes containing the suspended membranes was replaced by N_2, the tubes were capped, and the membrane suspensions were incubated at 37 C for 2 h. The samples were then dialyzed overnight at room temperature against 50 to 100 volumes of a solution of 0.1 M sodium phosphate buffer (pH 7.2) containing 8 M urea, 5×10^{-4} M EDTA, 0.1% SDS, and 0.1% 2-mercaptoethanol. This solution must be prepared fresh before use. Dialysis was done in screw-capped bottles in which the air had been replaced by N_2. After overnight dialysis, the samples were boiled for 5 min and, after addition of a 1:25 dilution of 1% bromophenol blue, they were ready for electrophoresis.

Gel electrophoresis. Gels were prepared by the procedure of Maizel (12). Acrylamide (3.75 g), urea (1.5 g), and bisacrylamide (0.10 g) were dissolved in 50 ml of gel buffer (0.1% SDS in 0.1 M sodium phosphate buffer, pH 7.2) at room temperature, and 10 mg of $(NH_4)_2S_2O_8$ was added. After the $(NH_4)_2S_2O_8$ dissolved, 30 μl of TEMED (*N,N,N',N''*-tetramethylethylenediamine) was added, and the resulting solution was degassed with a vacuum pump and pipetted (with a Pasteur pipette) into dry gel tubes (15 by 0.5 cm) that had been coated with Canalco Column-Coat. The acrylamide solution in the tubes was overlaid with distilled water. After polymerization (30 to 60 min), the gels were left undisturbed for about 18 h. The water was then removed and the tubes were filled to the top with gel buffer. A sample containing about 50 μg of protein was then injected onto the top of the gel with a Hamilton syringe, and electrophoresis was begun at 5 mA/gel and continued for 9 to 10 h. After the electrophoresis was complete, the gels were removed from the tubes and frozen. For slicing, the gels were barely thawed and then were cut (with a cheese-slicer type of gel slicer made by Earl Sandbeck at Johns Hopkins School of Medicine). Approximately 80 slices per gel were obtained and placed in scintillation vials containing 0.5 ml of a solution containing NCS solubilizer-water (9:1). The vials (tipped so that each slice was covered with NCS solution) were heated at 50 C for 2 h. A 10-ml volume of toluene-PPO-POPOP [PPO, 2,5-diphenyloxazole; POPOP, 1,4-bis-(5-phenyloxazolyl)benzene] counting fluid was added, and the samples were counted in a Beckman LS230 scintillation counter.

Analysis of results. The data obtained were analyzed routinely by computer, using a program developed by George Policello of the Biostatistics Department at the University of Florida. The actual counts per minute per slice were determined after correction for background and "spill," and the ^{3}H or ^{14}C radioactivity in each slice was then calculated as a percentage of the total ^{3}H or ^{14}C, respectively, in the entire gel. Such percentages are plotted against slice number. Difference plots are obtained by subtracting the percentage of ^{14}C counts from the percentage of ^{3}H counts in each slice.

RESULTS

Figure 1 shows the results of an experiment in which *E. coli* B was infected with wild-type T5 and labeled with tritiated leucine and tyrosine from 0 to 12 min after infection. Incorporation of radioactivity was stopped by mixing the infected cells with uninfected cells that had been labeled with [^{14}C]leucine and [^{14}C]tyrosine for the entire growth period and then chilled and treated with 50 μg of chloramphenicol per ml. Whole membranes were isolated from the mixture of infected and uninfected cells, and the proteins in them were solubilized and separated electrophoretically. Figure 1a shows the percentage of total counts in each slice; Fig. 1b shows the differences of these percentages. In both, a major new peak is seen (slices 31 through 36) (3a).

Figure 2 shows the results of an experiment in which *E. coli* B was infected with two prearly mutants of T5, T5 *am*H16d, and *am*H231. T5 *am*H16d has an amber mutation in the A1 gene and cannot transfer the whole DNA molecule to the infected cell nor induce the breakdown of host DNA. The T5-specific membrane protein is not synthesized in these cells (Fig. 2a and b). This would occur if gene A1 were the structural or a regulatory gene for the T5-specific membrane protein or if the protein were coded for by a gene on the 92% of the DNA that is not transferred to the cells when this mutant infects *E. coli* B. The latter possibility is eliminated by the finding that T5 *am*H231, which contains an amber mutation in the A2 gene and which also cannot transfer its whole DNA molecule, does induce T5-specific membrane protein biosynthesis normally (Fig. 2c and d).

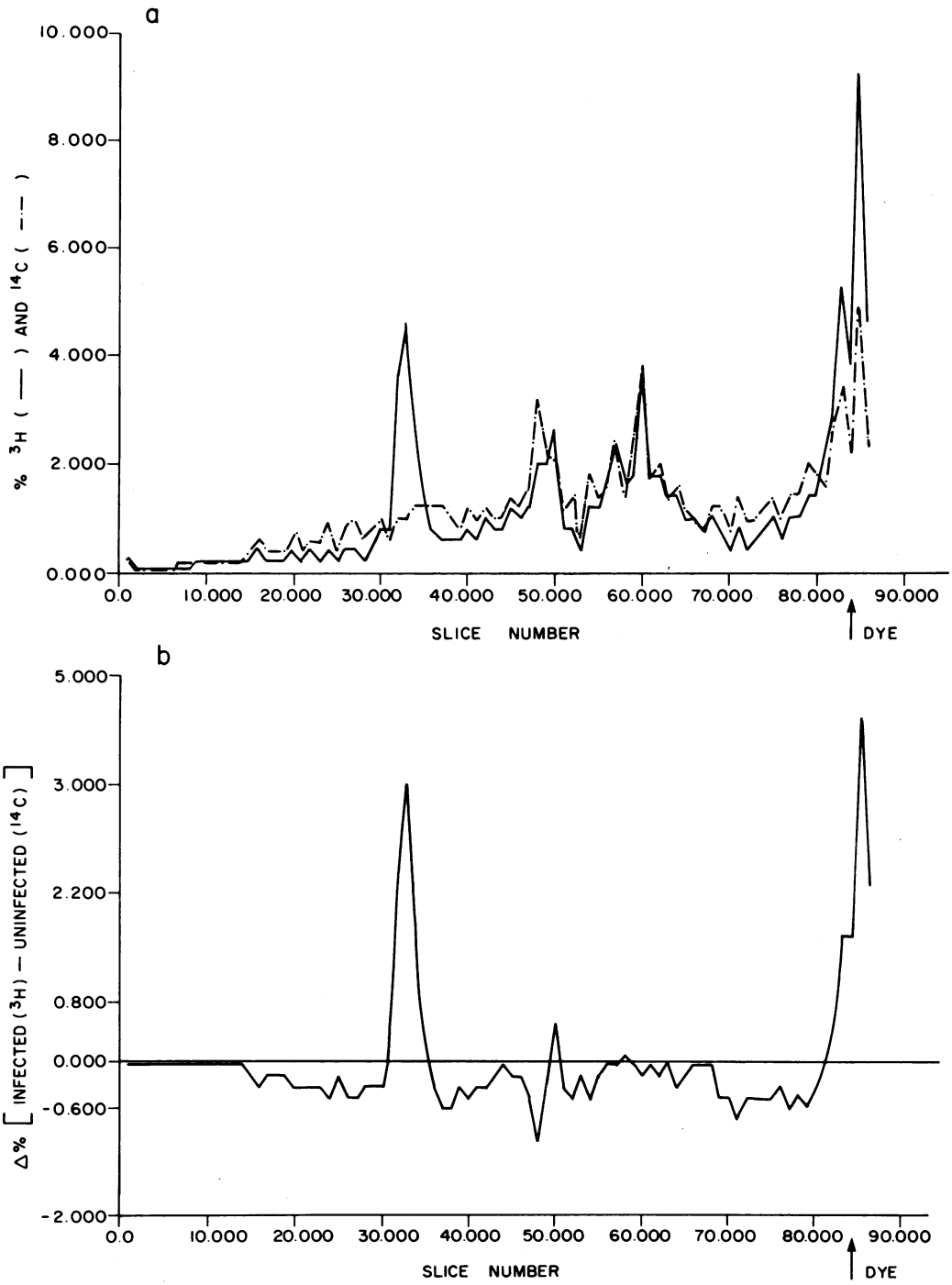

FIG. 1. *Comparison of membrane proteins in uninfected and T5-infected E. coli B. A 100-ml culture of E. coli B in M9 medium containing 0.5% glucose, 0.05% yeast extract, 5 × 10^{-4} M $CaCl_2$, and 10 μg each of leucine and tyrosine per ml was grown at 37 C to a concentration of 10^9 cells/ml. The cells were infected with 10 T5 phage per cell, and 50 μCi (each) of [³H]leucine and [³H]tyrosine was added. After 12 min the infected cells were mixed with 50 ml of chilled culture of uninfected E. coli B (10^9 cells/ml) that contained 100 μg of chloramphenicol per ml and that had been labeled in the same medium with 2.5 μCi of [¹⁴C]leucine and 2.5 μCi of [¹⁴C]tyrosine for the entire growth period of the culture. The envelopes from this mixture were isolated, and the proteins were solubilized and separated electrophoretically. The gels were sliced, and the counts per minute in each slice were determined. (a) Percentage of total counts in each slice in the infected (——) and uninfected (— · —) cells. (b) Differences in these percentages.*

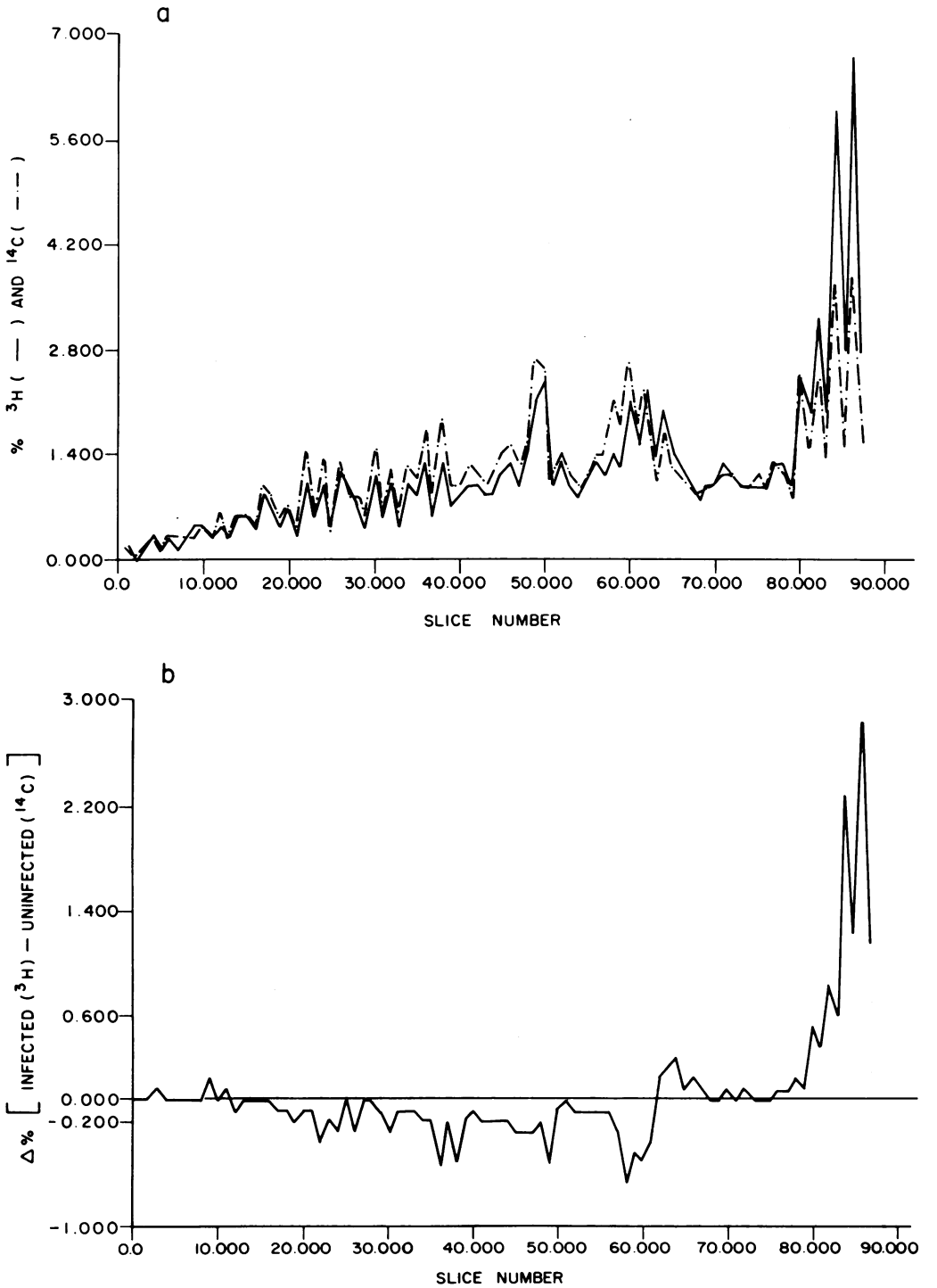

FIG. 2. *Membrane protein biosynthesis in E. coli B infected with two pre-early amber mutants of T5. E. coli B was grown as indicated in the legend of Fig. 1. (a and b) Results of an experiment in which the cells were infected with 10 T5 amH16d (A1⁻) phage per bacterium and labeled with 100 μCi each of [³H]leucine and [³H]tyrosine for 12 min. (c and d) Results of an experiment in which the cells were infected with 10 T5 amH231 (A2⁻) phage per bacterium and labeled with 50 μCi each of [³H]leucine and [³H]tyrosine for 12 min. In both cases, the infected cells were mixed with ¹⁴C-labeled, chloramphenicol-treated, chilled, E. coli B (as in the legend of Fig. 1). The envelopes from the mixtures were isolated, and the proteins were solubilized, separated electrophoretically, and counted.*

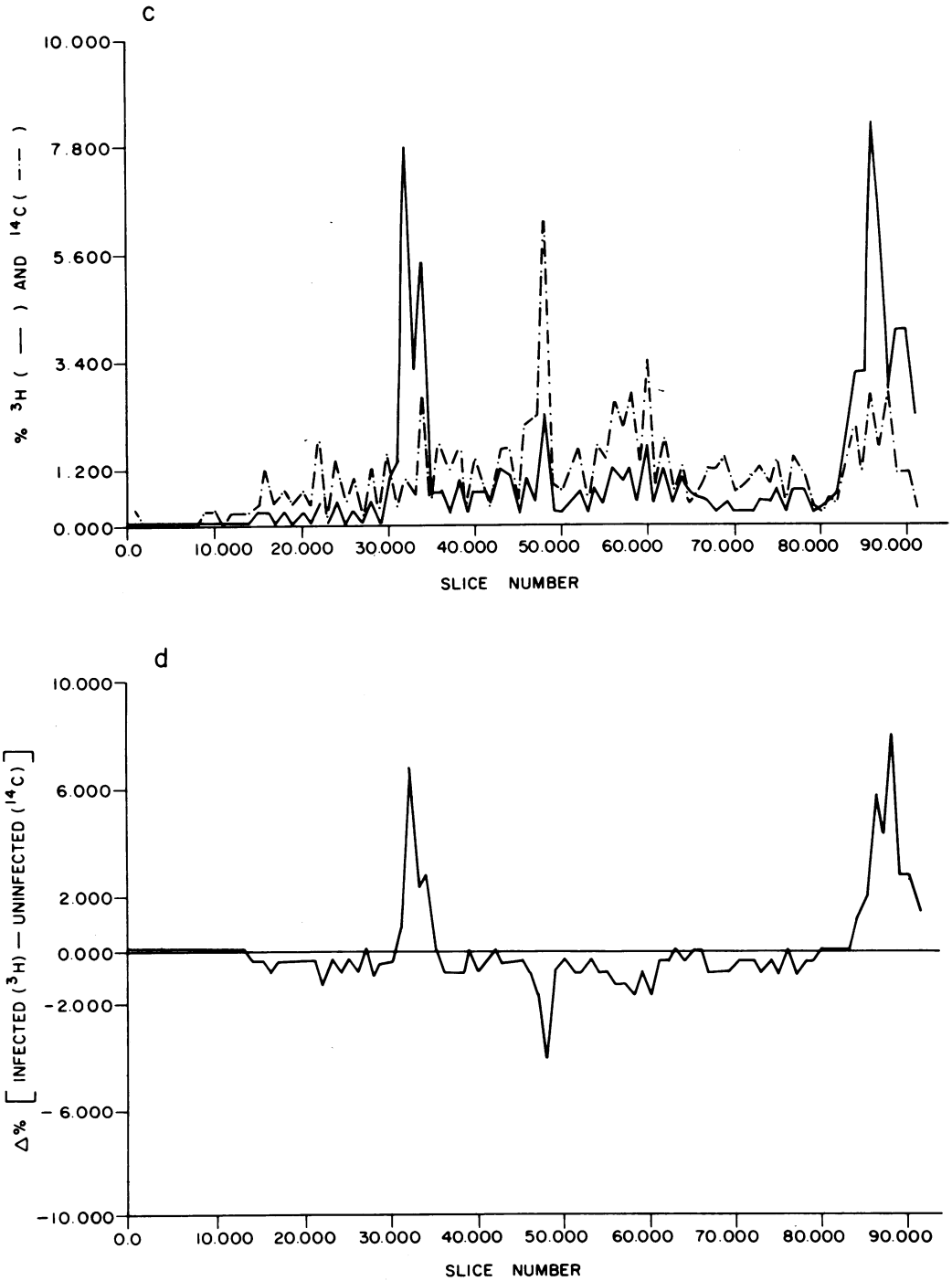

FIG. 3c and d

Two other mutants in gene A1, T5 *am*H27, and T5 *am*M118c, also did not induce the synthesis of the T5 membrane protein (data not shown) in *E. coli* B.

Figure 3 shows the results of an experiment in which T5 *am*H16d was used to infect *E. coli* CR63. The infected cells were labeled with tritiated amino acids for 15 min and then mixed

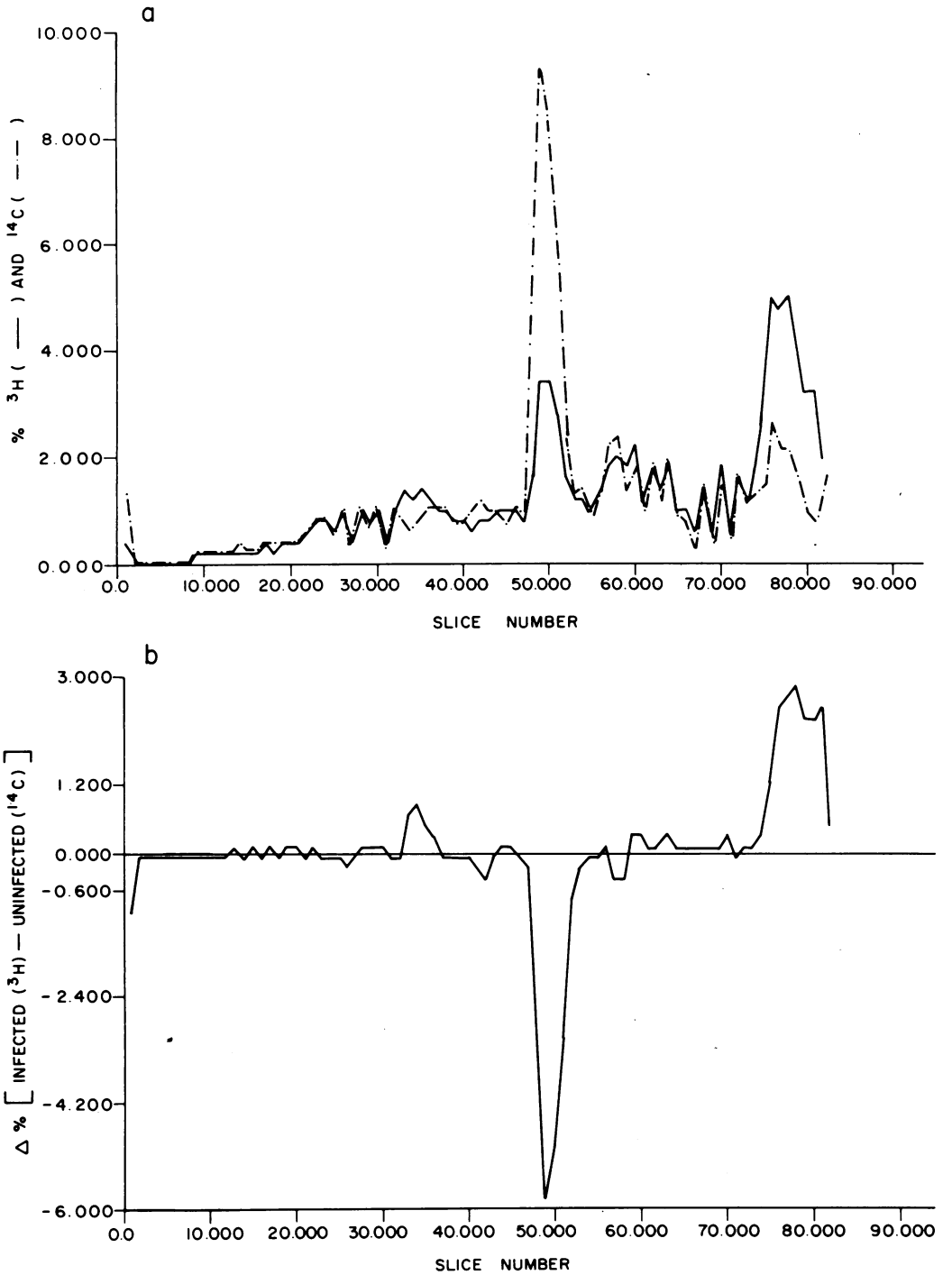

FIG. 3. *Membrane protein synthesis in E. coli CR63 infected by T5 amH16d. A 100-ml culture of E. coli CR63 in M9 medium containing 0.5% glucose, 0.05% yeast extract, 5 × 10^{-4} M $CaCl_2$, and 10 μg each of leucine and tyrosine per ml was grown to a concentration of 10^9 cells/ml. The cells were infected with 10 T5 phage per bacterium, and 50 μCi (each) of [³H]leucine and [³H]tyrosine was added. After 15 min the infected cells were mixed with 50 ml of a chilled culture of uninfected E. coli CR63 (10^9 cells/ml) that contained 100 μg of chloramphenicol per ml and that had been labeled in the same medium with 2.5 μCi of [¹⁴C]leucine and 2.5 μCi of [¹⁴C]tyrosine for the entire growth period of the culture. The envelopes from this mixture were isolated, and the proteins were solubilized, separated electrophoretically, and counted. (a) Percentage of total counts in each slice in the infected (——) and uninfected (— · —) cells. (b) Differences in these percentages.*

with chilled, chloramphenicol-treated, ^{14}C-labeled, uninfected *E. coli* CR63. A positive difference is seen in the area of the gels where the T5 membrane protein is found in *E. coli* B. The amount of the protein found is much less however. This is consistent with the finding that several *E. coli* K-12 strains synthesize less of the T5 major membrane protein than does *E. coli* B (3a). In two experiments with CR63 infected with wild-type T5, the T5 membrane protein was only 3% of the total membrane protein synthesized after infection, whereas in *E. coli* B this value averages about 10%. The amount synthesized by the A1$^-$ mutant in the suppressor-containing *E. coli* CR63 is very close to the 3% value found for wild-type T5 infection of this strain, in spite of the fact that this particular mutant is temperature sensitive. Apparently the temperature sensitivity does not affect the association of membrane with the "suppressed" protein, but only the functioning of the protein. Figure 3 also indicates that a greater percentage of the major host protein (slices 48 through 52) is found in *E. coli* CR63 than in *E. coli* B. Its synthesis is turned off slowly after infection by T5 in both *E. coli* B (3a) and in *E. coli* CR63. There may be some differences in this turnoff in the two strains, but accurate quantitative comparisons cannot be made without further work on strain CR63.

DISCUSSION

Previous studies on membrane protein biosynthesis after infection of *E. coli* by bacteriophage T5 showed that a major new protein species of an approximate molecular weight of 57,000 is made very early after infection. Because the protein appeared in the cell membrane when only the FST DNA had been injected, the remaining 92% of the DNA being removed by a Waring Blendor, and also because it appeared in the cell envelope within 5 min after phage infection, we concluded that it was a pre-early protein coded for, or at least regulated by, a gene on the FST DNA (3a). This conclusion was supported by the finding that the T5 membrane protein was also found in T5-infected *E. coli* containing the Col Ib factor. These abortively infected cells synthesize normal amounts of pre-early proteins of T5 (14) or of BF23 (a T5-related phage) (13) but synthesize little or no early or late proteins of either phage. Work reported here shows that the T5 membrane protein is, indeed, produced when only the FST DNA is present in the cell and, furthermore, that a functional A1 gene must be present for its synthesis.

It has been reported that four polypeptides are synthesized under the influence of the FST DNA: chains of molecular weights 11,000 15,000, 19,000, and 57,000 (3). A more recent investigation has revealed eight pre-early polypeptides with molecular weights of 10,500 11,500, 13,000, 15,000, 15,500, 29,000, 40,000, and 58,000 (G. Chinnadurai and D. J. McCorquodale, manuscript in preparation). Some of these polypeptides combine in various ways to form the four identified pre-early proteins found in extracts of infected cells (13). The polypeptide chain of molecular weight 58,000 has been reported to be a product of the A1 gene (3). Therefore, data agree very well with this work. The finding by Billmire and Duckworth (manuscript in preparation) that a BF23 mutant analogous to the A1 mutant of T5 also cannot produce a membrane protein of molecular weight 57,000 further substantiates the role of the A1 gene in membrane protein biosynthesis. It is possible that the products of other pre-early genes will also be found associated with the cell membrane. The gels we have used in these experiments do not resolve components of low molecular weight (below 20,000), however. In particular, the product of the A2 gene, which is necessary along with the product of the A1 gene for transfer of the whole T5 DNA molecule from the phage to the cell, may also be membrane associated.

The reason for the presence of the 57,000 molecular weight protein in both soluble cell extracts (13) and membranes (3a) is not known. It could exist normally in an unstable association with the membrane and be removed by the sonic treatment used to make the soluble cell extracts (13), or the soluble form could be a precursor to the membrane-bound form (3a).

Except for some phage and colicin receptor proteins and some "permease" proteins, the genes controlling the synthesis of bacterial membrane proteins are not known. Recently, several genes coding for membrane proteins in T4-infected cells have been identified (6, 15, 17). These are primarily genes associated with DNA functioning as is the A1 gene of T5. These findings, and the finding reported here of a gene that controls a protein that occurs in substantial amounts in cellular membranes, should greatly aid in the elucidation of membrane protein biosynthesis and the relationship between structure and function in biological membranes.

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