Intracellular Inactivation of Bacteriophage T4
Early Deoxyribonucleic Acid

CHRISTOPHER K. MATHEWS
Department of Biochemistry, University of Arizona, College of Medicine, Tucson, Arizona 85721

Received for publication 6 February 1970

As previously shown, a small amount of polynucleotide material is added to parental T4 deoxyribonucleic acid (DNA) molecules within the first 5 min of infection. I have asked whether this process is essential for phage replication. Two approaches—one involving decay of $^{32}$P incorporated into this "early DNA" and the other involving photoinactivation of bromodeoxyuridine-containing early DNA—indicate that it is.

Within the first 5 min of infection by bacteriophage T4, polynucleotide material is covalently added to parental deoxyribonucleic acid (DNA) molecules (4), to the extent of approximately 0.06 phage-equivalent units of new material per parental molecule. This process occurs before DNA replication has commenced, and occurs independently of the enzyme systems needed to support replication. To date, we know very little about the molecular nature of this "early DNA"—i.e., its overall nucleotide composition, whether it is added terminally or internally, whether it is added to both strands, the enzymatic route for its synthesis, and so forth. As a prelude to further studies on these questions, it seemed appropriate to ask whether the process of early DNA formation is biologically significant. The experiments reported in this note indicate that it is.

Bacterial strains used were Escherichia coli B and its thymine-requiring derivative, B3. Phage strains used were T4BO$_3$ and its thymidylate synthetase-negative derivative T4tdB8 (6). Methods for growth and assay of phages have been described (4). Other methods are presented in the legend to Fig. 1.

Two approaches were used, each one asking whether intracellular inactivation of early DNA in singly infected cells would lead to loss of the ability of an infected cell to release viable progeny. In the first approach, inactivation was accomplished by decay of radioisotopes incorporated within the first 5 min of infection of E. coli B by nonradioactive T4BO$_3$. As shown in Fig. 1A, the titer of infective centers decreased at a rate approximately one-fourth of that observed with phage fully labeled with $^{32}$P of the same specific activity. Very little loss of infective centers was observed in a control, in which $^{32}$P-phosphate at high specific activity was present in the cells for the 10 min immediately before infection, but was diluted by addition of excess $^{32}$P-phosphate at the time of infection.

As stated above, the slopes of the survival curves for the infective centers containing labeled early DNA and for the fully labeled whole phage are in the approximate ratio of 1:4 (this is uncorrected for the slow loss of infectivity seen in the controls). Since the size of the DNA target in fully labeled phage is about 16 times as great as that of the early DNA attached to a phage genome (early DNA constituting 0.06 of a phage-equivalent unit of early DNA), this means that a $^{32}$P decay event has four times the probability of inactivating a plaque-forming unit if it occurs in early DNA than in the rest of a phage genome. This is not unexpected, since some disintegrations occurring in a labeled phage DNA molecule would be occurring in genes which are nonessential for replication, or at least for the formation of a single viable progeny phage. Of course, the discussion neglects the possible contribution to the results of stabilization of infective centers to $^{32}$P decay (8). However, other experiments by us (not shown) have revealed that relatively little stabilization has occurred by 5 min after infection. On the other hand, even if one could easily correct for $^{32}$P stabilization, this would tend to steepen the inactivation slope for early DNA relative to that for fully labeled phage.

Of course, $^{32}$P-phosphate can be incorporated into many cellular substances other than DNA, and the possibility remained, however remote, that the lethal target of $^{32}$P decay in the cells labeled for 5 min was not DNA but some other species—conceivably some rare and essential pre-early messenger ribonucleic acid species which is not formed after 5 min (compare refer-
Fig. 1. Intracellular inactivation of early DNA. Part A, decay of incorporated 32P. E. coli B (20 ml) was grown at 37 C to a cell density of about 3 X 10^9/ml in tris(hydroxymethyl)aminomethane (Tris)-glucose medium containing 0.002 M phosphate. The cells were centrifuged, washed in Tris-glucose medium, and resuspended in 10 ml of Tris-glucose medium containing glucose, 0.0001 M phosphate, and 20 µg of L-tryptophan per ml as adsorption cofactor. Tris-glucose medium (1 ml) was placed in a growth tube containing 600 µc of carrier-free H^3PO_4 which had been evaporated to dryness; this mixture was incubated at 37 C for 10 min to label nucleotide pools. The culture (0.3 ml) was added to a growth tube containing 1.5 X 10^4 T4BOI particles (to give an average multiplicity of 0.01; culture a). Another 0.3-ml portion was added to a tube containing phage at the same multiplicity of infection, plus 0.1 ml of 0.1 M phosphate buffer, pH 7.0 (culture b). To the remaining cells were added phage at the same multiplicity of infection (culture c). At 5 min, 0.1 ml of phosphate buffer was added to culture a, and cultures a and b were both chilled, centrifuged to remove unadsorbed phage, and each culture was resuspended in 2 ml of cold glycerol-Casamino Acids medium (2). Culture c was incubated for 90 min, treated with chloroform, centrifuged at low speed, and the supernatant fraction was made up to 2.0 ml with glycerol-Casamino Acids medium. A fourth culture, d, was made up identically to culture b, except that isotopic phosphorus was never present. Samples (1 ml) from each culture were stored at -70 C and assayed for plaque-forming titer over the next few weeks. Symbols: (△) culture a, pre-labeled cells, labeled early DNA; (▼) culture b, pre-labeled cells, unlabeled early DNA; (○) culture c, fully labeled phage; and (●) culture d, unlabeled control. Part B, photoactivation of BU-containing early DNA. A 20-ml amount of E. coli B3 was grown at 37 C in Tris-glucose medium containing 10 µg of thymidine per ml to a cell density of approximately 2.5 X 10^9/ml. The cells were centrifuged, washed in Tris-glucose salts, and resuspended in 20 ml of Tris-glucose medium containing glucose but no thymidine. Three 3-ml cultures were incubated at 37 C. To culture a was added 10 µg of thymidine per ml; to cultures b and c, 10 µg of BUdR per ml. At 30 min, 20 µg of L-tryptophan per ml was added, and each culture was infected at an average multiplicity of infection of 0.02 as follows: cultures a and b with normal T4d8 phage, and culture c with BU-labeled T4d8. Five minutes after infection, each culture was centrifuged, and the cells were washed in Tris-glucose salts and resuspended in 5 ml each of Tris-glucose salts. Appropriate portions were diluted to 10 m each in Tris-glucose salts and irradiated as described by Eisenberg and Pardee (1a), with the exceptions that a Hanovia utility quartz lamp was used and the Corning 7-54 filter was omitted. Symbols: (▼) culture a, normal parental DNA, normal early DNA; (△) culture b, normal parental DNA, BU-labeled early DNA; and (○) culture c, BU-labeled parental DNA, BU-labeled early DNA.
undergo nicking after infection, the parental strands are still intact at 3 min after infection (3). In our laboratory, alkaline sucrose gradient centrifugation of parental DNA as late as 5 min after infection shows little degradation (unpublished data). (ii) Nicking of parental DNA would be expected to release acid-soluble material to the medium. Yet less than 1% of 32P in parental DNA is released within the first 5 min (5). (iii) Even if some damage and repair do take place, many of these events would occur at "silent" regions of the T4 genome, where inactivation would not destroy plaque-forming capacity. It seems impossible, in light of points (i) and (ii), that sufficient 32P, in the 32P suicide experiment, would be incorporated into essential genes by repair for the inactivation slope to be one-fourth of that observed for fully labeled phage.

In summary, the results of two types of experiments involving intracellular inactivation of early DNA are in agreement with the idea that formation of this material is an essential process in T4 multiplication.

This work was supported by Public Health Service grant AI-08230 from the National Institute of Allergy and Infectious Diseases and grant 68 811 of the American Heart Association. I thank Mildred Ceizyk for capable technical assistance and A. P. Pardee for making available details of his irradiation procedure before publication.

LITERATURE CITED