JOURNAL OF VIROLOGY, Sept. 1971, p. 275-285
Vol. 8, No. 3

T-Even Bacteriophage-Tolerant Mutants of *Escherichia coli* B

II. Nucleic Acid Metabolism

CHRISTOPHER K. MATHEWS AND MARTINEZ J. HEWLETT
Department of Biochemistry, University of Arizona, College of Medicine, Tucson, Arizona 85724

Received for publication 1 June 1971

T-even bacteriophage-tolerant mutants are strains of *Escherichia coli* which can adsorb T-even phages but cannot support the growth of infective virus. Under some conditions, the infected cells are not killed. Mutant cells infected by phage T6 are able to carry out several metabolic functions associated with normal virus development, including arrest of bacterial nucleic acid and protein synthesis, incorporation of isotopic precursors into viral nucleic acids and proteins, synthesis of early enzymes of deoxyribonucleic acid (DNA) metabolism, formation of rapidly sedimenting DNA intermediates, and formation of normal levels of early and late messenger ribonucleic acid species. Phage are unable to mutate to forms capable of growth on these mutants. The nature of the biochemical alteration leading to tolerance is still unknown.

As an approach to understanding the contribution of host cell metabolic functions to virus development, our laboratory has isolated T-even bacteriophage-tolerant (*tet*) mutants of *Escherichia coli* B (12). These strains can adsorb T-even phages but are unable to form infective progeny. Under some conditions, the cells are not killed. Preliminary investigation showed that some viral genes are expressed after infection of these mutants by phage T6. Early enzymes of deoxyribonucleic acid (DNA) metabolism are synthesized, albeit in limited amounts (12), and some phage DNA replication occurs.

The objectives of the work described in the present paper were, first, to devise experimental conditions suitable for extensive analysis of metabolic events in abortive infections of *tet* mutants, and, second, to explore events in nucleic acid metabolism which might provide clues to the primary biochemical alteration in *tet* mutants. We find that even though *tet* mutants are absolutely defective in the ability to propagate infective virus they are able to support a wide range of virus-directed metabolic processes.

MATERIALS AND METHODS

All of the present work was carried out with T6 phage and *E. coli* B, along with its derivatives *E. coli tet* 1 and *tet* 2, both described previously (12). T6 was used because of its rapid adsorption to all three bacterial strains. Culture and assay techniques and culture media were also as described in earlier communications from this laboratory, except that glycerol-Casamino Acids (GCA) medium contains 5 mg of Casamino Acids per ml rather than 15. Except where otherwise indicated, all experiments were carried out in GCA medium.

Because of the tendency of *tet* mutants to revert to wild type, stocks were reisolated from single colonies at frequent intervals. Moreover, in each experiment reported herein, the *tet* phenotype was checked as follows. At 6 min after infection, samples were removed to dilution tubes either containing chloroform for determination of unadsorbed phage or to tubes containing no chloroform for measurement of total infective centers. At 90 min, another sample was removed to a dilution tube containing chloroform for determination of total progeny. Suitable dilutions of the above were plated, with *E. coli* B as the plating host. The following were taken as criteria for satisfactory expression of the *tet* phenotype: infective center titer $\leqslant$ unadsorbed phage titer and infective progeny titer $\leqslant$ unadsorbed phage titer. Thus, in each experiment reported, there was no detectable formation of infective phage in infection of *tet* mutants.

For studies on β-galactosidase induction, 10-ml cultures were induced as described in the legend to Fig. 4 and then chilled and centrifuged. The pellets were resuspended in 5 ml each of 0.02 M sodium phosphate buffer (*p*H 7.5) and sonically treated for 30 sec. β-Galactosidase activity was measured in these sonically treated extracts as described by Duckworth and Bessman (4).

Incorporation of ^{14}C-uracil into DNA and ribonucleic acid (RNA) and incorporation of ^{14}C-leucine into protein were followed as described previously (10, 11). Techniques for isolation of RNA and for DNA-RNA hybridizations were also as described

earlier (11), except that in isolating RNA diethyl pyrocarbonate (at 1%) was present in the extracting buffer for inhibition of nucleases rather than polyvinylsulfate.

In studies on DNA metabolism, the methods used for DNA labeling, cell lysis, sucrose gradient centrifugation, and radioisotope counting were as described by Murray and Mathews (14, 15). The technique used for DNA-DNA hybridization was the membrane filter method of Denhardt (3), as modified in this laboratory by M. Sisson (M. Sisson and C. K. Mathews, *in preparation*). Incorporation of parental phage DNA into "replicating DNA complexes" was measured by the M-band technique of Earhart and his colleagues (5, 6).

Further experimental details are provided in individual figure legends.

RESULTS

Effects of host cell survival. The basis for isolation of *tet* mutants is their ability to grow on agar plates in the presence of a large excess of two different T-even phages. Under these conditions, infection is not lethal to the host cell. However, in our early studies we found that considerable cell killing occurs in infection of *tet* mutants in liquid media, even though infection is always abortive. It is now clear that the ability of a *tet* cell to survive infection depends upon its environment; cell survival is higher in solid media than in liquid media, and in liquid survival is higher in nutritionally rich media than in minimal or other defined media. Figure 1 presents data on the ability of *tet* cells to form colonies when plated in the presence of various amounts of T6. The mutants did lose the ability to form colonies ultimately but only at an input of phage some 100-fold greater than that necessary to abolish the colony-forming ability of *E. coli* B plated under the same conditions. Similarly, in liquid media *tet* cells could lose the ability to form colonies when plated after infection at high multiplicities, but the difference between *E. coli* B and the mutants was considerably less under these conditions. Figures 2 and 3 show bacterial survival curves at various multiplicities of infection by T6 in nutrient broth and GCA medium, respectively. Note that the slopes of the *tet* survival curves are considerably steeper in GCA medium than in nutrient broth. Similar results are seen in infection by T2 (data not shown). In all cases, the survival curves are approximately linear, indicating that killing of the *tet* mutants is a one-hit process; infection by a single phage particle is a lethal event, although this occurs with lower probability in infection of *tet* mutants than in infection of wild-type bacteria.

Because the environment on an agar plate is considerably more anaerobic than that of a

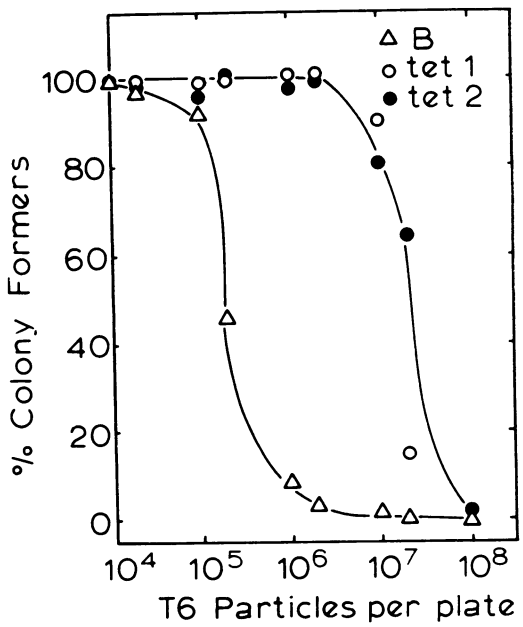

FIG. 1. *Expression of the tet phenotype on agar plates. For each strain, a constant number of colony-formers was plated on each of a series of nutrient agar plates in the presence of the indicated numbers of T6 particles.*

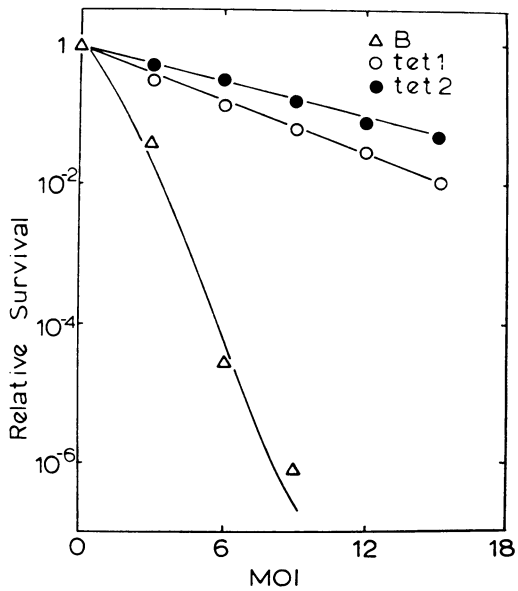

FIG. 2. *Survival curves for bacteria infected in nutrient broth. Bacterial cultures at about 5×10^8 per ml were infected with T6 at the indicated multiplicities. At 6 min, samples were removed, diluted, and plated for determination of colony-forming ability. MOI, multiplicity of infection.*

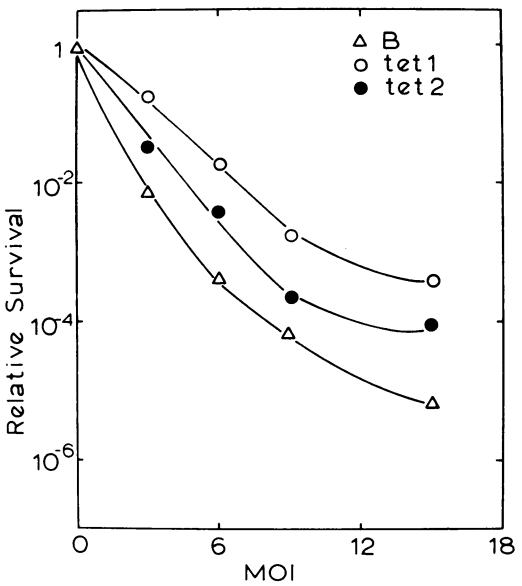

FIG. 3. *Survival curves for bacteria infected in glycerol-Casamino Acids (GCA) medium. Except for the substitution of GCA medium for broth, the protocol was identical to that described in the legend to Fig. 1. MOI, multiplicity of infection.*

vigorously bubbled liquid culture, it seemed possible that there is a relationship between anaerobiosis and cell killing: the more anaerobic the culture, the lower the probability that a particular infection will be lethal. This was tested by comparing cell survival in GCA medium either aerated or bubbled with nitrogen (data not shown). Cell survival was no greater in the anaerobic than in the aerobic culture and indeed was somewhat less, indicating the absence of a direct relationship between anaerobiosis and cell survival.

Inability of phage to overcome tolerance. We wished to know whether T-even phages could undergo mutations which would allow them to overcome tolerance, i.e., to plate on either *tet* 1 or *tet* 2. The map positions of such "host-range" mutations, at least in the genetically well-characterized T4, might provide clues to the bacterial functions altered in the original *tet* mutations. In our previous study, we found that T2, T4, and T6 were all unable to plate on either *tet* mutant when as many as 10^6 viable phage were added to a single plate. We have now extended this work by subjecting both T4 and T6 to chemical mutagenesis by nitrous acid, hydroxylamine, 2-aminopurine, 5-bromodeoxyuridine, and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Even when as many as 10^8 plaque-forming units from mutagenized stocks were plated on *tet* 1 or *tet* 2, no plaques were seen. Thus, phage seem quite unable to circumvent the effects of the *tet* mutations by extending their own host range. In this context, it is of interest that phage T7 plates on *tet* 1 with an efficiency equal to that seen on *E. coli* B (W. C. Summers, *personal communication*).

By analogy with temperate phages, we considered whether tolerance in the *tet* systems is at all akin to prophage immunity. In other words, could the *tet* character result, not from mutation, but from a stable association of phage DNA with the bacterial genome either as an episome or a plasmid. Although this seemed improbable, we tested it by attempted hybridization between bacterial DNA, fixed on membrane filters, and ^{14}C-labeled T6 DNA. Fewer than 0.2% of the input counts were bound to bacterial DNA from either *E. coli* B, *tet* 1 or *tet* 2, and no differences were seen among the three strains. We conclude, therefore, that the *tet* genomes do not contain significant amounts of phage-specific DNA.

Effects on bacterial polymer synthesis. One of the most powerful biochemical research techniques is the use of radioisotopes for labeling metabolic intermediates, whereas one of the most attractive features of T-even phages as objects for metabolic studies is the fact that infection completely arrests the synthesis of macromolecules coded by the bacterial genome. Thus, one can label T-even phage-infected cells with isotopic protein or nucleic acid precursors secure in the knowledge that only virus-specific polymers will be labeled. In our earlier work in *tet* mutants, we had no such security; since bacterial survival was high, the level of bacterial nucleic acid and protein synthesis almost certainly was also high, and a number of informative biochemical experiments could not be performed. However, once we had learned that infection at high multiplicity in chemically defined media leads to extensive cell killing, it seemed that these approaches might be available after all. First, of course, we had to establish that host cell gene expression is arrested in the *tet* mutants in a fashion similar to that seen in wild-type cells. As a prerequisite to these experiments, we needed to know that phage adsorb to *tet* cells as rapidly as to *E. coli* B. This was indeed the case, at least under our conditions of infection (5×10^8 to 8×10^8 cells/ml, in GCA medium, and multiplicities of 6 to 10). With each bacterial strain, more than 60% of added phage was adsorbed by 2 min after infection and over 85% by 5 min.

As a first approach to host cell gene expression, we examined the ability of infected cells to synthesize β-galactosidase after induction by iso-

propylthiogalactoside (IPTG). The specific question asked is the following: does every cell which is killed (i.e., loses colony-forming ability) also lose the capacity to synthesize β-galactosidase? This was asked by infecting cultures at different multiplicities. Six minutes after infection, samples were removed for determination of viable count. IPTG was added to the remainder of each culture for a 12-min period, after which the cells were harvested and assayed for β-galactosidase activity. Now, if every cell which is killed also loses the capacity for induced enzyme synthesis, we expect a plot of residual enzyme induction versus bacterial survival to be a straight line with a 45° slope. However, if some killed cells do not lose enzyme inducibility with the same kinetics, then we expect significant upward deviation from a 45° line. As seen in Fig. 4, the data most closely approximated 45° lines for all three strains. In other experiments (Fig. 5), no differences were observed among the three strains in enzyme inducibility when IPTG was added earlier in infection, i.e., at 3 min. Thus, it appears that the kinetics of loss of induced enzyme synthesis are the same in the mutants as in the wild-type strain and that every killed cell loses the capacity for bacterial enzyme induction.

We have investigated the kinetics of arrest of host-specific RNA synthesis indirectly by following the rate of phage-specific RNA synthesis early in infection. RNA was pulse-labeled with uracil-*5-*^3H, either in uninfected cells or at early times after T6 infection. After purification, the RNA samples were hybridized to T6 DNA. The RNA to DNA input ratio was adjusted 1:20, a condition favorable for complete hybridization of all RNA species which are homologous to phage DNA (9). Thus, the percentage of input counts bound to phage DNA represents the percentage of the total labeled RNA which is phage-specific. Our results for T6-infected *E. coli* B (Fig. 6) are simialr to those reported by Kennell (9) for T4-infected *E. coli*; a relatively large fraction of the RNA being synthesized as late as 5 min after infection is not phage-specific and hence is host-specific. The important point from Fig. 6 is that the data for *E. coli* B, *tet* 1, and *tet* 2 are identical. In other words, the rate of replacement of host transcription by virus-directed transcription is the same in all three strains.

The kinetics of arrest of host cell DNA synthesis were studied in similar fashion (Fig. 7). Thymidine-*methyl-*^3H was used to label DNA for 2-min periods either before or at the indicated periods after infection. Figure 7A shows the total radioactivity incorporated into acid-insoluble material during each labeling period. The differences among the three strains in counts incorporated before infection may well represent not true differences in rates of DNA synthesis but rather differences in thymine nucleotide pool sizes or some other factor. At any rate, the data on total counts incorporated during each post-infection labeling period are quite similar, suggesting that the rates of host DNA shutoff are similar, if not identical, in the three strains. The upturn in rate of total thymidine incorporation seen with each strain between 5 and 7 min represents the initiation of phage DNA synthesis, since DNA labeled during this period hybridizes efficiently with T6 DNA (data not shown).

Hybridization of DNA isolated from each labeled culture against unlabeled *E. coli* B DNA

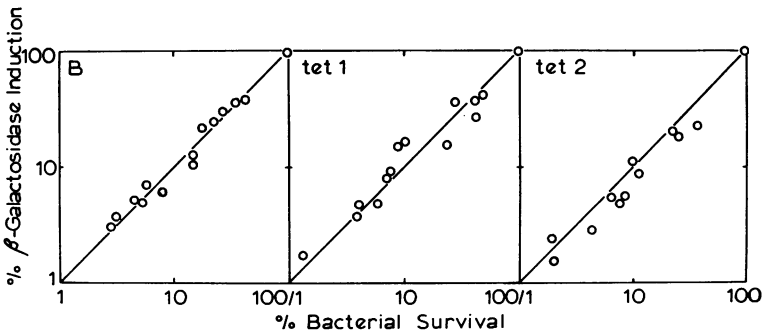

FIG. 4. *Comparison of cell killing with β-galactosidase inducibility. Log-phase bacterial cultures at about* 8×10^8 *per ml in glycerol-Casamino Acids medium were subdivided into 10-ml amounts, each of which was infected with T6 over a range of multiplicities between 0 and 5. At 6 min, samples were removed for determination of viable count, and isopropylthiogalactoside was added to the remainder of each culture to a level of* 5×10^{-4} *M. Twelve minutes after addition of inducer, each culture was chilled and centrifuged. The pellets were resuspended, and β-galactosidase was assayed as described in Materials and Methods. The figure presents combined data from three separate experiments on each bacterial strain.*

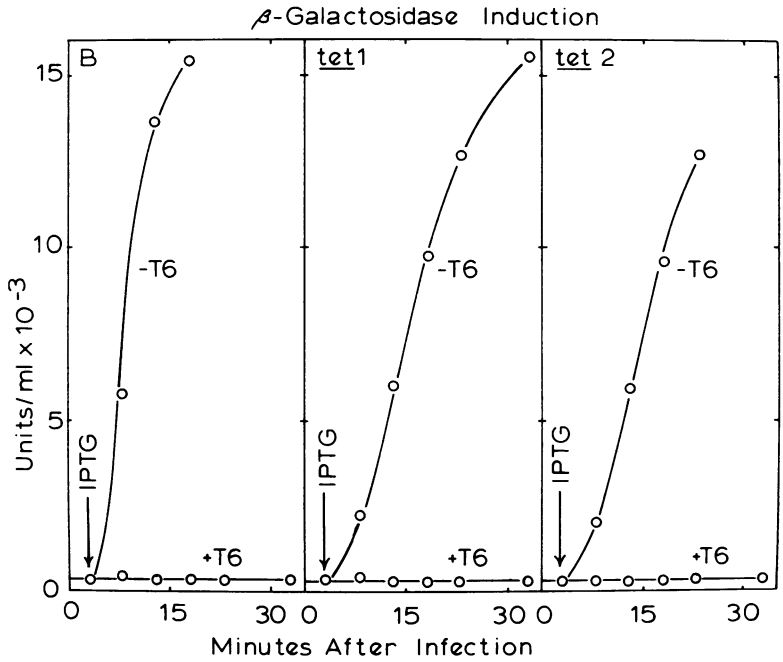

FIG. 5. *β-Galactosidase induction after T6 infection. Log-phase bacterial cultures in glycerol-Casamino Acids medium were infected with about 6 T6 particles per bacterium. Isopropylthiogalactoside (IPTG) was added to 5 × 10⁻⁴ M at 3 min, and samples were removed at the indicated times and assayed for β-galactosidase as described in the legend to Fig. 4. Results are compared with control values from uninfected cultures.*

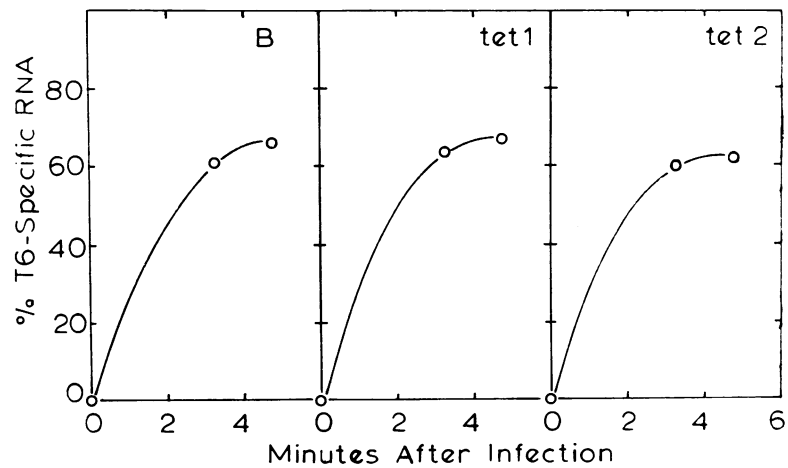

FIG. 6. *T6-specific RNA synthesis. Bacterial cultures (100-ml) were grown in glycerol-Casamino Acids (GCA) medium plus 20 μg of uracil per ml. At a turbidity corresponding to about 8 × 10⁸ cells/ml, each culture was centrifuged, washed in GCA medium, and resuspended in 100 ml of fresh GCA medium containing no uracil. Pulse-labeling was carried out for 1.5-min periods with 50 μCi each of uracil-5-³H on uninfected cells and T6-infected cultures (multiplicity of infection = 6), beginning at 2.5 min and at 4 min after infection. RNA was isolated as described in Materials and Methods. Labeled RNA samples containing 2.5 μg each were hybridized against 50 μg each of T6 DNA immobilized on membrane filters. Each point in the figure corresponds to the midpoint of a labeling period. The ordinate denotes the percentage of labeled RNA which is phage-specific in each preparation.*

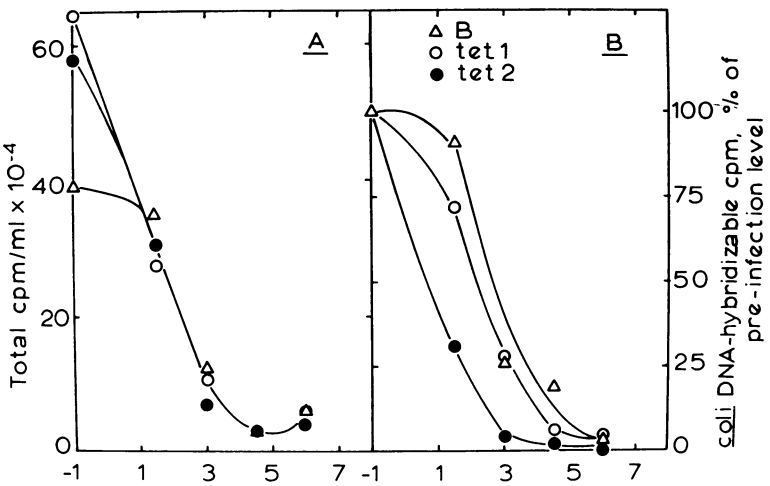

FIG. 7. *Incorporation of thymidine-methyl-^2H into E. coli DNA. Bacterial cultures (20 ml) were infected with T6 at a multiplicity of about 6. At various times, 1-ml samples were removed to small incubation tubes containing 10 μCi each of ^3H-thymidine. After 2 min of labeling, metabolism was halted by addition of cyanide-ethylenediaminetetraacetic acid-lysozyme lysing fluid (14). Lysis was carried out by a high-temperature procedure (14). Each lysate was extracted twice in succession with water-saturated phenol, and the solutions were dialyzed overnight against 0.02 M sodium phosphate buffer, pH 6.8. A 0.5-ml amount of each preparation was mixed with 0.5 ml of 1 N NaOH, and all preparations were heated in a boiling-water bath for 7 min. Each preparation was neutralized and made up to 3.0 ml in 3× SSC (SSC = 0.15 M NaCl plus 0.015 M sodium citrate). Duplicate 0.5-ml samples of each were hybridized against membrane filters containing 20 μg each of E. coli B DNA. Hybridized counts were corrected for the small number of counts bound to blank filters. (A) Total counts incorporated into acid-insoluble material. Each point on the graph represents the midpoint of a pulse-labeling period. (B) Hybridizable counts. These are plotted as per cent of E. coli DNA-hybridizable counts relative to the labeled uninfected cultures. Again, each point represents the midpoint of a labeling period.*

provides more specific information on the relative rates of host DNA shutoff (Fig. 7B). These data make it appear that host DNA synthesis is actually turned off faster in infection of the *tet* mutants than in normal infection. The differences are more apparent than real, since the *E. coli* DNA-hybridizable counts in each labeling period are plotted relative to the counts incorporated in the uninfected cultures. Thus, the apparent differences derive in part from the differences in total counts per minute incorporated among the three uninfected cultures. However, it does appear that host cell DNA synthesis is arrested at least as fast in the *tet* mutants as in the parent strain, if not faster. Of course, quantitative studies of DNA metabolism must await the isolation of thymine auxotrophs of the *tet* mutants, and these are currently being prepared in our laboratory.

DNA metabolism. Our earliest work on DNA metabolism in T6 infection of *tet* mutants involved studies on the fate of labeled parental phage DNA. We find (Table 1) that parental DNA does become attached to the "M band," a complex described by Earhart and his colleagues (5, 6), which apparently contains replicating DNA and the membrane material present at the replicating site. This, of course, is not unexpected, since we had earlier found (12) that *tet* mutants support T6 DNA replication.

In our previous study, we found that parental phage DNA is not degraded to acid-soluble

TABLE 1. *Incorporation of parental T6 DNA into M bands*[a]

Bacteria	Total counts/min in M band	Per cent of total parental counts/min in M band
E. coli B.....	1.48×10^4	25.1
E. coli tet 1....	1.78×10^4	30.2
E. coli tet 2....	1.47×10^4	24.8

[a] Bacterial cultures (5.0 ml) growing in tryptone broth medium were infected at a multiplicity close to 5.0 with T6 containing ^{14}C-labeled DNA. At 7 min after infection, the cells were harvested and M bands were prepared and visualized on magnesium-sarkosyl gradients as described by Earhart (5). Each M band was removed from its gradient centrifuge tube by lowering the collection tube of a Buchler gradient collector to the level of the band and pumping out the white, viscous material by suction.

material in infection of *tet* cells. In the present study, we asked whether any breakdown is detectable. Again, parental DNA was labeled by growth of phage in the presence of ^{14}C-uracil (10). Cells were harvested at 4 min and lysed by a low-shear, low-temperature process (7, 15); the lysates were subjected to sucrose gradient analysis in the presence of ^{32}P-labeled mature T6 DNA present as a sedimentation marker. At 4 min, no breakdown is evident, as shown by the fact that the sedimentation profiles of marker and parental DNA are virtually identical in neutral gradients (Fig. 8). The same results are seen when portions of the same lysates are sedimented through alkaline sucrose gradients (Fig. 9); parental and marker profiles are identical. Thus, within the first 4 min of infection, parental phage DNA is neither broken nor subjected to significant endonucleolytic cleavage.

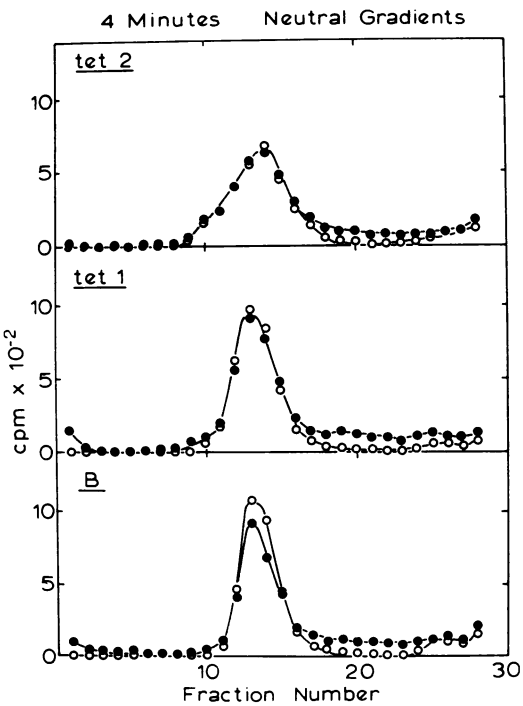

FIG. 8. *Neutral sucrose gradient centrifugation of parental T6 DNA at 4 min after infection. Bacterial cultures (about 8 × 10^8 per ml) were infected with T6 phage containing ^{14}C-labeled DNA (15). At 4 min, cells were lysed by a low-temperature procedure (7) in the presence of ^{32}P-labeled T6 DNA added as a sedimentation marker. Portions (0.2 ml) were layered onto 5.0-ml neutral 5 to 20% sucrose gradients, which were then centrifuged at 32,000 rev/min for 35 min. Symbols: ○, ^{32}P marker counts per minute; ●, ^{14}C parental counts per minute.*

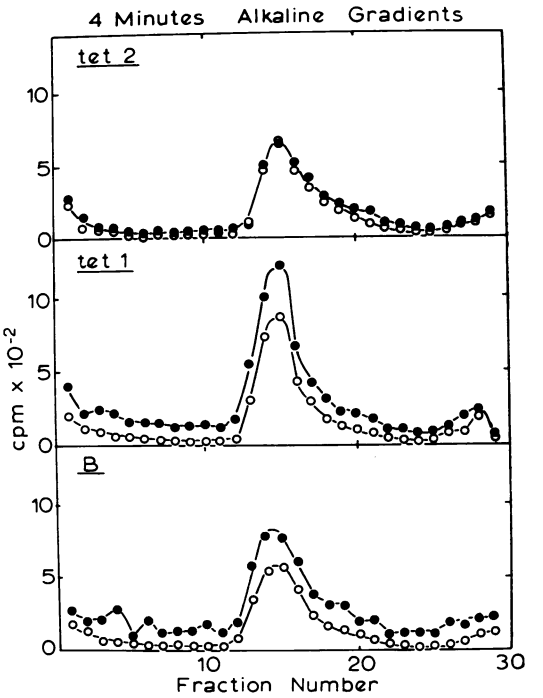

FIG. 9. *Alkaline sucrose gradient centrifugation of parental T6 DNA at 4 min after infection. Samples of the lysates prepared for the experiment described in Fig. 8 were adjusted to 0.3 N with NaOH, and 0.2-ml portions were layered onto 5.0-ml alkaline sucrose gradients (14), which were centrifuged at 40,000 rev/min for 60 min. Symbols: ○, ^{32}P marker counts per minute; ●, ^{14}C parental counts per minute.*

Analysis of the fate of parental DNA later in infection reveals differences between the wild and mutant strains. As shown by Frankel (7), much of the parental DNA during periods of active DNA replication in infection by T4 is present in a form which sediments more rapidly than does mature DNA and which contains individual DNA strands longer than those of mature DNA. As shown in Fig. 10, much of the parental DNA at 13 min after T6 infection of *E. coli* B sediments through neutral gradients heterogeneously but more rapidly than mature T6 DNA. Infected *tet* cells also form rapidly sedimenting intermediates containing parental DNA. However, this material does not sediment as rapidly as that seen in infection of *E. coli* B, and a smaller proportion of the total parental DNA sediments rapidly. The true magnitude of the difference between the wild strain and the mutants is probably underestimated in this figure. Recovery of input parental counts in the *tet* 1 and *tet* 2 gradients was 87 and 96%, respectively, whereas that in the *E. coli* B

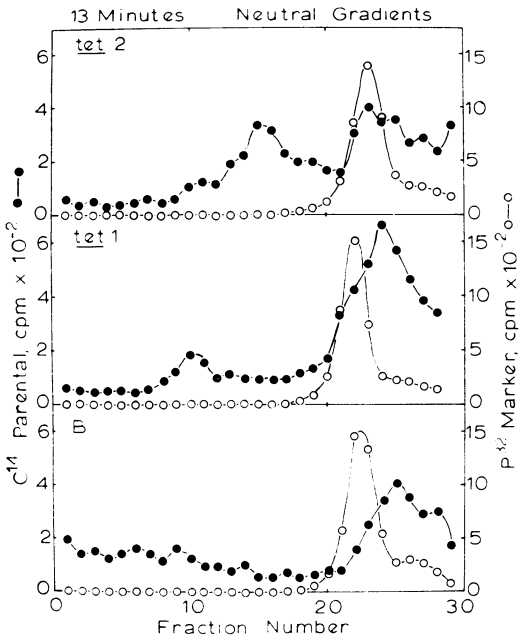

FIG. 10. *Neutral sucrose gradient centrifugation of parental T6 DNA at 13 min after infection. Except for the time of infection, the protocol was identical to that described in the legend to Fig. 8.*

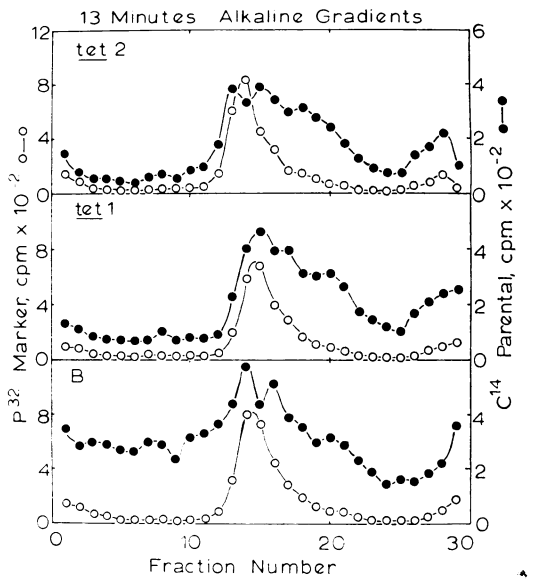

FIG. 11. *Alkaline sucrose gradient centrifugation of parental T6 DNA at 13 min after infection. Except for the time of infection, the protocol was identical to that described in the legend to Fig. 9.*

gradient was only 78%. It is probable in the latter case that considerable parental DNA sedimented to the bottom of the tube and was lost in this gradient.

When the above lysates, obtained at 13 min, were analyzed in alkaline gradients, an even more striking difference was seen. As shown in Fig. 11, parental DNA strands in infection of *E. coli* B sediment heterogeneously, but most of the strands sediment as though they are longer than mature DNA strands. By contrast, virtually no long-stranded DNA was seen in infection of the *tet* mutants. This implies that parental DNA is subject to considerable endonucleolytic breakage or nicking. Whether this is a direct or indirect consequence of the *tet* alteration remains to be determined.

Once we had established that arrest of host cell gene expression occurs normally in T6 infection of the *tet* strains, it became possible to design isotope incorporation experiments for measuring relative rates of nucleic acid and protein synthesis. When cells are grown in the presence of high levels of uracil, such that they are repressed in the pyrimidine biosynthetic pathway, it becomes possible to use ^{14}C-uracil for following accumulation of both DNA and RNA (10, 11). When such an experiment was performed (Fig. 12), we found, somewhat to our surprise, that the rates of accumulation of isotope in both DNA and RNA were comparable in infected *tet* cells to those seen in normal infection. Similarly, rates of incorporation of ^{14}C-leucine into protein reveal no gross defects in protein synthesis in infection of the mutants (Fig. 13). Similar results for T4 infection of these mutants have been observed by A. Kaji (*personal communication*). We have relatively little information on the synthesis of specific phage proteins. However, it is of interest that infected *tet* cultures incubated for several hours ultimately undergo partial lysis. Thus, even though the synthesis of phage lysozyme is subnormal (12), some expression of this late gene does occur. We have not yet investigated the synthesis of other specific late phage proteins.

RNA synthesis. When *tet* mutants were originally isolated, an attractive hypothesis to explain their inability to support phage replication was an alteration in the enzymatic machinery for transcription: perhaps an alteration in RNA polymerase which prevented the replacement of the host cell sigma factor by comparable phage factors (17, 18). If this were true, then one would expect to see a failure of infected *tet* cells to carry out the normal program of phage gene transcription (for review, see reference 13). We have used competitive DNA-RNA hybridization to try to detect this. To ask whether any abnormalities

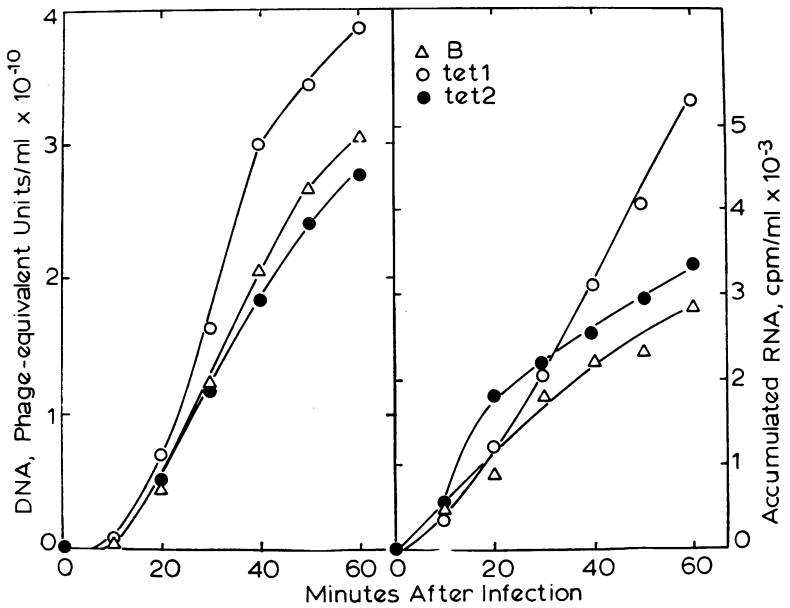

FIG. 12. *Accumulation of phage DNA and RNA. Bacterial cultures (20 ml) in glycerol-Casamino Acids medium were grown in the presence of 20 μg of uracil per ml, centrifuged, resuspended in fresh medium, and infected in the presence of* ^{14}*C-uracil, as described previously (10, 11). Removal of samples, determination of the radioactivity incorporated into DNA and RNA, and calculation of the radioactivity in DNA as phage-equivalent units of DNA per milliliter of culture were also as described previously (11). Note the different ordinates for the two panels.*

exist in transcription of early phage genes, we hybridized early pulse-labeled (2 to 5 min at 37 C) RNA from T6-infected *E. coli* B to T6 DNA in the presence of unlabeled early RNA species prepared from cultures of *E. coli* B, *tet* 1, and *tet* 2 harvested 5 min after infection by T6. As shown in Fig. 14, both the initial slopes and the final plateau values of all three competition curves are similar. Hence, we conclude that at 5 min infected *tet* cells contain the same messenger RNA (mRNA) species, and in the same relative abundance, as those present 5 min after infection of wild-type bacteria.

The ability of infected *tet* cultures to form mRNA transcripts normally synthesized late in infection was tested by using unlabeled 20-min RNA preparations as competitors against T6-B RNA labeled from 15 to 20 min. The left panel of Fig. 15 shows competition curves for unlabeled 5- and 20-min RNA. This merely confirms for T6 what has long been known for T2 and T4 (1, 8, 11), namely, that there are some mRNA species which are synthesized late but not early in infection. If the *tet* mutants were unable to carry out part or all of the transitions involved between early and late patterns of transcription, one might expect competition curves with late unlabeled *tet* RNA to resemble those seen with normal early RNA. However, as shown in Fig. 15, the competition curves for late infected *tet* RNA species are virtually identical to the corresponding curve for late T6-B RNA. Thus, within the limits of resolution of this technique, we can see no difference in the ability of infected *tet* cells to carry out the normal program of viral gene transcription. It is possible that one or a small number of critical late genes are not transcribed in infected *tet* cells, but no gross defects in transcription patterns exist.

DISCUSSION

The genetic alteration in *tet* mutants which prevents phage replication seems to affect a relatively late viral function in view of the large number of phage-directed processes which occur in infection of *tet* cells. These include adsorption, cell killing, arrest of host cell nucleic acid and protein synthesis, early changes in phospholipid metabolism (L. I. Pizer, *personal communication*), initiation of DNA replication, formation of rapidly sedimenting DNA intermediates, accumulation of newly synthesized nucleic acid and protein, formation of specific early enzymes, formation of early and late mRNA species in normal amounts, and partial lysis of infected cultures. Thus far, the chief distinctive properties of *tet* cells relative to wild-type include slightly slower growth rate in defined media (12), relative

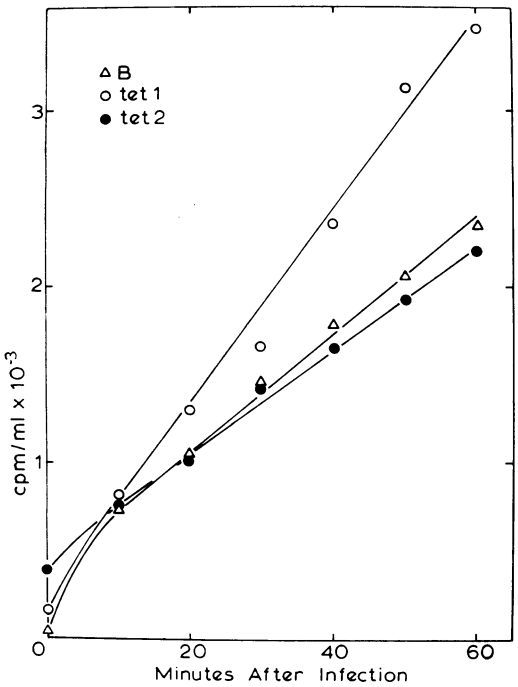

FIG. 13. *Incorporation of ^{14}C-leucine into protein after T6 infection. Bacteria were grown in M9 medium containing leucine, centrifuged, washed, resuspended in fresh medium, infected with T6, and labeled with ^{14}C-leucine as described previously (10). Determination of the amount of radioactivity incorporated into protein was also as described previously (10). The higher rate of incorporation seen in tet 1 relative to B was noted in three separate experiments.*

resistance to ultraviolet light (12), subnormal levels of both early and late enzymes (12), extensive endonucleolytic cleavage of intracellular DNA, high survival to infection in solid media, and, of course, failure of infective progeny phage to be formed under any conditions yet devised. An additional difference relates to the ability to adsorb T4. When the mutants were originally isolated, both *tet* 1 and *tet* 2 could adsorb T4, although more slowly and to a lesser extent than the adsorption of either T2 or T6. However, with increasing time of cultivation in the laboratory, both strains gradually lost the ability to efficiently adsorb T4, although T2 and T6 are both adsorbed at rates comparable to those seen with wild-type bacteria. Whether this is a result of the primary *tet* mutation or some secondary mutation we do not know. We are presently trying to isolate new *tet* derivatives capable of adsorbing T4.

We still have very little idea of the biochemical difference between wild-type and *tet* bacteria. A difference in membrane structure and function seems an attractive possibility, first, because so many phage-directed processes, including tail morphogenesis (16), occur at membrane attachment sites and, secondly, because bacterial cells with membrane defects can lose the ability to support T-even phage growth (2). We plan to analyze further intracellular phage DNA intermediates, since the extensive endonucleolytic cleavage of intracellular parental phage DNA may point towards a defect in DNA metabolism as the primary basis for explaining the *tet* phenotype. Finally, we plan to analyze the process of phage

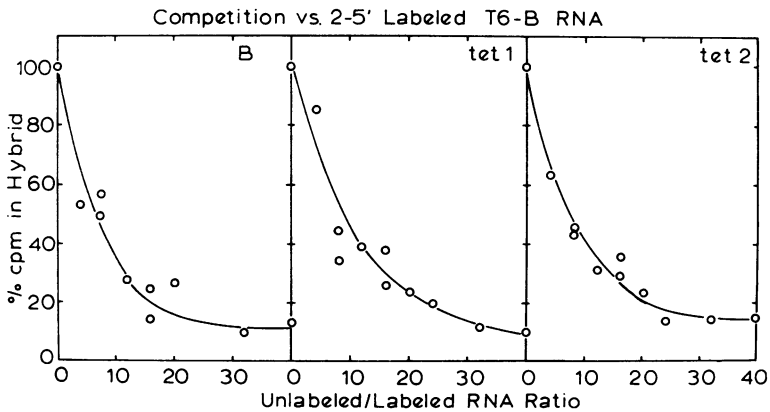

FIG. 14. *Competitive DNA-RNA hybridization: early RNA. Pulse-labeled RNA was prepared as described in the legend to Fig. 6, with labeling carried out from 2 to 5 min after infection of E. coli B with T6. Unlabeled RNA samples for competition curves were prepared from cells harvested at 5 min after infection of E. coli B, tet 1, or tet 2 with T6. Hybridization mixtures contained 5 μg each of T6 DNA immobilized on filters, either 2.5 or 5 μg each of labeled early T6-B RNA, and amounts of unlabeled RNA species sufficient to give the indicated ratios of competing (unlabeled) to labeled RNA. The figure contains combined data from two separate experiments.*

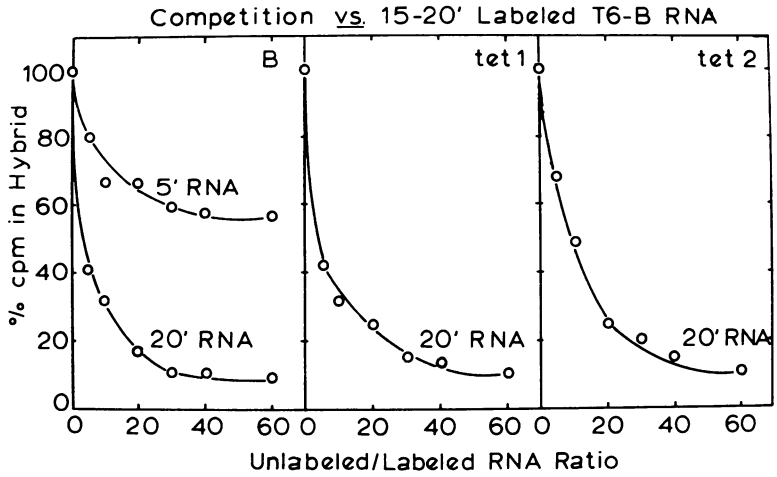

FIG. 15. *Competitive DNA-RNA hybridizations: late RNA. The experimental protocol was similar to that described in the legend to Fig. 14, except that labeled RNA was obtained in a period between 15 and 20 min after infection at 37 C, and unlabeled competitor RNA species were isolated from cells harvested at 20 min. A control hybridization with unlabeled 5-min T6-B RNA is also presented in the left panel. This figure presents combined data from two separate experiments.*

morphogenesis, since the high rates of phage nucleic acid and protein synthesis seen throughout, plus the normal operation of the transcription program, suggest that the *tet* phenotype, at least in liquid media, is expressed at a very late stage. At present, we have no idea why *tet* cells survive infection under some conditions but are efficiently killed under others. Further investigation of this point will be deferred until we have a better understanding of why these cells are so totally unable to form infective phage progeny.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service research grant AI-08230 from the National Institute of Allergy and Infectious Diseases and research grant 70 1013 from the American Heart Association.

We thank Mildred Ceizyk and Celta Lacambra for capable technical assistance.

LITERATURE CITED

1. Bolle, A., R. H. Epstein, W. Salser, and E. P. Geiduschek. 1968. Transcription during bacteriophage T4 development: synthesis and relative stability of early and late RNA. J. Mol. Biol. 31:325–348.
2. Cronan, J. E., Jr., and P. R. Vagelos. 1971. Abortive infection by phage T4 under conditions of defective host membrane lipid biosynthesis. Virology 43:412–421.
3. Denhardt, D. T. 1966. A membrane-filter technique for the detection of complementary DNA. Biochem. Biophys. Res. Commun. 23:641–646.
4. Duckworth, D. H., and M. J. Bessman. 1965. Assay for the killing properties of T2 bacteriophage and their "ghosts." J. Bacteriol. 90:724–728.
5. Earhart, C. F. 1970. The association of host and phage DNA with the membrane of *Escherichia coli*. Virology 42:429–436.
6. Earhart, C. F., G. Y. Tremblay, M. J. Daniels, and M. Schaechter. 1968. DNA replication studied by a new method for the isolation of cell membrane-DNA complexes. Cold Spring Harbor Symp. Quant. Biol. 33:707–710.
7. Frankel, F. R. 1968. DNA replication after T4 infection. Cold Spring Harbor Symp. Quant. Biol. 33:485–493.
8. Hall, B. D., A. P. Nygaard, and M. H. Green. 1964. Control of T2-specific RNA synthesis. J. Mol. Biol. 9:143–153.
9. Kennell, D. 1968. Inhibition of host protein synthesis during infection of *Escherichia coli* by bacteriophage T4. I. Continued synthesis of host RNA. J. Virol. 2:1262–1271.
10. Mathews, C. K. 1966. DNA metabolism and virus-induced enzyme synthesis in a thymine-requiring bacterium infected by a thymine-requiring bacteriophage. Biochemistry 5:2092–2100.
11. Mathews, C. K. 1968. Biochemistry of DNA-defective amber mutants of bacteriophage T4. I. RNA metabolism. J. Biol. Chem. 243:5610–5615.
12. Mathews, C. K. 1970. T-Even bacteriophage-tolerant mutants of *Escherichia coli* B. I. Isolation and preliminary characterization. J. Virol. 6:163–168.
13. Mathews, C. K. 1971. Bacteriophage biochemistry. American Chemical Society Monograph No. 166. Van Nostrand-Reinhold Books, New York.
14. Murray, R. E., and C. K. Mathews. 1969. Addition of nucleotides to parental DNA early in infection by bacteriophage T4. J. Mol. Biol. 44:233–248.
15. Murray, R. E., and C. K. Mathews. 1969. Biochemistry of DNA-defective amber mutants of bacteriophage T4. II. Intracellular DNA forms in infection by gene 44 mutants. J. Mol. Biol. 44:249–262.
16. Simon, L. D. 1969. The infection of *Escherichia coli* by T2 and T4 bacteriophages as seen in the electron microscope. III. Membrane-associated intracellular bacteriophages. Virology 38:285–296.
17. Travers, A. A. 1969. Bacteriophage sigma factor for RNA polymerase. Nature (London) 223:1107–1110.
18. Travers, A. A. 1970. Positive control of transcription by a bacteriophage sigma factor. Nature (London) 225:1009–1016.