Inhibition of Host Protein Synthesis During Infection of *Escherichia coli* by Bacteriophage T4

I. Continued Synthesis of Host Ribonucleic Acid

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The ribonucleic acid (RNA) synthesized at specified intervals during infection of *Escherichia coli* K-12 by bacteriophage T4 was hybridized to denatured *E. coli* or T4 deoxyribonucleic acids (DNA). The reactions were performed under conditions that maximized the yield and at RNA/DNA inputs such that excess DNA sites were available for all RNA species. Most of the RNA synthesized at any time during the first 3 min of infection was host-specific. The fraction declined rapidly as infection progressed; host RNA represented about half that made between 3 and 4 min. It is unlikely that this represented RNA synthesized by bacteria that had escaped infection, as judged by the kinetics of adsorption and killing as well as by the rapid inhibition of β-galactosidase induction after infection. The nature of the host RNA was also examined. Part of the RNA synthesized during infection of cells rendered sensitive to actinomycin was stable in the presence of this inhibitor. This RNA was essentially all host-specific and it sedimented as ribosomal and transfer RNA; most of the ribosomal RNA was incorporated into 30S and 50S ribosomes. Hybridization analyses suggested that unstable *E. coli* messenger RNA was also synthesized for several minutes after infection; the proportion of unstable to stable host RNA synthesized appeared to be similar in infected and uninfected cells. Thus, it is concluded that significant amounts of *E. coli* RNA are synthesized during the first minutes of T4 infection. Host messenger RNA initiated after infection may not be translated into enzymes; alternatively, it is conceivable that continued bacterial messenger RNA synthesis only reflects the completion of transcription of operons whose reading was initiated prior to infection.

Infection of *Escherichia coli* by certain viruses such as the T-even phages causes an immediate block in the induction of host-specific enzymes (4, 6, 24, 27, 31). This inhibition has generally been ascribed to a sudden decline in host nucleic acid synthesis (2, 7, 10, 28, 33). However, it will be shown in this paper that for several minutes after infection a large fraction of the ribonucleic acid (RNA) being synthesized is still host-specific, although this fraction decreases rapidly with time. The nature of this host RNA has been studied in order to identify the processes inhibited by phage infection. The results suggest that transfer RNA (tRNA), ribosomal RNA (rRNA), and messenger RNA (mRNA) are all synthesized at about the same rates relative to each other as they are in uninfected cells.

**Materials and Methods**

*Organisms and conditions for growth and infection.* *E. coli* K-12 was grown at 37°C with a mass doubling time of 55 min in an inorganic salts medium (17) plus 0.2% glucose (MMG). At the time of infection, L-tryptophan was present (MMTC) at about 10 μg/ml. To label with 32P, bacteria were grown in the presence of 8 × 10⁻⁴ M 32P-labeled, sodium potassium phosphate with citrate as the buffer (19).

Bacteriophage T4 B was provided by Subir Bose (St. Louis University Medical School). Ten volumes of exponentially growing bacteria (5 × 10⁸ cells/ml) were infected with phage contained in about one volume of growth medium to give a multiplicity of infection (MOI) of eight particles per bacterium. The latent period was about 27 min. Plaque assays were performed by the top agar technique (1) with *E. coli* B as the indicator strain. Phage-diluting fluid (PDF) contained, per liter: 3.0 g of NaCl, 1.0 g of peptone, and 0.15 g of MgSO₄. Actinomycin treatment. The cells were sensitized by the ethylenediaminetetraacetic acid (EDTA) treatment of Leive (23). The treated cells (5 × 10⁸/ml) were infected 1 min later. At indicated times after infection, actinomycin D (2 mg per ml of ethyl alcohol) was added to give a concentration of 20 μg/ml.
Under these conditions, the inhibition of RNA synthesis was not complete, especially when the inhibitor was added at late times during infection. However, when added as late as 13 min after infection, the inhibitor allowed only 3 to 4% as much uracil-5-hydration incorporation during the subsequent 20 min as occurred in its absence. However, the inhibition was not complete and this fact is important. When added after 19 min of infection, the inhibition was only about 50%. Leive (23) noted that there is measurable repair of the permeability properties of E. coli cultures by 20 min after EDTA treatment. Increasing the EDTA concentration to 10-4 M did increase the sensitivity somewhat at the late times of infection. However, in agreement with the observations made on uninfected cells (23), this more drastic treatment also decreased the incorporation of 3H-uracil to a significant extent during infection (no actinomycin).

Thus, the lower concentration of EDTA was used to preserve, to a greater extent, the normal metabolism of the infected cell.

Hybridization procedures. The reaction conditions have been described in detail (19). Calf thymus deoxyribonucleic acid (DNA) was used as a control in all experiments. To estimate the fraction of RNA that is specific to E. coli or T4, the following conditions are necessary. First, the efficiencies of hybridization reactions must be comparable for the two species with respect to such variables as concentration dependence or competition from DNA-DNA reannealing (19). Second, to hybridize a maximal fraction of the RNA, the input RNA/DNA ratio must be low enough so that there are excess DNA sites available for all homologous RNA species.

It was observed earlier that there is a very marked concentration dependence for the reaction of T4-specific RNA to T4 DNA (19), i.e., at a given RNA/DNA input, the fraction of RNA bound can be a function of the concentration of the reactants. This effect is more marked with the filter procedure (8) than when both DNA and RNA react in solution (19). When both nucleic acids were reacted in solution, the maximal fraction of T4 RNA that could be bound was observed within RNA labeled late in infection at an RNA/DNA input between 1 and 0.2; this value (65 to 70%) was also the maximal fraction bound when RNA from uninfected E. coli was reacted to its DNA under our conditions (16). At lower RNA/DNA inputs, the fraction bound declined due to competition from reannealing DNA, whereas at higher RNA/DNA inputs the fraction also became lower because of the saturation of DNA sites. With the filter procedure, the maximal fraction bound was about the same. Thus, with our conditions, the efficiencies of reaction for the E. coli and T4 nucleic acids were very similar.

Regarding excess DNA sites, the amount (μg) of T4-specific RNA present per microgram of cells was very small (less than 6%) at all times of infection; unpublished observations; the most abundant T4 RNA species saturated their DNA sites only at RNA/DNA inputs > about 0.1. In fact, as might be expected, this ratio was close to that at which the most abundant mRNA species present in uninfected E. coli saturate their E. coli DNA sites (16). However, very much lower RNA/DNA inputs (about 1:1,000) were required in order to provide excess E. coli DNA sites for all E. coli RNA species; the bulk of the RNA in the infected cell (micrograms/micrograms of cells) is rRNA and tRNA coded by a small fraction of the genome, and, as will be seen, these species were synthesized during infection.

Other procedures. The following procedures have been previously described in detail: β-galactosidase assay (20); preparation of RNA and DNA, the hybridization procedures, labeling of RNA (19); estimations of radioactivity in a scintillation spectrometer, normalization procedures to estimate losses of stable RNA during infection, and preparation of extracts and RNA for sucrose gradient centrifugation (17).

**RESULTS**

**Synchrony of infection and its initial effects on macromolecule synthesis.** In the next sections, evidence will be presented for the continued synthesis of host RNA for several minutes after infection by T4 bacteriophage. Obviously, this observation would be trivial if a significant fraction of the bacteria escaped infection during this time.

Various treatments have been used in the past to insure the simultaneous infection of all bacteria (1) before dilution with several volumes of growth medium to allow phage development; e.g., preadsorption in incomplete media (9) or in the presence of cyanide (5), or at 0 C. How-

![FIG. 1. Synchrony of infection. (A) One volume of MMTG (37°C) containing enough phage to give an MOI of 7.3 particles per bacterium was poured into ten volumes of rapidly swirling bacterial culture (37°C) at time 0. At the indicated times, 1-ml samples were mixed with 100 ml of PDF containing 10 ml of chloroform (0°C) to prevent further adsorption and to inactivate infective centers. Unadsorbed particles were estimated by plaque count. (B) Induction of β-galactosidase. Bacteria were infected as described above (△, □) or not infected (●). IPTG was added to a final concentration of 5 × 10-4 M at -30 sec (□) or at time 0 (●, △).](http://jvi.asm.org/)
ever, good synchrony can be achieved without interfering with cell metabolism.

When nine volumes of rapidly swirling culture (5 x 10^6 bacteria/ml) was infected with one volume of growth medium containing T4 to give an MOI of 7.3, 99% of the bacteria had irreversibly absorbed one or more virions within 1 min (Fig. 1A). When plated at 10 min after infection, <0.1% of the bacteria formed colonies; i.e., the killing was >99.9%.

An even more sensitive criterion of successful infection is its immediate effect on the synthesis of certain host enzymes such as β-galactosidase (4, 27). One can detect as little as 30 sec of pre-induced mRNA for the enzyme (12). This dramatic effect is shown in Fig. 1B, to demonstrate the immediate response when bacteria were infected by the procedure described above.

The net accumulation of labeled precursors in total nucleic acids, DNA, and protein during T4 infection is shown in Fig. 2. Infection did not cause an immediate cessation of host DNA synthesis; DNA synthesis continued at an almost normal rate for about 2 min before stopping and then resumed again at about 5 min after infection.

Protein synthesis, as measured by 

\[ ^{14}C-L-\text{valine}, \] 

\[ ^{14}C-L-\text{leucine,} \] 

or \[ ^{14}C-L-\text{arginine incorporation,} \] 

was close to that of uninfected cells for at least 5 min after infection, as has been observed in many studies (7). RNA synthesis was more difficult to estimate; \[ ^{14}C-\text{guanine or uracil-5-}\text{H} \] incorporation declined gradually from the rate in uninfected cells to a value close to 30% at 3 to 4 min. Rates of RNA synthesis relative to uninfected cells might be somewhat higher or lower as a result of changes in the specific activity of the nucleic acid precursor pool compounds.

By prelabeling during the 10-min period of exponential growth before infection, the nucleoside triphosphate pool reached a specific activity similar to the mRNA nucleoside. Under these conditions, incorporation of exogenous precursor would be proportional to total RNA synthesis except for changes either in the relative contribution of mRNA turnover to the pool or in the relative contributions to the nucleoside triphosphate from exogenous base compared to endogenous synthesis, e.g., as occurs upon prolonged Mg^{2+} starvation (18). The major change is probably in the contribution of mRNA turnover. Since virtually all of the phage-specific RNA is unstable, the fraction of synthesized RNA that is unstable must increase during the first minutes of infection. Therefore, relative to exponential growth, the actual rate of RNA synthesis during infection (micromoles of nucleotide polymerized per sec) is probably higher than that indicated by uracil incorporation; e.g., if the only RNA made is unstable RNA and all breakdown products are reutilized, then exogenous incorporation could be zero while the actual rate of RNA synthesis could be very high due to turnover (21).

In fact, if the mRNA of infected cells were translated at the same average efficiency as the mRNA of uninfected cells, RNA synthesis would be 60 or 70% of the normal rate at 3 to 4 min, rather than 30%, in order to account for equal rates of protein synthesis before and after infection.

**Host-specific RNA synthesis during T4 infection.** Figure 3 shows the partial titrations of the T4 and E. coli DNA by RNA pulse-labeled
postinfection (3H-labeled) and RNA labeled between 3 and 4 min postinfection (3H-labeled) to E. coli and T4 DNA. Bacteria were grown in the presence of 8 \times 10^{-4} \text{ M} inorganic \(^3\)P-phosphate for at least five generations before infection with phage in the same medium. \(^3\)H-uracil was added 3 min after infection and the culture was brought to 0°C at 4 min. Fraction of \(^3\)P-RNA input hybridized to E. coli DNA (■) and to T4 DNA (□). Fraction of \(^3\)H-RNA input hybridized to E. coli DNA (○), to T4 DNA (△), and to calf thymus DNA (▲).

with \(^3\)H-uracil between 3 and 4 min after infection as well as by the total \(^3\)P-labeled RNA present at 4 min. Almost all of the total RNA was E. coli-specific. More than 60% of the \(^3\)P-RNA hybridized to E. coli DNA; this value was close to the maximum achievable under these reaction conditions (16). In contrast, <1\% of the \(^3\)P-RNA hybridized to T4 DNA. Of the RNA synthesized between 3 and 4 min, about 20\% hybridized to E. coli DNA and 26\% to T4 DNA. To complex a maximal fraction of either the \(^3\)H or \(^3\)P radioactivity to E. coli DNA, it was necessary to react the fraction with RNA/DNA inputs of <1:1,000, in agreement with previous results (16). However, all sites of T4 DNA were in excess at any RNA/DNA input <1:10.

Also, the shapes of the titration curves for both \(^3\)P- and \(^3\)H-RNA to E. coli DNA were similar to those observed with RNA from uninfected cells (16): a steep slope for \(^3\)P-RNA and a shallow slope for \(^3\)H-RNA at RNA/DNA inputs >1:1,000. This suggested that both the stable and unstable RNA species of E. coli are synthesized between 3 and 4 min postinfection. Further evidence for this inference will be presented later.

The fraction of \(^3\)H-RNA that is E. coli-specific at this time can be estimated by three independent methods. (i) The maximal fractions of \(^3\)H-RNA hybridized to E. coli DNA compared to T4 DNA suggests that about 55\% of the RNA synthesized is T4-specific and 45\% is E. coli-specific. (ii) The ratio of \(^3\)H to \(^3\)P hybridized to E. coli DNA at RNA/DNA ratios <1:1,000 suggests that about 35\% of the RNA being synthesized is E. coli-specific. (iii) The \(^3\)H hybridized to T4 DNA (25\%) is about 40\% of the maximal fraction that can be hybridized from RNA labeled very late in infection. Thus, from these three independent comparisons, it is estimated

\[ \text{Fraction of newly synthesized RNA that is T4-specific at different times after infection of E. coli K-12 by phage T4. Hybridization analyses were similar to those shown in Fig. 3. The points are plotted for times that correspond to the mid-time of the pulse-labeling period, e.g., a 3- to 4-min pulse is plotted at 3.5 min.} \]

\[ \text{Fig. 4. Sedimentation (5 to 20\% sucrose) of RNA synthesized in the preceding period of exponential growth and then purified from the bacteria at certain times after T4 infection. Bacteria were grown in the presence of \(^{14}\)C-adenine (16 \text{ µg/ml}) for at least five generations before infection in the presence of \(^{14}\)C-adenine (100 \text{ µg/ml}). Cultures were brought to 0°C at 14 min (△) or 29 min (■). The zero-time culture (○) was exposed to excess \(^{14}\)C-adenine for 10 min without phage. Acid-precipitable counts/min recovered from fractions corrected for losses after gradient centrifugation in a SW 39 rotor (Spinco) for 7 hr at 4°C.} \]
that between 35 to 60% of the RNA being synthesized between 3 and 4 min after infection is E. coli-specific.

Similar analyses were made with RNA from bacteria labeled at different times during infection; the results are summarized in Fig. 4. Even though the resolution is most poor at either extreme (0 or 100%), it is clear that for the first few minutes of infection the bulk of the RNA being synthesized was E. coli-specific. However, during this period, the synthesis of some or possibly all host proteins was blocked.

Stability of preformed rRNA and tRNA during infection. Before considering characteristics of the RNA synthesized during infection, it should be noted that the 30S and 50S ribosomes, as well as the 16S and 23S rRNA and tRNA that were synthesized before infection, appeared to be completely stable during infection. Analyses of RNA prelabeled during exponential growth are shown in Fig. 5. After the appropriate normalizations, there was no observable loss of 4S, 16S, or 23S material.

Synthesis of stable E. coli RNA. The shape of the titration curve in Fig. 3 suggested that both stable and unstable E. coli RNA are synthesized after infection. More definitive evidence was sought for the formation of the stable species. Bacteria were pulse-labeled with 3H-uracil for 1 min during exponential growth or infection. The pulse-labeling period was terminated by the addition of a great excess (>1,000 fold) of unlabeled uracil in order to enrich stable components with label. During exponential growth, breakdown of mRNA was masked by the efficient reutilization of the products; however, during T4 infection, purine and pyrimidine products were only partially reutilized (Fig. 6), as is also true of RNA phosphorus (2).

The species specificity of the “chased” 3- to 4-min RNA was examined by use of hybridization titrations as shown in Fig. 3. The results are summarized in Table 1 (top two lines). All values were derived from fractions of RNA hybridized when all DNA sites were in excess. The chase actually increased the fraction of 3H-RNA that is T4-specific. At the same time, the level of E. coli RNA was somewhat lower after 20 min of chase than at 10 min.

In the presence of actinomycin, reutilization of breakdown products can be almost completely suppressed by blockage of all DNA-dependent RNA synthesis. Almost all labeled RNA remaining after a suitably long exposure to the drug would then be in a stable species. With the aid of this inhibitor, it could be seen that 30% of the RNA synthesized between 3 and 4 min was

![Graph](http://jvi.asm.org/Downloaded from http://jvi.asm.org)
stable (Fig. 7). Furthermore, with the accumulation of T4-specific RNA now prevented, the bulk of the stable RNA was seen to be E. coli-specific (line 4, Table 1).

These results suggested the following. During the chase, both T4 and E. coli $^3$H-mRNA are breaking down with most of the products reutilized. Since most of the RNA synthesized during the chase period is T4 RNA (Fig. 4), the fraction of $^3$H-RNA that is T4-specific actually increases from this turnover as well as from the slow incorporation of the remaining $^3$H from the acid-soluble pools, a process that requires about 10 min in uninfected cells (Fig. 6). Nonetheless, one would expect the fraction that is stable E. coli RNA to be somewhat >5% at the end of the chase. Apparently, some step in the formation of mature ribosomes is at least partially interrupted by infection and the rRNA of the incompletely particles is destroyed (32). However, when the normal process of infection is blocked by actinomycin, it remains stable.

Is any of the stable RNA T4-specific? Recently, Hsu et al. (11) reported evidence for the existence of a T4-specific tRNA. If stable T4 species exist, they must be an extremely small fraction of the T4 RNA labeled at this time. This is indicated by the results shown in Table 1 (lines 3 and 4); only a small fraction of the $^3$H-RNA could be bound to T4-DNA (<4% of the total). Even more significant, this fraction was the same whether actinomycin was present during the entire chase period or added later; i.e., there was no enrichment of T4-specific RNA during the 4- to 11-min period when almost all of the RNA made was T4-specific. The small but equal amount of T4 $^3$H-RNA present in the two cases undoubtedly represented the low level of RNA synthesized, even with inhibitor present. Thus, most, if not all, of the stable RNA is E. coli-specific. This does not exclude the possibility of an extremely small fraction of stable T4 RNA (certainly <1% of the T4 RNA labeled in this period).

A larger fraction of the $^3$H-RNA could form hybrids from the cells treated for 11 to 50 min with actinomycin than from those exposed from 4 to 50 min. This may reflect the breakdown of incomplete RNA polymers in cells treated with this inhibitor. Several years ago, evidence was presented which suggested that unfinished rRNA and mRNA, as well as finished mRNA molecules, break down in cells exposed to actinomycin (15); recently, Zimmermann and Levinthal (35) were forced to the same conclusion. The latter authors now estimate that a very large fraction of the 30-sec pulse-labeled RNA that decays in the presence of actinomycin D is composed of unfinished RNA molecules (Appendix of reference 35). In cultures that were "chased" (4 to 11 min) before the addition of actinomycin, only a very small fraction of the $^3$H-RNA was in the form of unfinished molecules on the DNA; therefore, very little of the $^3$H-RNA would be degraded to pieces large enough to be acid precipitable but at the same time too small to form a stable hybrid. With the inhibitor present during the entire chase (4 to 50 min), a larger fraction of $^3$H would be in unfinished RNA molecules which could be degraded to these sizes.

**Synthesis of rRNA and tRNA during infection.**

A large fraction of the stable E. coli RNA synthesized during T4 infection had the specificity of rRNA. When pulse-labeled bacteria were chased in the presence of actinomycin, 86% of the remaining $^3$H-RNA could be eliminated from hybrid formation by the presence of excess unlabeled rRNA (Table 2, lines 3 and 5). Are complete rRNA molecules synthesized during infection? Sucrose gradients of the RNA labeled after a 3- to 4-min postinfection pulse followed by a 10-min chase did not show a significant amount of labeled 16S or 23S RNA. Again, however, the results are misleading because about
95% of the label is in T4 RNA (Table 1). When the chase included actinomycin to prevent further rounds of RNA synthesis, the T4 RNA that had masked the presence of E. coli species was lost. Label could then be seen in completed 16S and 23S RNA as well as in 4S RNA (Fig. 8a). Even more significant, at least some of this RNA became a part of 30S and 50S ribosomal particles (Fig. 8b). In both cases, some labeled RNA sedimented at abnormal rates especially from cells labeled at very early times of infection. Presumably, this represented either unfinished or partially degraded rRNA molecules.

**E. coli mRNA synthesis during infection.** The shallow slope for the titration of pulse-labeled E. coli 3H-RNA (Fig. 3) suggested that it was composed of stable and unstable E. coli RNA (16). This conclusion was tested by hybridization-competition experiments. RNA, pulse-labeled at any of a number of times after infection (even as late as 18 min), still retained the hybridization characteristics of RNA made by uninfected bacteria; i.e., the fractions of unstable and stable host RNA being synthesized were comparable. In Table 2, the hybridization yields with or without rRNA competitor are shown for pulse-labeled RNA from bacteria before and after their exposure to actinomycin after pulse-labeling. 3H-RNA of very high specific activity was used for these experiments. When unlabeled rRNA competed with pulse-labeled RNA from uninfected cells, the hybrid yield was reduced to about 65% since approximately half of the label was in mRNA (16). In Table 2, competition with the pulse-labeled RNA preparations resulted in figures between 60% and 77%, indicating a composition similar to that in uninfected bacteria.

**DISCUSSION**

There is an abrupt block in the induction of certain host enzymes when E. coli is infected by bacteriophage T2 (12, 24, 31), T6 (6, 12), or T4 (this paper); there is also an abrupt block in the synthesis of constitutive β-galactosidase after T6 infection (13). Thus, it seems likely that synthesis of many, if not all, host proteins terminate soon after infection by the T-even phages. Therefore, the conclusion that host RNA synthesis, and mRNA synthesis in particular, continues for several minutes may be of interest with respect to the reactions involved in the coupling of transcription to translation. First, the evidence for synthesis of significant amounts of host RNA will be evaluated.

**Synthesis of E. coli RNA.** At 3 to 4 min after infection, rates of uracil and guanine incorporation were 30% of those just before infection. Half of the RNA synthesized at this time was
with both DNA and RNA in solution (12, 30), there is competition between reannealing DNA and the DNA/RNA hybridation at RNA/DNA inputs of less than about 10 (19). Even more important, to hybridize all molecules of the stable E. coli species, it is necessary to use RNA/DNA inputs that are very low (≤1:1,000).

However, in a recent study, Landy and Spiegelman (22) also reported that E. coli RNA is made at early times after T4 infection. They avoided the problem of reannealing DNA by use of the filter procedure (8), and estimated that 13 to 16% of the RNA labeled between 3 and 5 min after infection was E. coli-specific. This value is lower than that reported in this paper (50% for a 3- to 4-min labeling); however, the input RNA/DNA ratios came closer to providing sufficient E. coli DNA sites than had those in previous studies. Their three successive hybridizations with 20 μg of DNA and 0.5 μg of RNA (Table 3 in reference 22) corresponded to one hybridization at an RNA/DNA input of 1:120 or 0.0083. Of course, it makes no difference whether the reactions are performed with three filters in sequence or one filter once, since the critical variable is the total DNA reacted with the input RNA. As can be seen in the present paper (Fig. 3), at an RNA/DNA input of 0.0083, about half of the H-RNA and one-third of the ^32P-RNA that can be bound have sufficient DNA sites available. Thus, at this RNA/DNA input there is good agreement.

What host functions are inhibited? Soon after infection by T-even phages, the host chromosome loses its characteristic morphology (14, 26); loss of the functioning chromosomes could account for the arrest of RNA and protein synthesis (25). However, sedimentation analyses suggest that breakdown of the host chromosome probably occurs quite slowly (29, 34). Even after 15 min of infection, about half of the host DNA still sediments at a rate (78S) consistent with the largest size that could be extracted by very mild procedures (about 200 × 10^6 daltons; 34). Therefore, for several minutes after infection very large fragments of the host DNA are still intact and continue to serve as a template for transcription.

Host enzymes cannot be induced at any time during infection, but those induced just prior to infection continue to be made for a short time, presumably by "run-off" of the ribosomes from the mRNA, the synthesis of which had been initiated before infection (Fig. 1). This led Kaempfer and Magasanik to propose that initiation of host RNA synthesis is blocked by infection (12). In this paper, it has been shown that

### Table 2. RNA synthesized during T4-infection of E. coli

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<th>Culture conditions</th>
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<td>Pulse (min)</td>
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* Hybridization (with or without excess unlabeled rRNA) of RNA to E. coli DNA trapped to a filter at an approximate ratio of ^3H-RNA to DNA of 1:100. At this ratio, DNA sites for stable RNA are not in excess so that the fraction of RNA bound is not maximal. However, the DNA sites for all mRNA species are in great excess, so that the competition is only for rRNA sites even if some mRNA were present in the unlabeled rRNA.

* The time gives the minute after infection. The pulse of ^3H-uracil was terminated by the presence of excess ^3H-uracil, and the chase was terminated by bringing the culture to 0 °C.

* Unlabeled rRNA was present, where indicated (+), at a weight ratio to ^3H-RNA of 10:1.

E. coli-specific; this would indicate a rate of host RNA synthesis that is 15% of the normal rate. However, for reasons discussed above, this is a minimal estimate and RNA synthesis is probably closer to 35% of the normal rate at this time. If the rate of E. coli RNA synthesis decreased linearly from 100% at infection to 20% at 4 min, then there would have been more RNA made in that 4 min than is made in 2 min of exponential growth. This is also a minimal estimate, since the fraction that is E. coli-specific appeared to decrease even more slowly with time of infection (Fig. 4).

Since previous studies also used hybridization to detect host-specific RNA, it can be asked why so little of this RNA was observed. Without examining each case in detail, it is not possible to exclude strain differences as a factor; e.g., T2 infection may result in more rapid inhibition than does T4 infection. However, the technical aspects of the hybridization conditions used could account for the lower values. For example,
host RNA synthesis continues at a decreasing rate for several minutes after infection. This synthesis apparently includes not only the synthesis of 4S, 16S, and 23S stable RNA but also that of host mRNA. It is possible that this mRNA synthesis involves only the completion of molecules initiated before infection. A large fraction of E. coli mRNA may be made as large polycistronic units that require several minutes to transcribe completely; the cistrons distal to the operator would be transcribed and translated several minutes after initiation of transcription of the operon. It has been estimated that in E. coli, growing at a similar rate, transcription of the five contiguous genes of the prophage operon requires about 6.5 min (3). In this paper, I estimate that host mRNA synthesis is 15 to 30% of normal between 3 to 4 min after infection. It remains to be shown whether such a large fraction of the E. coli mRNA requires more than 3 min to transcribe completely.

Alternatively, the initiation of transcription may occur during infection but the resulting mRNA molecules are not translated into active enzymes. Several subsequent steps could be affected. These include: (i) the association of one or more ribosomes to the host mRNA, (ii) the translation of host mRNA into peptides, and (iii) the formation of active enzymes from component peptides. Further experiments aimed at identifying the processes inhibited by phage infection are in progress.

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LITERATURE CITED


