Synthesis of Soluble Ribonucleates in *Escherichia coli* Infected with Bacteriophage R17

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Received for publication 23 October 1967

Measurements were made on the level of synthesis of soluble ribonucleates (sRNA) in *Escherichia coli* K-12 infected with the ribonucleic acid (RNA)-containing bacteriophage R17. Little or no decrease in sRNA synthesis was found to occur under conditions of infection which result in a 70 to 80% inhibition of ribosomal RNA synthesis.

It was shown in previous communications that infection of *Escherichia coli* with phage R17 results in a marked inhibition of ribosomal ribonucleic acid (rRNA) synthesis (7), and that this inhibition is paralleled by a loss of ribosome biosynthetic capacity (3). Basic to the understanding of the mechanism by which phage R17 causes this inhibition of rRNA synthesis is the question of whether the inhibition is specific for the synthesis of rRNA, or whether it is a more general phenomenon involving other forms of cellular ribonucleic acid (RNA) such as messenger RNA (mRNA) and the soluble ribonucleates (sRNA).

The present study attempts to answer this question in part by examining the level of synthesis of sRNA in phage R17-infected cells. The results demonstrate that the synthesis of sRNA continues in the infected cell at a level equal to or only slightly less than that in uninfected cells, indicating that phage R17 infection does not give rise to a generalized blockage of host-specific RNA synthesis.

MATERIALS AND METHODS

Bacteria and bacteriophage. Bacteriophage R17 and its host bacterium *E. coli* K-12, Hfr-1, have been previously described (15). The tris(hydroxymethyl)-aminomethane (Tris)-maleate synthetic growth medium (TMM) used was as described by Paranchych (14).

Measurement of acid-insoluble and alkali-stable nucleates. The incorporation of radioactive isotopes into acid-insoluble nucleates was determined by the filter paper disc method of Mans and Novelli (10). Alkali-stable acid-insoluble nucleates were measured as previously described (7).

Labeling and extraction of sRNA. A culture of *E.*

coli was grown in TMM to a density of 2×10^8 to 3×10^8 bacteria/ml and divided into two portions, one of which was infected with phage R17 (about 20 plaque-forming units per bacterium). After a further 15 min of growth, both cultures were incubated with the radioactive isotope for an additional 15 min, followed by rapid chilling in an ethyl alcohol-dry ice bath. The bacteria were immediately harvested by centrifugation, washed once in 0.01 M Tris (pH 7.3) containing 0.01 M MgCl₂, and resuspended in 1.0 ml of the same buffer. The cold bacterial suspension was then mixed with 1.0 ml of cold buffer-saturated phenol for 3 min on a vortex mixer to extract sRNA (1). Additional mixing did not liberate additional labeled material. The aqueous phase was recovered after centrifugation of the mixture and was extracted three times with ether to remove residual phenol. Excess ether was removed by bubbling nitrogen gas through the solution, and the mixture was then chromatographed on a 2.0 by 15 cm methylated albumin-kieselguhr column (9). The peak sRNA fractions from this column were pooled and used for specific radioactivity determinations or hybridization experiments.

Labeling and extraction of high-molecular-weight RNA. A culture of *E. coli* was grown, infected, and labeled as described in the previous section. After concentrating the culture by centrifugation, the bacterial pellet was resuspended and extracted for RNA as described previously (7). The RNA was then fractionated by sucrose density gradient centrifugation, and the fractions sedimenting in the 23S and 16S regions were pooled and assayed for specific radioactivity.

DNA-sRNA hybridization. The purification of *E. coli* DNA and its adsorption to nitrocellulose filters were carried out by the procedures of Gillespie and Spiegelman (5). The filters, containing 90 µg of denatured *E. coli* deoxyribonucleic acid (DNA), were incubated in scintillation vials with various concentrations of purified sRNA in 1.0 ml of hybridization buffer (0.01 M Tris, pH 7.3, containing 0.5 M NaCl and 10^{-3} M ethylenediaminetetraacetic acid) at 67°C for 10 hr. The vials were then cooled to 30°C and

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the NaCl concentration was reduced to 0.3 M. To the solutions were added (per ml) 20 μ g of pancreatic ribonuclease and 5 units of T1 ribonuclease (Sankyo), followed by incubation at 30 C for 60 min. The filters were then washed with hybridization buffer containing 0.3 M NaCl, dried, and assayed for radioactivity. The inclusion of T1 ribonuclease (13) was found to reduce significantly the level of apparent hybrid detected. This level was not decreased further by a 10-fold higher concentration of T1 ribonuclease, nor by prolonged incubation (up to 2 hr) at 30 C. Under the conditions described, it was found unnecessary to wash the filters before the ribonuclease treatment.

Radioactivity measurements. The liquid scintillation fluid used contained 6 g of 2,5-diphenyloxazole and 0.5 g of 1,4-bis-2-(5-phenyloxazolyl)-benzene per liter of toluene. Radioactive counting was carried out in a Beckman model LS100 scintillation spectrometer.

RESULTS

Purity of sRNA. In principle, the relative synthesis of sRNA in uninfected and infected cultures can be compared by labeling with a suitable RNA precursor and measuring the specific radioactivities of the purified sRNA samples. Such an approach, however, is dependent upon the complete assurance that the final sRNA product is totally devoid of contaminant ribonucleates, such as degradation products from mRNA, rRNA, or phage-specific RNA.

To test the purity of the sRNA being used in the present investigation, samples of sRNA from uninfected and infected cultures were prepared, purified, and then hybridized to *E. coli* DNA (Fig. 1). If the preparation from the infected culture contained any phage-specific RNA, then its hybridization capacity (defined as fraction

of input RNA hybridized per microgram of DNA) would be less than that for the corresponding uninfected sRNA preparation. It may be seen in Fig. 1 that the two preparations contained approximately equal proportions of host-specific sRNA, thus showing no contamination from phage-specific RNA. Furthermore, since the hybridization curves showed saturation of binding sites at about 0.04% of the DNA, a value which is in close agreement with published values for *E. coli* sRNA (4, 6, 12), there could be no significant contamination by degraded host cell high-molecular-weight ribonucleates, since such contamination would lead to greatly increased values for saturation of DNA-binding sites.

It was thus concluded that the method being used for purification of sRNA was adequate to allow the comparison of specific radioactivities as a means of determining the relative amounts of sRNA synthesis in infected and uninfected cells.

Specific radioactivities of sRNA from uninfected and R17-infected *E. coli*. In comparing the specific radioactivities of purified sRNA samples from infected and uninfected cultures, certain factors which can influence the rate of incorporation of a particular precursor into RNA must be considered. For example, the overall base composition of the RNA synthesized in the two cultures may be sufficiently different to alter the rate of incorporation into RNA. Also, infection may change the pool size of certain RNA precursors, again giving rise to an altered rate of incorporation into RNA. Moreover, infection may change the rate of turnover of end groups of RNA molecules so that one no longer measures relative de novo synthesis. Finally, a small fraction of RNA precursor is incorporated into DNA, the synthesis of which decreases after R17 infection (15).

In view of these possibilities, it was decided to compare the relative levels of sRNA synthesis by using several different RNA precursors. Infected and uninfected cultures were therefore labeled with ^{32}P , ^3H -uracil, or ^{14}C -guanine, after which the sRNA was extracted and purified. The specific radioactivity of the sRNA samples was determined, and the resulting ratios of infected to uninfected samples were calculated (Table 1).

When ^{32}P or ^3H -uracil was the RNA precursor used, no significant difference between sRNA synthesis in infected and uninfected cells was evident. The use of a guanine label, however, gave rise to an apparent phage-induced decrease in sRNA synthesis of approximately 30%.

The possibility thus arose that the decrease in guanine incorporation represents a real decrease

FIG. 1. Hybridization of *Escherichia coli* DNA with sRNA from uninfected and R17-infected cells. The cultures were labeled with ^{14}C -guanine (0.5 $\mu\text{C}/\text{ml}$; 3×10^{-5} M) between 15 and 30 min after infection. Symbols: ●, sRNA from uninfected culture; ○, sRNA from R17-infected culture.

in de novo synthesis of sRNA, whereas the use of ^{32}P and ^3H -uracil as precursors reflects both de novo synthesis and an increased turnover of sRNA end groups. Alternatively, the reduced incorporation of guanine may reflect a reduction of guanine incorporation into all the RNA synthesized in the cell.

To resolve this question, a comparison was made of the overall rates of incorporation of radioactive guanine and uracil into acid-insoluble nucleates of infected and uninfected cultures. The rate of incorporation of uracil was only slightly affected by phage R17 infection, whereas guanine incorporation was approximately 20 to 25% lower in the infected culture (Fig. 2).

These results were taken to mean that phage infection causes a general decrease in the rate of guanine incorporation into most or all ribonucleates of the cell, rather than a specific inhibition of sRNA synthesis. Further support for this contention was obtained by determining the specific radioactivity of ^{14}C -guanine-labeled high-molecular-weight RNA. The specific radioactivity of this RNA, sedimenting in the 23S and 16S regions of a sucrose density gradient, was found to be about 20% less in infected cultures than in the control.

It is clear, therefore, that phage R17 infection causes a general lowering of the rate of incorporation of ^{14}C -guanine into RNA. This can be explained at least partly by the overall change in base composition accompanying the replacement of rRNA synthesis by R17-RNA synthesis, the latter RNA possessing a lower guanine content (11, 15). It is not clear, however, why the sRNA should reflect this same decrease in guanine incorporation. Another possibility is that infection may give rise to an increase in the amount of guanosine triphosphate (GTP) in the cell, thus

FIG. 2. Incorporation of ^3H -uracil and ^{14}C -guanine by uninfected and phage R17-infected cells. Log-phase cultures of Hfr-1 (2×10^8 bacteria/ml) were treated with either labeled uracil or guanine and then were split into two equal portions. One portion was then infected with phage R17 at a multiplicity of infection of 20 plaque-forming units per bacterium, and the cultures were allowed to continue growth. Samples (0.1 ml) were periodically withdrawn from each portion for measurement of acid-insoluble radioactivity. Isotope concentrations used: ^3H -uracil, $0.5 \mu\text{C}/\text{ml}$, $5 \times 10^{-5} \text{ M}$; ^{14}C -guanine, $0.1 \mu\text{C}/\text{ml}$, $3 \times 10^{-5} \text{ M}$. Symbols: $\bullet$, uninfected culture; $\circ$, R17-infected culture.

causing a dilution of the ^{14}C being used. Whatever the reason, however, it is evident that phage infection does not affect sRNA synthesis to a degree comparable to its effect on rRNA synthesis.

DISCUSSION

These results show that phage R17 causes little or no decrease in the level of sRNA synthesis. Under the same conditions, a 70 to 80% decrease in rRNA synthesis occurs (7). Although the use of ^{32}P or ^3H -uracil indicated no significant decrease in sRNA synthesis, guanine incorporation into sRNA did decrease by about 30% after infection. According to the arguments outlined earlier, this decrease in guanine incorporation could reflect a change in the GTP pool size of the cell or a change in sRNA base composition. Alternatively, it could represent a real decrease in de novo synthesis, whereas the other

TABLE 1. Ratio of specific radioactivities of sRNA from uninfected and phage R17-infected cultures^a

Expt	Radioisotope used	Ratio of specific activity of sRNA (infected/uninfected)
1	^{32}P	1.05
2	^3H -uracil	0.97
3	^3H -uracil	1.17
4	^{14}C -guanine	0.61
5	^{14}C -guanine	0.75

^a The sRNA was labeled and purified. Isotope concentrations used: ^{32}P , $10 \mu\text{C}/\text{ml}$, 10^{-3} M ; ^3H -uracil, $0.5 \mu\text{C}/\text{ml}$, $5 \times 10^{-5} \text{ M}$; ^{14}C -guanine, $0.5 \mu\text{C}/\text{ml}$, $1.5 \times 10^{-5} \text{ M}$.

isotopes measured an increased turnover of end groups. Cannon (2) and Rosset and Monier (17) have shown that the terminal adenosine common to most of the sRNA population turns over at the rate of about 20% per generation. Thus, it is conceivable that about 30% of the ^{32}P administered to the infected cultures was incorporated into the end groups, while the de novo synthesis decreased by this amount. In addition, since a large fraction of the uracil is converted intracellularly to cytosine (8), this isotope could be partly turning over as the subterminal cytosine groups. In this connection, however, it should be mentioned that Rosset and Monier (16) failed to detect turnover of the subterminal cytosine in exponentially growing yeast. Thus, it cannot be stated with certainty whether there is a small decrease in sRNA synthesis or whether there is no decrease at all. It is evident, however, that phage R17 infection does cause markedly different effects on the synthesis of rRNA and sRNA. This poses an interesting problem with regard to the regulatory mechanisms involved.

The evidence assembled to date appears to rule out a direct interaction of the phage RNA with one of the components of the host transcription complex (J. B. Hudson, Ph.D. Thesis, Univ. of Alberta, Edmonton, Alberta, Canada). Furthermore, if a phage-induced inhibitor is produced, then it must be endowed with the ability to inhibit the synthesis of two species of RNA (23S and 16S rRNA) while permitting the synthesis of numerous species of sRNA.

This consideration also argues against a direct competition between host and phage genomes for a common intermediate or precursors involved in RNA synthesis, unless rRNA and sRNA are regulated in a different manner in the cell.

One possible scheme which could explain the results derives from the hypothesis of Stent (18), whereby the synthesis of mRNA and rRNA is regulated by a negative feedback mechanism, the processes of translation and transcription being coupled. According to this scheme, if ribosomes are no longer available to effect release of nascent rRNA from the template (e.g., by virtue of binding to phage ribonucleates), then further synthesis of rRNA ceases. On the other hand, if sRNA continues to be utilized (for phage protein synthesis) and degraded, then further synthesis of sRNA will continue.

ACKNOWLEDGMENTS

This investigation was supported by grants from the Medical Research Council of Canada (MT-1610) and the National Cancer Institute of Canada.

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