Ribosomal Ribonucleic Acid Maturation and Synthesis after Ribonucleic Acid Bacteriophage Infection†

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ABSTRACT: The effect of RNA bacteriophage infection upon synthesis and maturation of ribosomal RNA precursors was studied. The synthesis and turnover of the 16S rRNA precursor is similar in infected and uninfected *Escherichia coli* D-10. Only one-third of this precursor, however, matures in the infected cell, and the remainder appears to be degraded. In RNA bacteriophage infected *E. coli* D-10 the RNA thought to be the 23S rRNA precursor is synthesized at a slightly slower rate than in the uninfected cell and does not turn over. MS2 infection of *E. coli* MRE 600, in contrast to infection of *E. coli* D-10, causes a 90% reduction in the incorporation of label into ribosomal RNA precursors.

Ribonucleic acid bacteriophage infection causes a decrease in ribosomal RNA synthesis (Hudson and Paranchych, 1967; Watanabe *et al.*, 1968; Spangler and Iglewski, 1972). In all these studies, infected cells were given a pulse of label, and the amount of ribosomal RNA made was determined after a chase. Thus, these studies did not distinguish between inhibition of rRNA synthesis and inhibition of rRNA maturation.

In this work it is shown that in MS2-infected *Escherichia coli* D-10 rRNA precursors are synthesized normally, but are not matured normally. The fate of the different ribosomal RNAs in MS2-infected *E. coli* D-10 was followed to determine what could be learned about rRNA maturation. These studies show that the block in maturation results in the degradation of the 16S rRNA precursor. The behavior of the 23S rRNA precursor suggests the presence of another rRNA precursor which has not yet been separated or identified.

In another host, *E. coli* MRE 600, MS2 infection causes a 90% reduction of the incorporation of label into rRNA precursors. Therefore, whether MS2 infection alters synthesis or only maturation of rRNA depends upon the host. This suggests a possible relationship between rRNA maturation and synthesis.

Experimental Procedures

Bacteria and Bacteriophage. MS2 was originally obtained from Alvin Clark. *E. coli* D-10 (Hfr, met$^-$, Sm^s, rel$^-$, RNase I$^-$) was derived from AB301 by Dr J. A. Steitz and was cured of λ by Dr Raymond Devoret. AB301 is a derivative of the Lederberg strain W 6, the bio$^+$, met B$^-$, rel$^-$ derivative of 58–161 (Alföldi *et al.*, 1962). *E. coli*, MRE 600 (F$^+$, RNase I$^-$, rel$^+$) was isolated by H. E. Wade and came from NTCC 8164.

Media. Modified TPG and 0.15% Casamino acids have been described previously (Haywood *et al.*, 1969).

Chemicals. 5-[^{3}H]Uracil, 25.4 Ci/mmol, and 2-[^{14}C]uracil, 54.9 mCi/mmol, were obtained from Schwarz Bioresearch, Inc. Deoxyribonuclease I (electrophoretically purified) was obtained from Mann Research Laboratories.

Prelabeling of Stable RNA. One doubling time prior to infection [^{3}H]uracil, 20–50 nCi/ml and 1–2.4 ng/ml, was added to the culture. At these low concentrations uracil is completely incorporated into ribosomal RNA in less than half a doubling time. The ^{3}H was used to check the yields of RNA isolated, so that the RNA put on the gels represented equal number of cells (7×10^8) for every gel. Control experiments showed that ^{3}H at the levels used could not be seen on the radioautographs.

Lysis and Extraction of the RNA. Harvesting and lysis of the cells have been described (Haywood, 1971). Ten minutes after the addition of 10 μg/ml of DNase, 0.1 vol of 5% sodium dodecyl sulfate was added, and the RNA extracted at 0–4° by the method of Kirby (1965). The material at the phenol–water interface was reextracted with 0.1 M Tris, pH 7.6, and 0.5% sodium dodecyl sulfate, and added to the aqueous phase prior to the second phenol treatment.

Polyacrylamide Gel Electrophoresis of the RNA. The acrylamide gels were made as described by Summers (1969) except that they were chilled for 30 sec in a beaker of ice water immediately after pouring. Buffer G (0.04 M Tris base, 0.02 M sodium acetate, 0.002 M EDTA, 0.2% sodium dodecyl sulfate, and enough glacial acetic acid to bring the pH to 7.5) (Summers, 1969) was used. All electrophoresis was carried out at room temperature. The gels were sliced longitudinally (Fairbanks *et al.*, 1965). To determine the position of the 23S and 16S rRNA bands, one slice was fixed in 1 M acetic acid, stained with 0.2% Methylene Blue in 0.2 M acetic acid and 0.2 M sodium acetate, and destained by leaving it overnight in a nylon mesh bag suspended in a beaker of water with a magnetic stirrer. The radioautographs were made with Kodak single emulsion blue sensitive medical X-ray film. The radioactivity in each band was determined by the method described by Adesnik and Levinthal (1969). The radioautographs were scanned with a Joyce Loebl recording microdensitometer. Several exposures of a single gel were made when it was necessary to get all the bands into a range where the response of the film was linear. The paper under the microdensitometer tracing of each band was cut out and weighed on an analytical balance. This weight was corrected according to the time of exposure, *e.g.*, in Figures 2 and 3 multiplied by the ratio of the days exposed to 2 days since most of the bands were exposed 2 days. The radioactivity was then expressed as "arbitrary units" (Adesnik and Levinthal, 1969) which is the corrected

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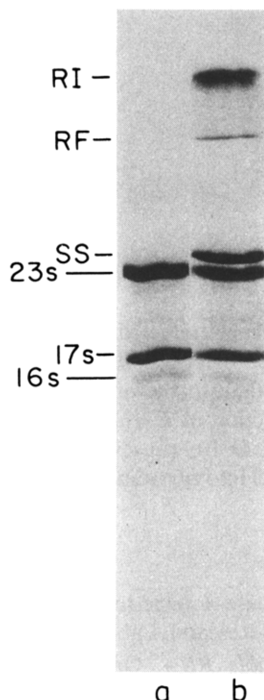

FIGURE 1: Radioautographs of gels containing RNA from MS2-infected and uninfected *E. coli* D-10. A culture of *E. coli* D-10 at a cell concentration of 1×10^8/ml was divided in half, and one-half was infected with MS2 at a multiplicity of 10. Twenty minutes after infection 0.2 μCi/ml and 0.4 μg/ml of [^{14}C]uracil were added to both the infected and uninfected cultures. Two minutes after the addition of label, the cultures were harvested. The RNA was isolated and subjected to electrophoresis on 2.4% acrylamide–0.5% agarose gels at room temperature for 3 hr at 5 mA/gel and 5 V/cm. The position of mature 23S and 16S RNA was determined by staining with Methylene Blue. The film was exposed 3 days: RI, MS2 replicative intermediate; RF-MS2 replicative form; ss-MS2 single-stranded viral RNA; a, uninfected; b, infected.

weight of the tracing. An arbitrary unit in Figures 2 and 3 is is nearly 62,000 dpm of ^{14}C, and in Figure 4, where the gels were exposed ten times as long, is a little over 6,200 dpm of ^{14}C.

Results

Within 20 min after infection the effect of RNA bacteriophage infection upon rRNA is established (Hudson and Paranchych, 1967; Watanabe *et al.*, 1968). Furthermore, after 16 min host RNA synthesis is no longer required for MS2 production (Haywood and Harris, 1966). At this time there is active MS2 RNA and protein synthesis, and some intracellular virus has been made. If 20 min after infection *E. coli* D-10 are labeled for 2 min with [^{14}C]uracil, the amount of label incorporated into 17S precursor rRNA is nearly equal in infected and uninfected cultures (Figure 1).

To determine the fate of the rRNA precursor in MS2-infected *E. coli* D-10, one-half of a culture of *E. coli* was infected with MS2 at a multiplicity of 10, and 22 min after infection equal amounts of [^{14}C]uracil were added to the infected and uninfected cultures. After a 2-min pulse, aliquots were removed, a 900-fold excess of unlabeled uracil was added, and further aliquots were removed after an additional 3, 7, and 14 min. Samples (0.1 ml) from the pulse and 3-, 7-, and 14-min chase aliquots showed 4.9, 6.8, 8.9, and 8.3 $\times 10^3$ Cl_3CCOOH precipitable counts, respectively, in the uninfected culture and 6.7, 8.9, 9.8, and 10.9 $\times 10^3$ Cl_3CCOOH precipitable counts, respectively, in the infected culture. Thus, there was continued uptake after the chase as a result of the residual label in the uracil pools, but there appears to be no gross difference between infected and uninfected cells in subsequent uptake from this source. The RNA from the infected and uninfected cultures was extracted and subjected to electrophoresis on acrylamide gels of several concentrations.

Figure 2 shows the results of the calculations from the densitometer tracings of the 16S and 17S RNA which had been subjected to electrophoresis on 2.4% acrylamide–0.5% agarose gels. Figure 2a shows the amount of 17S precursor rRNA labeled after a 2-min pulse of radioactivity is nearly equal in both cultures, and that after the chase it levels off and then drops. Figure 2b shows that the 16S RNA made in the infected culture is about one-third that made in the uninfected culture. Since in the infected cell the amount of 17S RNA decreases without a concomitant increase in the 16S RNA, the 16S RNA appears to be degraded.

Kinetic data imply that RNA which has a slightly lower mobility than 23S RNA on 3–4% gels is a precursor to 23S RNA (Adesnik and Levinthal, 1969). It has been suggested that maturation of this 23S RNA precursor involves only alterations in secondary and tertiary structure (Dahlberg and Peacock, 1971). The RNA from the cultures described above was subjected to electrophoresis on 3.7% acrylamide–0.25% agarose gels. Figure 3a shows the amount of an RNA migrating a little slower than 23S RNA in the infected and uninfected cultures. The amount of 23S RNA precursor RNA is slightly decreased in the infected cell but the most striking difference between the two cultures is that the amount of RNA levels off in the infected culture shortly after the addition of excess unlabeled uracil, whereas the amount in the uninfected culture decreases, presumably the result of maturation. The amount of mature RNA could not be measured in the infected culture because single-stranded MS2 RNA migrates with mature 23S RNA at gel concentrations which separate precursor 23S RNA from mature RNA. Therefore, the total amount of 23S RNA (mature and precursor) was measured on the 2.4% acrylamide–0.5% agarose gels. At the end of the experiment the amount of total 23S RNA made in the infected cell is just a little more than one-third that made in the uninfected cell. As can be seen a large fraction of the total 23S RNA in the infected cell is 23S precursor. Since in the infected cell the amount of 23S RNA precursor labeled after 2 min is only slightly decreased and since the 23S RNA pre-precursor RNA does not appear to be degraded, it is hard to account for the decrease in total 23S RNA. This suggests there may be another 23S RNA precursor which has not been separated or identified.

In this and previous work (Haywood, 1971) special care was taken to reextract the membrane-bound RNA at the phenol–water interface. 30S and 35S membrane-bound RNAs have been described (Haywood, 1971). Their nature is unknown but they fit the criteria which would be expected for the large ribosomal RNA precursor postulated recently (Jacobson, 1971; Doolittle and Pace, 1971; Kossman *et al.*, 1971). Autoradiography for 3 weeks showed that the 30S RNA is really two RNA bands migrating close together, but only the slower of the two bands appears in the infected culture. Inspection showed the slow 30S band appears to behave very much like the 23S precursor RNA in that, after the addition of cold uracil, it remained apparently unchanged in the infected cell, but it (and the faster 30S band) decreased by 12 min to barely visible in the uninfected cells.

To investigate the fate of 5S RNA in MS2-infected *E. coli*,

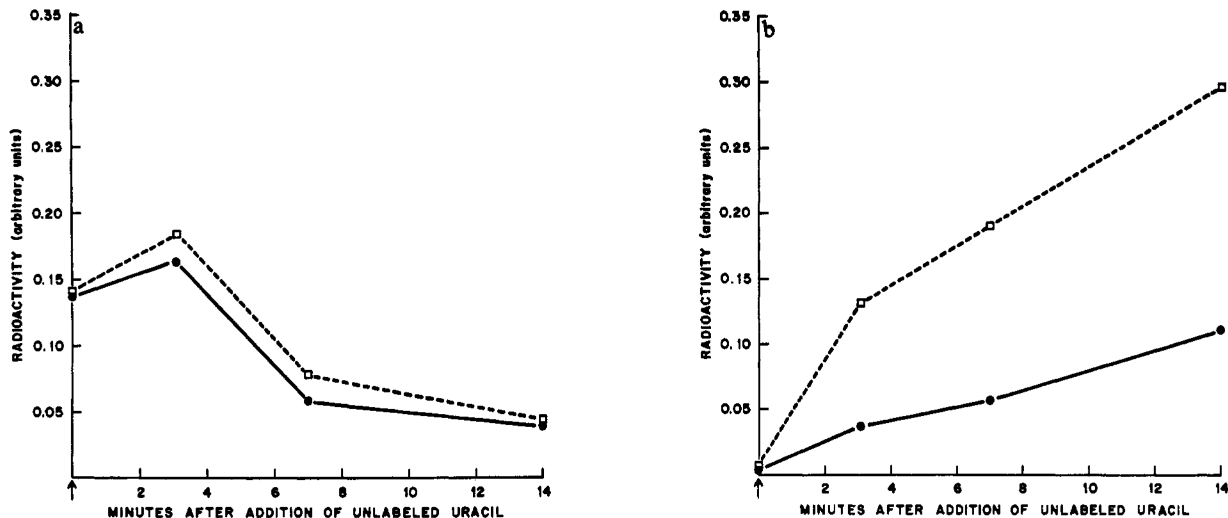

FIGURE 2: 17S and 16S RNA from MS2-infected and uninfected *E. coli* D-10. A culture of *E. coli* D-10 was grown to 1 × 10^8 cells/ml, and half was infected with MS2 at a multiplicity of 10. Twenty-two minutes after infection 0.14 μCi/ml and 0.27 μg/ml of [^{14}C]uracil were added to both the infected and uninfected cultures. After 2 min, an aliquot was harvested from each culture, and 250 μg/ml of unlabeled uracil was added. At 3, 7, and 14 min after the addition of unlabeled uracil, further aliquots were harvested. The RNA was extracted and subjected to electrophoresis at room temperature on 2.4% acrylamide–0.5% agarose gels for 3 hr at 5 mA/gel and 5 V/cm. The radioactivity in each band was calculated from radioautographs as described under Experimental Procedures: (a) 17S RNA; (b) 16S RNA; (□) uninfected; (●) infected.

RNA was subjected to electrophoresis on 9.1% gels. About one-third as much 5S RNA is made in the infected cell as in the uninfected (Figure 4a) which corresponds well to the decrease in the amount of 16S RNA made. The same 9.1% gels show the amount of label incorporated into 4S RNA is decreased in infected *E. coli* D-10 (Figure 4b). Since a large fraction of uracil is converted intracellularly to cytosine, this kind of experiment does not differentiate between labeling of the tRNA CCA end groups and synthesis of tRNA. Hudson and Paranchych (1967), using very different methods of tRNA isolation and identification, concluded there was little or no decrease in tRNA synthesis in R17 infected cells despite a notable inhibition of ribosomal RNA synthesis. The differences in methodology may account for the different results, and more work is needed to resolve the question. Degradation of 16S RNA in the infected culture might result in small segments that would be seen as additional bands on the 9.1% gels. No such bands were seen.

In an experiment similar to that described in Figure 1, infected and uninfected *E. coli* MRE 600 were labeled for 2 min with [^{14}C]uracil 20 min after infection. Figure 5 shows that the amount of label incorporated into precursor rRNA in *E. coli* MRE 600 is greatly decreased in the infected culture. Densitometry shows the amount of precursor rRNA made in the infected culture to be one-tenth that made in the uninfected culture. The amount of 5S ribosomal RNA synthesized

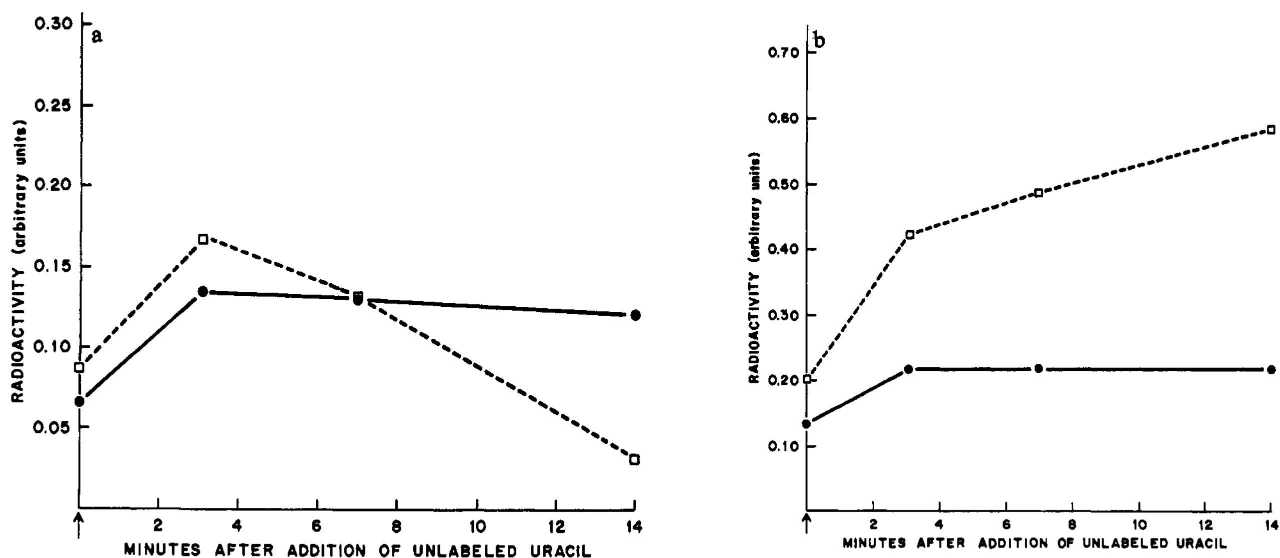

FIGURE 3: Precursor 23S RNA and 23S RNA from MS2-infected and uninfected *E. coli* D-10. The sum of the precursor 23S RNA and mature 23S RNA labeled in the experiment described in the legend to Figure 2 was determined from the radioautographs of the 2.4% acrylamide–0.5% agarose gels. To separate the 23S precursor from the mature 23S RNA, the RNA was subjected to electrophoresis on 3.7% acrylamide–0.25% agarose gels for 12 hr at 5 mA gel and 5 V/cm. The radioactivity in each band was determined from radioautographs as described under Experimental Procedures: (a) precursor 23S RNA; (b) precursor 23S RNA and mature 23S RNA; note difference in scales; (□) uninfected; (●) infected.

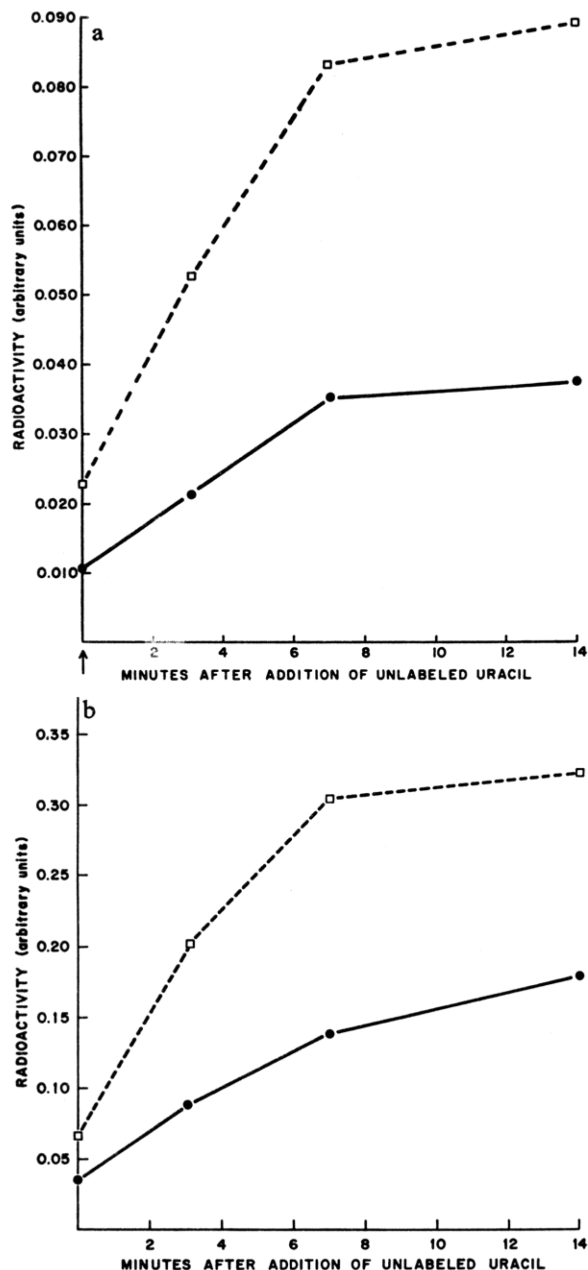

FIGURE 4: 5S and 4S RNA from MS2-infected and uninfected *E. coli* D-10. The RNA from the experiment described in the legend to Figure 2 was subjected to electrophoresis on 9.1% acrylamide gels for 5.5 hr at 5 mA/gel and 6 V/cm to separate 5S and 4S RNA. The radioactivity in each band was determined from radioautographs: (a) 5S RNA; (b) 4S RNA; (□) uninfected; (●) infected.

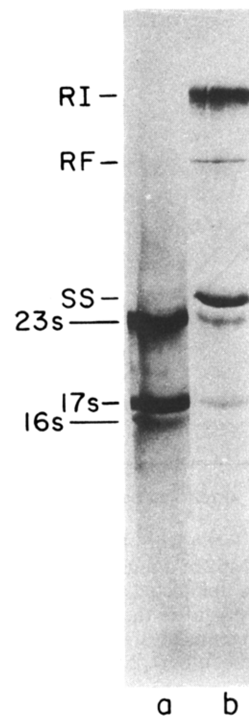

FIGURE 5: Radioautographs of gels containing RNA from MS2-infected and uninfected *E. coli* MRE 600. The procedure was identical with that described in the legend to Figure 1 except that *E. coli* MRE 600 was used: RI, MS2 replicative intermediate; RF-MS2 replicative form; ss-MS2 single-stranded viral RNA; a, uninfected; b, infected.

was determined on 9.1% gels and also found to be one-tenth that made in uninfected cells. Therefore, the host determines whether MS2 infection results in an alteration of rRNA synthesis or maturation.

Discussion

How 23S rRNA matures and whether a large common precursor RNA exists are at present not determined. The changes in RNA maturation caused by RNA bacteriophage suggest that there is a yet unidentified 23S RNA precursor.

That 17S RNA is the direct precursor to 16S RNA has been established. MS2 infection causes 17S precursor RNA to become susceptible to degradation after its synthesis. Maturation of 17S RNA occurs after it has been assembled into a 26S ribonucleoprotein particle and involves removal of nucleotides probably by RNase II (Corte *et al.*, 1971). A defect in assembly of this ribosome precursor particle should leave the 17S RNA vulnerable to further degradation by RNase II or some other RNase. Nashimoto and Nomura (1970) have shown that assembly of 50S subunits does not occur in the absence of assembly of 30S ribosomal subunits. Thus, the decrease in 23S RNA in MS2-infected cells may be secondary to the decrease in 16S RNA. The effect of MS2 infection upon ribosomes is not due to a general decrease in host protein synthesis, since previous work (Haywood and Harris, 1966) has shown that 20 min after MS2 infection of *E. coli* D-10 host protein synthesis is decreased only about 25%. Spangler and Iglewski (1972) have recently shown that the label incorporated into 16S RNA in R17 infected *E. coli* 3000 (Hfr, rel$^-$), after a 10-min chase is about one-third that incorporated in uninfected *E. coli* 3000, and that this reduction does not occur after infection with R17 polymerase mutants. Since they did not find an accumulation of precursor RNA and did not consider the possibility that the precursor could be degraded, they concluded transcription not maturation was altered by R17. Since the amount of mature ribosomal RNA synthesized after a 10-min chase is reduced two-thirds in *E. coli* 3000 as it is in *E. coli* D-10, it is likely that this conclusion is erroneous and the result of RNA bacteriophage infection and the importance of the viral polymerase is similar in the two strains.

After a very short labeling period, the amount of rRNA precursor labeled in MS2-infected *E. coli* MRE 600 is greatly reduced, so it seems probable that rRNA synthesis is greatly reduced, although it is impossible to eliminate the possibility of extremely rapid degradation. The effect of MS2 infection

upon rRNA maturation is, of course, only demonstrable in those strains where the precursor is made. It seems likely that the effect of infection upon rRNA maturation in *E. coli* D-10 and the effect of infection upon rRNA synthesis in *E. coli* MRE 600 are related. Both effects could be due to a common cause such as an alteration in a membrane site (Haywood, 1971; Hunt *et al.*, 1971). Alternately, the inhibition of rRNA synthesis could be purely secondary to a defect in ribosomal assembly. Sykes (1966) has reviewed evidence for the theory that ribosomal assembly is necessary for ribosomal synthesis. Michaels (1972) has shown that not only the rate of ribosomal RNA synthesis but also the rate of ribosomal assembly varies with growth rate. One possible way ribosomal assembly could be linked to rRNA synthesis and be altered by phage infection would be if one of the ribosomal proteins were utilized by both the host and viral polymerases. Kuo and August (1972) have suggested that a ribosomal protein may serve as a factor for Qβ polymerase. Leavitt *et al.* (1972) have shown that *in vitro* RNA synthesis can be stimulated by some ribosomal proteins.

Why *E. coli* MRE 600 and *E. coli* D-10 respond differently to RNA bacteriophage infection is not known. It is tempting, however, to postulate that the difference is related to the RC locus, since *E. coli* MRE 600 is rel^+ and *E. coli* D-10 is rel^-.

The effect of MS2 infection upon rRNA maturation and synthesis provides a tool for the study of rRNA maturation and contributes to the recent body of evidence which suggests a link between RNA synthesis and assembly.

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