ON THE RNA SYNTHESIZED IN ESCHERICHIA COLI INFECTED WITH T_2 PHAGE*

S. Osawa** and I. Watanabe

Institute for Virus Research, Kyoto University, Kyoto, Japan

A. Ishihama and H. Mitsui

Biological Institute, and Institute for Molecular Biology,

Faculty of Science, Nagoya University, Nagoya, Japan.

Received November 28, 1961

It was reported that the base ratio of the RNA synthesized in T_2-phage infected E. coli is reflected by the base ratio of T_2 DNA (Volkin and Astrachan, 1957; Watanabe and Kiho, 1957). The intracellular distribution of this T_2 specific RNA has been investigated by various workers (Volkin and Astrachan, 1957; Nomura et al, 1960; Brenner et al, 1961) mainly by means of ultracentrifugal procedures. The present communication describes a study on the localization and some properties of the T_2 specific RNA as fractionated by DEAE column chromatography described previously (Otaka et al, 1961).

T_2 infected cells received guanine-8-C^{14} or inorganic P^{32} between 1 min and 10 min after infection. About 90% of radioactive RNA was found in the ribosomal pellet, and the remaining 10% in the supernatant fraction. When the ribosomal fraction was chromatographed, most of the radioactivity was separated from the ribosomal particles and recovered in the fraction eluted with alkali (PIII). Some activity was detected in the peak (indicated by "control" in Fig. 1c) where small ribosomes are located (PII). Whether this represents the synthesis of small ribosomes could not be decided, because

* Supported by grants from Rockefeller Foundation, the Jane Coffin Childs Memorial Fund for Medical Research and the Asahi Press.

** On leave from Institute for Molecular Biology, Faculty of Science, Nagoya University, Nagoya, Japan.

exact nature of this radioactive peak was not established. In accordance with the previous reports (Nomura et al, 1960; Brenner et al, 1961), RNA in typical ribosomal particles (70s and 100s) was not formed (PI).

The radioactive RNA in the 105,000 x g supernatant could be separated by the column into two fractions: one is nearly superimposed on the second peak (SII) in which most of the DNA (except in the phage particles) is localized. The other was eluted with alkali (SIII). That about 10% of radioactivity in the SII peak was due to RNA was disclosed by (1) the liberation of radioactivity after alkaline hydrolysis or after ribonuclease treatment of this peak and (2) nucleotide composition analyses on the alkaline hydrolysate with Dowex column (see below). The result strongly suggests that a considerable part of the RNA in phage infected cells is present in the form of a DNA-RNA complex (cf. Otaka et al, 1961). Another point of interest is that upon infection the synthesis of transfer RNA (SI) virtually ceased. No radioactive transfer RNA was formed when cells were exposed to C^{14}-guanine even for 25 min from the beginning of phage infection.

An experiment was conducted in which the infected cells received 50 µg/ml chloramphenicol (CM) and C^{14}-guanine simultaneously between 1 min and 10 min period. One of the striking points in T_2 infected and CM treated cells is that while the general pattern of radioactive RNA is essentially the same (except for transfer RNA) as in the control cells without CM, an increase of the activity in SII, SIII, PII and PIII peaks was observed. This result may be explained by the fact that CM causes an apparent increase of RNA content after phage infection by inhibiting breakdown of the RNA (Watanabe and Kiho, 1957; Astrachan and Volkin, 1958). Here again, a large amount of radioactivity due to RNA was localized in the position of DNA (SII). In contrast to the case of normally infected cells, a considerable amount of transfer RNA is synthesized even after T_2 phage infection when CM is present.

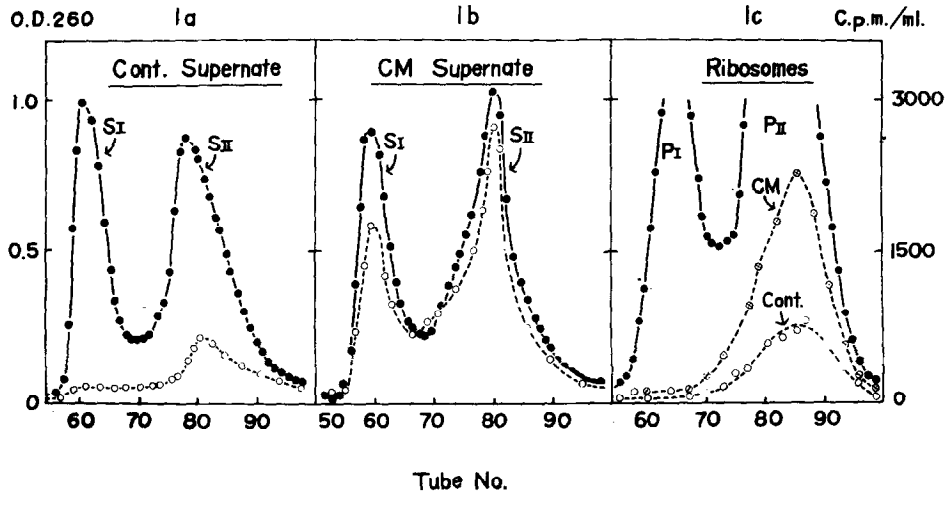

Fig. 1a - 1c. Elution pattern on a DEAE column of the RNA synthesized in E. coli infected with T_2 phage. Exponentially growing E. coli B(H) in 200 ml synthetic medium were infected with T_{2r}+ phage at multiplicity of about 5 at cell conc of 5 x 10^8. Infected cells received 20 μc=0.9 mg guanine-8-C^{14} between 1 to 10 min period. The reaction was stopped by adding crushed ice. Chilled suspension of uninfected E. coli was added as carrier. Cells collected were then ground with quartz sand and extracted with 3 vol of 0.01 M tris-0.001 M Mg acetate at pH 7.6 The extract was centrifuged at 15,000 x g for 30 min to remove sand and cell wall. The supernatant was recentrifuged at 105,000 x g for 120 min to obtain the ribosome and supernatant fraction, each of which was chromatographed with 15 x 1.5 cm DEAE (0.44 meq/g) column as previously described (Otaka et al, 1961). —●—: O.D. 260 mμ; ---o---: radioactivity.

Radioactive phage type DNA present in Peak SII of Fig. 1a was not indicated. Radioactivity due to RNA plotted in the figure was estimated as follows; to each tube content was added carrier RNA and DNA. Cold TCA precipitate was dried and dissolved in 0.005 M tris- 0.0001 M Mg^{++}(pH 7.6), containing 100 μg/ml ribonuclease. It was incubated at 37 C for 3 hrs. Acid-soluble radioactivity was measured. Almost no radioactivity due to DNA was found in Peak SII of Fig. 1c. SIII and PIII which were not eluted with NaCl but only with alkali are not shown in the figures.

Next, infected cells were exposed to inorganic P^{32} with or without CM. Essentially the same chromatographic patterns as those of the C^{14}-guanine experiment were obtained. Nucleotide composition of each peak was determined by counting the radioactivity of 2',3'-ribomononucleotide after alkaline hydrolysis of RNA. The result indicates that all the RNA, except transfer RNA, has a similar, if not identical, nucleotide composition to that of T_2 DNA irrespective of the presence or absence of CM.

Our working hypothesis derived from these results is that the specific RNA is first formed on DNA(SII), liberated from it (now in SIII) and finally deposited on the old ribosomal particles. The active RNA attached to the

Table 1. Nucleotide composition of RNAs derived from T_2 phage infected *Escherichia coli*

Adenylic acid = 1.0

RNA	Guanylic	Cytidylic	Uridylic
SII ("DNA")	0.80	0.64	0.93
SIII (alkali eluted fraction)	0.69	0.54	0.87
PII (Small ribosome fraction)	0.73	0.72	0.98
PIII (alkali eluted fraction)	(0.93)*	0.69	0.92
SII-CM ("DNA")	0.81	0.63	0.99
SIII-CM (alkali eluted fraction)	0.70	0.60	1.00
PII-CM (small ribosome fraction)	0.84	0.72	1.07
PIII-CM (alkali eluted fraction)	0.80	0.68	.0,87
SI-CM (transfer RNA)	1.24	1.76	1.15
T_2 phage DNA	0.56	0.51	1.00
E. coli RNA	1.06	0.96	0.91
E. coli transfer RNA	1.55	1.43	0.96

* contaminated with inorganic P^{32}
S: 105,000 x g supernatant; P: 105,000 x g pellet

old ribosomes is peeled off from the particles with DEAE column and recovered in Peak PIII.

The nucleotide composition of the transfer RNA formed after phage infection in the presence of CM was definitely different from the phage type composition, but is rather close to that of *E. coli* transfer RNA. These results, together with the complete inhibition of transfer RNA synthesis in normally infected cells, strongly suggest that, upon phage infection, a

proteinic factor which inhibits transfer RNA synthesis can be formed under the control of phage DNA; in the presence of CM no such factor can be formed, thus allowing the synthesis of bacterial transfer RNA even after the infection of T_2 phage. The hypothesis is further strengthened by the finding that transfer RNA is not formed if CM is added 10 min after phage infection.

References

Astrachan,L., and Volkin,E., Biochim.Biophys.Acta, 32. 449 (1959).

Brenner,S., Jacob,F., and Meselson,M., Nature, 190, 576 (1961).

Hall,B., and Spiegelman,S., Proc.Nat.Acad.Sci.U.S., 47, 137 (1961).

Nomura,M., Hall,B.D., and Spiegelman,S., J.Mol.Biol., 2, 306 (1960).

Otaka,E., Osawa,S., Oota,Y., Ishihama,A., and Mitsui,H., Biochim. Biophys. Acta, in press (1961).

Volkin,E., and Astrachan,L., In "the Chemical Basis of Heredity", p.686, Baltimore: Johns Hopkins Press (1957).

Watanabe,I., and Kiho,Y., Proc.Intern.Symp.Enzyme Chem., Tokyo and Kyoto, p.418 (1957).