THE ROLE OF PHAGE SPECIFIC RNA AS MESSENGER *

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According to the messenger hypothesis (Jacob and Monod, 1961), only a small fraction of the cellular RNA acts as template for the proper assembly of amino acids in protein synthesis. The RNA synthesized in phage infected cells in many respects behaves as such a messenger. In addition to the criteria found so far (Astrachan and Volkin 1958, Hall and Spiegelman 1961), T4 phage-specific RNA should exhibit a high level of activity in mediating amino acid incorporation in a cell free system. With the introduction of DNA-cellulose columns it has become possible to separate T4-specific RNA from E. coli RNA (Bautz and Hall 1962). Improving this method further, we have succeeded in obtaining T4-specific RNA free from any detectable amounts of contaminating DNA.

The finding that T4-specific RNA, purified in this manner, shows high messenger activity in a cell free system is the subject of this communication.

E. coli B cells were isolated 8 minutes after infection with T4 wild type phage. (Multiplicity = 5). Although the lifetime of the E. coli

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messenger RNA might be considerably longer than 4 to 6 seconds as estimated by Gros et al. (1961), practically no residual E. coli messenger activity should be encountered at this time after infection. The cells were washed in 0.01 M Tris buffer (pH 7.2) + 0.01 M Mg^{++}, frozen, thawed, resuspended in cold 0.01 M acetate buffer pH 5.1, containing 2 μg/ml polyvinylsulfate. Sodiumdodecylsulfate (final conc. 0.5%) was added and the RNA was extracted with hot phenol as described by Scherrer and Darnell (1962). The redissolved RNA was dialyzed overnight against 1000 volumes of distilled water and applied to a DNA-cellulose column with dimensions 25 x 1.5 cm, containing approximately 200 mg of T4 wild type DNA. The column had been prepared as described previously (Bautz and Hall, 1962). In addition the remaining condensing agent had been extracted with petroleum ether. The RNA samples were applied to the column in about 1 ml of 0.3 M NaCl + 0.03 M Na citrate (2 x Standard Saline Citrate = 2 x SSC) and incubated at 51°C for 30 minutes in the top part of the column; then 1.5 ml 2 x SSC was added to the column, allowing the RNA to move down into a zone of new DNA-cellulose; incubation and addition of 2 x SSC were repeated for a total period of 5 to 8 hrs until the RNA sample was incubated with every part of the column. After removal of the unadsorbed RNA (peak 1, Fig. 1) with 2 x SSC, T4-specific RNA (peak 2, Fig. 1) was eluted with 0.01 x SSC at the same temperature. The two peaks obtained were concentrated, dialyzed against conc. solutions of polyethyleneglycol (Carbowax 6000) to reduce their volumes, followed by overnight dialysis in the cold against 1000 volumes of distilled water.

Assay for amino acid incorporation

The two RNA fractions obtained as described above and also the unfractionated RNA were assayed for stimulation of amino acid

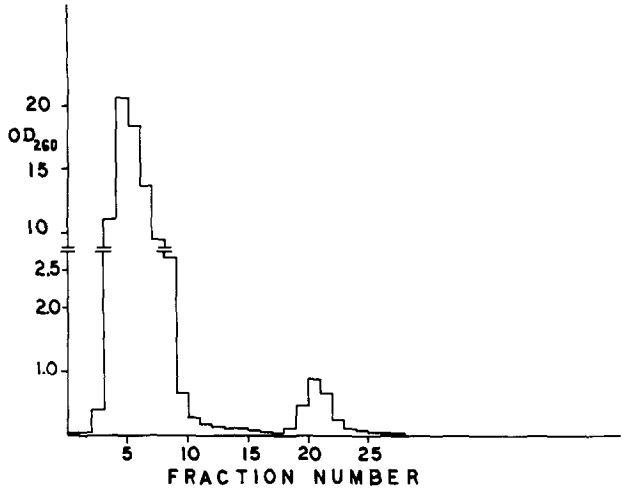

Fig. 1

Purification of T4-specific RNA on a T4-DNA cellulose column. 10 mg of RNA isolated 8' after infection were incubated stepwise for 5 hours at 51°C. The upper half of the scale was reduced 5 fold. Fraction size = 2.6 ml.

incorporation in the Nirenberg system (Nirenberg and Matthaei 1961), containing per ml: 12 μM Mg acetate; 0.1 ml of a preincubated 30,000 x g supernatant fraction from E. coli B cells (OD_{260} = 150), 7 mg E. coli B S-RNA, 0.03 μc C^{14}L-leucine (specific activity: 21 mc/mM), or 0.04 μc C^{14}L-valine (spec. act.: 8 mc/mM), or 0.12 μc C^{14}DL-alanine (spec. act: 2 mc/mM). The other additions for preincubation and for the incorporation assay as well as the assay procedure itself were the same as described by Nirenberg and Matthaei (1961) with the exception that the samples were filtered through Millipore filters after hot TCA-hydrolysis and were counted in a Packard Model EX liquid scintillation counter, having a counting efficiency of 55 to 60%.

Results

Fractionation of RNA isolated from T4-infected E. coli B cells cells on the DNA-column yielded two UV-absorbing peaks (Fig. 1).

The smaller 2nd peak had an absorption max. at 260 mμ and showed a positive orcinol and a negative diphenylamine test. Together with the criteria previously established (Bautz and Hall, 1962) this nucleic acid can be considered to consist to 90% or more of T4-specific RNA. The combined fractions containing the T4-specific RNA were concentrated and the activity was compared with the activity of equal amounts of the unfractionated RNA in the cell free system (Table 1). With all three amino acids tested, T4-specific RNA stimulated amino acid incorporation at a much higher rate than did the unfractionated RNA. Omission of a mixture of the other 19 cold amino acids greatly diminished incorporation of C^{14}L-leucine. Addition of 3 μg of DNAase slightly reduced the amount of incorporation whereas addition of 3 μg of RNAase resulted in a complete loss of activity.

In another experiment, C^{14}L-leucine incorporation was measured with the addition of various amounts of unfractionated RNA, unadsorbed RNA and adsorbed RNA (Fig. 2). It was found that the messenger activity of the T4-specific RNA showed an eight fold increase over the unfractionated RNA. Figure 2 also shows that this increase in activity of the T4-specific RNA was gained at the expense of the unadsorbed RNA eluted in the column front. By rechromatography of this unadsorbed RNA an additional fraction of active T4-specific RNA was obtained.

Due to a small but steady loss of messenger activity during the chromatographic procedure, it has not been possible to recover quantitatively all the activity of the unfractionated RNA in the purified T4-messenger fraction. Nevertheless, it can be concluded from these experiments, that in the case of T4-infected cells, most if not all of the

Table 1

Stimulation of amino acid incorporation by RNA isolated from T4 infected cells.

C^{14} amino acid			
C^{14} L-leucine	Complete	None	354
"	"	+ 40 μg unfractionated RNA	588
"	"	+ 40 μg T4-specific RNA	2336
"	"	+ 40 μg T4-specific RNA -C^{12} mixture	863
C^{14} L-valine	"	None	131
"	"	+ 40 μg unfractionated RNA	280
"	"	+ 40 μg T4-specific RNA	1158
C^{14} DL-alanine	"	None	10
"	"	+ 40 μg unfractionated RNA	91
"	"	+ 40 μg T4-specific RNA	394

The reaction mixtures were incubated for 60 min. at 35°C. The assay volumes were 1 ml. The composition of the mixtures and the counting procedure were as described in the text.

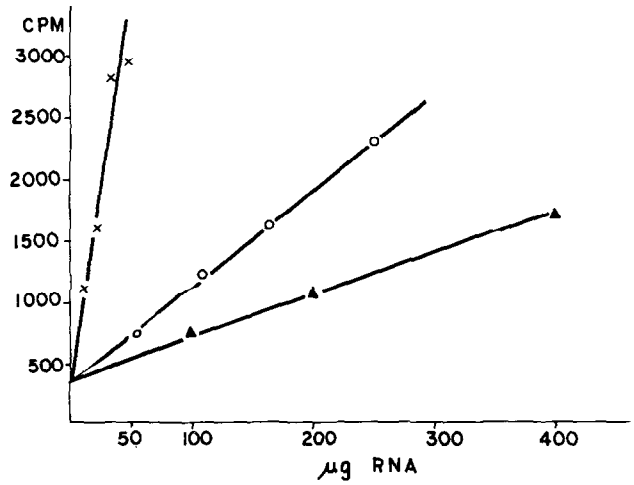

Fig. 2

C^{14} L-leucine incorporation per ml reaction mixture with various amounts of three RNA samples: 0 = unfractionated RNA from T4-infected cells; ▲ = RNA not adsorbed by the DNA column; X = T4-specific RNA.

messenger activity resides in the RNA which is complementary in sequence to the phage DNA and which therefore carries the genetic information of the corresponding DNA regions.

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