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Translation of *Pseudomonas aeruginosa* Bacteriophage PP7 RNA by a Cell-Free Amino Acid Incorporating System from *Escherichia coli*

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We have compared the activities of the RNA genomes of *Pseudomonas aeruginosa* phage PP7 and coliphages Qβ and f2 in a cell-free amino acid incorporating system derived from *Escherichia coli*. The rate of incorporation of [^{14}C]leucine in the PP7 RNA-directed system is greater than in the systems directed by either Qβ or f2 RNA. The response to changes in phage RNA concentrations is similar in all the systems, reaching a saturation level at 0.75 to 1.0 mg of RNA per ml of reaction mixture. Analysis of complete reaction mixtures of the PP7 RNA and of the Qβ RNA systems by sucrose gradient centrifugation shows generally similar patterns for both RNAs. The principal differences are that in the PP7 system a slightly higher percentage of RNA forms ribosome complexes and that the polysomes are somewhat smaller. PP7 RNA is also degraded more extensively during the reaction than is Qβ RNA. Analysis of the products of the reactions by acrylamide gel electrophoresis shows that PP7 coat protein is the only identifiable product of the PP7 RNA-directed system, suggesting that only the coat protein cistron is translated by *E. coli* ribosomes.

The translation of coliphage RNAs in cell-free systems by extracts derived from bacterial species other than *Escherichia coli* is restricted. Extracts of *Bacillus subtilis* (10), *Clostridium pasteurianum* (3, 12, 13), and several species of *Enterobacteriaceae* (5) have been reported to be unable to translate coliphage RNAs or to do so at low levels compared with *E. coli* extracts. Two psychrophilic bacteria (14) effectively translate MS2 RNA. *Bacillus stearothermophilus* ribosomes have been reported to translate only the A-protein cistron of f2 RNA (8, 9). We have shown previously that the translation of coliphage RNAs by *Salmonella typhimurium* extracts is strongly dependent on the growth conditions used for the cells (1).

These restrictions indicate that investigations of the translation of phage RNAs by nonhost bacterial extracts may provide insights into the general mechanisms of ribosome-mRNA initiation site recognition. Studies of the activities of two independent host-phage systems and their cross-reactions are of particular interest in this respect. Leffler and Szer (7) have recently investigated one such system using *E. coli* and MS2 phage and *Caulobacter crescentus* and Cb5 phage. In these systems, translation of the phage RNAs occurs only when 30*S* subunits of the natural host for the phage are present.

We report here the results of an investigation of the activity of RNA of *Pseudomonas aeruginosa* phage PP7 (2) in a cell-free system derived from *E. coli*.

MATERIALS AND METHODS

Preparation of phage RNAs. The preparation of Qβ RNA and Qβ RNA labeled with [^{3}H]uridine has been described previously (1). f2 RNA was prepared by the same procedures. PP7 lysates were prepared in the same medium and under the same growth conditions used for Qβ. *P. aeruginosa* strain 01 was used as the host for PP7. Lysate titers of 10^{12} to 2×10^{12} PFU/ml were obtained routinely. Three hours after infection, the lysates were treated with 1 μg of DNase I (Sigma)/ml, and Mg^{2+} was added to a concentration of 2 μM. Shaking was then continued for 30 min more at 37 C. The lysate was chilled to 5 C, and the PP7 phage were precipitated with 6% (wt/vol) polyethylene glycol (Schwarz-Mann, Carbowax 6000) and 3% (wt/vol) NaCl. After a few hours in the cold, the suspension was centrifuged for 15 min at 8,000 × *g*. The pellets were resuspended by shaking 2 h at 5 C in 0.01 M Tris-hydrochloride, pH 7.4, the volume equal to about 2% of the original lysate volume. If the preparation was still too viscous to handle conveniently at this point, it was treated again with DNase as above. The suspension was centrifuged for 15 min at 12,000 × *g*. The supernatant fluid was dialyzed overnight at 5 C against 100 volumes of 0.01 M Tris-hydrochloride, 0.01 M EDTA, pH 7.4. Subse-

quent purification and the extraction of RNA was carried out as described for Qβ RNA.

Phage particles used for the electrophoresis of structural proteins were purified further after banding in CsCl by centrifugation in a 2.5 to 20% linear sucrose gradient for 2.5 h at 40,000 rpm in the SB-283 rotor of the International B-60 centrifuge and isolation of the phage-containing zone.

Amino acid incorporating system. The preparation of *E. coli* A19 S30 extracts, the phage RNA-directed amino acid incorporating system, and techniques for sucrose gradient analysis have been described in detail previously (1). Briefly, the amino acid incorporating system consisted of 10 mM Tris-hydrochloride, pH 7.8, 10 mM magnesium acetate, 50 mM NH_4Cl, 10 mM KCl, 1.5 mM dithiothreitol, 1.5 mM ATP, 0.3 mM GTP, 6 mM phosphoenol pyruvate, 50 μg of pyruvate kinase per ml, 0.1 mM 20 common amino acids except lecine, 0.02 mM [^{14}C]leucine (approximately 300 mCi/mmol, Schwarz-Mann), 40 A_{260} units of *E. coli* S30 extract per ml, and the indicated amounts of phage RNA. For sucrose gradient analyses, ^{3}H-phage RNA was added to the reaction mixtures at a concentration of 10^6 counts per min per ml. The amount of amino acid incorporation was determined by adding 3 ml of 5% trichloroacetic acid to 40-μliter portions of the reaction mixture after the appropriate period of incubation at 37 C. The suspension was heated for 10 min at 90 C, and the hot trichloroacetic acid-insoluble material was collected on cellulose nitrate membrane filters, washed with 1% trichloroacetic acid, dried, and counted in a liquid scintillation spectrometer.

Acrylamide gel electrophoresis. Samples were prepared for electrophoresis by the following procedure. At the end of the appropriate incubation period, leucine was added to the amino acid incorporation reaction mixture at a concentration of 10 mM, and the incubation was continued for 10 min more. This chase procedure reduced the amount of nonspecific, polydisperse material present in the gels. The reaction was then stopped by the addition of an equal volume of a solution of 0.1 M EDTA, 0.2 M 2-mercaptoethanol, 2% (wt/vol) sodium dodecyl sulfate (SDS), 0.02 M sodium phosphate buffer, pH 7.2. This mixture was incubated for 1 h at 37 C. Samples were layered onto 10% acrylamide-bisacrylamide (15) gels whose dimensions were 6 mm in diameter and 60 mm in length. Electrophoresis buffer consisted of 1% (wt/vol) SDS, 0.01 M sodium phosphate buffer, pH 7.2. Samples were run for 3 h at 10 mA per column at room temperature. After electrophoresis, the gels were sliced into approximately 1.2-mm thick slices, and each slice was heated individually for 20 min at 90 C in 3 ml of 5% trichloroacetic acid. Slices were removed from the acid and treated for 30 min at room temperature in 0.5 ml of tissue solubilizer (Eastman) in a scintillation vial. Ten milliliters of scintillation fluid (Omnifluor, New England Nuclear Corp.) was then added, and after equilibration the radioactivity was determined in a liquid scintillation spectrometer.

This technique offers some advantages over previously reported procedures. Treatment of the gel slices with hot acid quantitatively removes unincorporated amino acids and hydrolyzes amino acyl-tRNA without reducing the radioactivity incorporated into polypeptides. This makes time-consuming steps such as dialysis or repetitive precipitations of the reaction mixtures prior to electrophoresis unnecessary.

Electrophoretic analysis of nonradioactive structural proteins from purified phage particles was carried out according to the procedures of Osborn, Weiner, and Weber (11).

RESULTS

We have compared the kinetics of [^{14}C]leucine uptake in *E. coli* cell-free amino acid incorporating systems directed by PP7, Qβ, and f2 RNAs (Fig. 1). The rate of incorporation in the PP7 RNA-directed system is greater than in the systems directed by either of the coliphage RNAs. The maximum rates of incorporation are approximately 0.5 nmol per min per ml of reaction mixture for PP7 RNA and 0.35 and 0.25 for Qβ, and f2 RNA, respectively. The initial lag in the incorporation of [^{14}C]leucine is marked in the f2 RNA reaction and somewhat less obvious in the Qβ RNA reaction. The PP7 RNA reaction is initiated without a detectable lag period. The reaction rates decline rapidly after 15 min of incubation in all the reaction systems, and uptake stops completely after 20 to 25 min. Comparing the total amino acid incorporation in each of the systems after 30 min of incubation

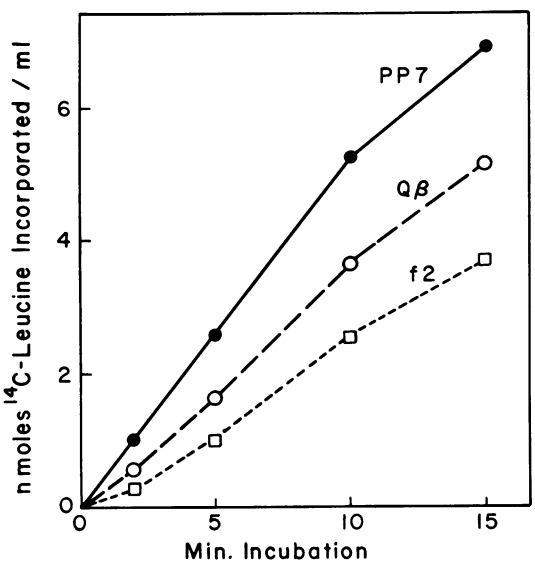

FIG. 1. *Leucine incorporation directed by PP7, Qβ, and f2 RNAs. Reaction mixtures (40 μliters) containing 500 μg of phage RNA per ml were prepared and incubated at 37 C for the times indicated. The reactions were stopped with 5% trichloroacetic acid, and the radioactivity was determined as described in Materials and Methods.*

gives approximately the same relative values as those shown above. However, the maximum incorporation rates early in the reactions express more accurately the activities of the RNAs than the total incorporation in the systems incubated to exhaustion because of the complex interaction of factors which influence the decline and stoppage of the reaction.

The relationship between phage RNA concentration and activity is shown in Fig. 2. The rate at each concentration represents the maximum rate of incorporation, typically between 5 and 10 min of incubation, obtained from kinetics experiments such as those described above. The response with all three RNAs is similar. The reactions are approximately 80% saturated at an RNA concentration of 500 μg per ml of reaction mixture, and essentially no additional stimulation of activity is obtained at phage RNA concentrations greater than 1 mg/ml. The residual activity of the system without added phage RNA is less than 0.005 nmol per min per ml of reaction mixture. The ratio of phage RNA strands to ribosomes in these systems is approximately one to one at a phage RNA concentration of 500 μg per ml of reaction mixture. This concentration was selected for use in the experiments described below.

We have analyzed complete reaction mixtures of the systems directed by Qβ RNA (Fig. 3A) and by PP7 RNA (Fig. 3B) by sucrose gradient centrifugation. The general pattern is similar for both species of RNA. Phage RNA is found in monosome complexes at about 70S and in polysome fractions at progressively higher sedimentation values. All of these fractions are associated with acid-insoluble [^{14}C]leucine activity, indicating polypeptide synthesis. A slightly higher percentage of PP7 RNA than Qβ RNA is bound to ribosomes. In the PP7 RNA reaction, there is a higher proportion of monosomes and disomes than larger polysomes. More, larger polysomes are formed in the Qβ RNA reaction, with polysomes as large as pentasomes clearly resolved.

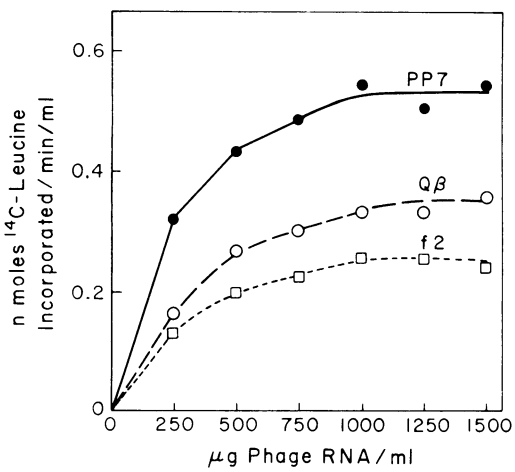

FIG. 2. *Effect of phage RNA concentration on leucine incorporation. Reaction mixtures (40 μliters) containing the indicated amounts of phage RNA were incubated for 2, 5, 10, and 15 min at 37 C and stopped with 5% trichloroacetic acid, and the radioactivity was determined as described in Materials and Methods. Uptake kinetics were plotted, and the maximum rate (nanomoles per minute per milliliter), usually between 5 and 10 min, was used as the measure of activity.*

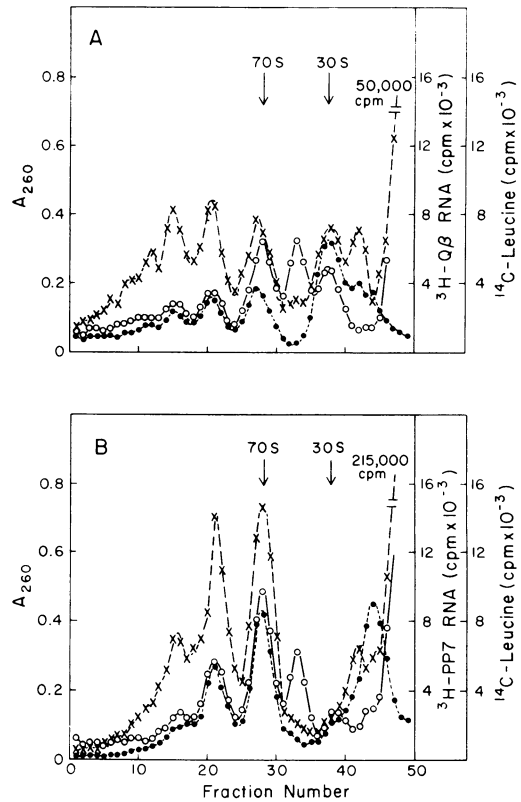

FIG. 3. *Sucrose gradient analysis of complete reaction mixtures directed by Qβ RNA (A) and PP7 RNA (B). Reaction mixtures (200 μliters) containing 500 μg of phage RNA per ml were incubated for 10 min at 37 C, layered onto 10 to 40% exponential sucrose gradients, and centrifuged for 3 h at 40,000 rpm in the SB-283 rotor of the International B-60 centrifuge. Fractions were collected by puncturing the bottoms of the tubes. The absorption at 260 nm was determined, fractions were then precipitated with cold 5% trichloroacetic acid, and the radioactivity was determined as described in Materials and Methods except that the heating step was omitted, and the washings were carried out at 0 C. Symbols: ○, A_{260}; ●, ^{3}H-phage RNA; ×, [^{14}C]leucine.*

PP7 RNA is degraded more extensively than Qβ RNA during the protein synthesizing reaction. This can be seen in the smaller amounts of both A_{260}-absorbing material and ^{3}H-phage RNA in the 30*S* region of the gradient analysis of the PP7 reaction system. Most of the ^{3}H-PP7 RNA not bound to ribosomes is found in a rather broad peak close to the top of the gradient. Some fragmentation of the Qβ RNA not bound to ribosomes can also be observed, but, even after 10 min of incubation, most of this RNA sediments at approximately 30*S*. We have run similar experiments at shorter reaction times (data not shown.). The amount of ribosome-bound RNA in both the PP7 and the Qβ reaction systems is approximately the same in both 2- and 10-min reactions, although the degradation of the free RNA is much less extensive in the 2-min reactions. This indicates that the ribosome-bound PP7 RNA may be protected from degradation since fragmentation as extensive as that observed in the free RNA would result in a substantial reduction in the amount of PP7 RNA sedimenting with the monosome and polysome fractions.

In the Qβ RNA system, [^{14}C]leucine is associated with the free phage RNA. It is observed with both the complete RNA at about 30*S* and with the larger fragment closer to the top of the gradient. Some [^{14}C]leucine activity can be seen extending into the upper region of the gradient at less than 30*S* in the PP7 reaction. This peak is in the same region as the leading edge of the fragmented PP7 RNA, but it does not appear to be associated with a specific fraction. This suggests that PP7 RNA may form complexes with its translational products as the coliphage RNAs do, but no estimate of the extent or type of complex formation can be made.

Qβ RNA directs the synthesis of three distinct products (Fig. 4A). The major product (III) has an electrophoretic mobility which is the same as that of Qβ coat protein from purified phage particles. The intermediate product (II) co-electrophoreses with one of the minor structural components of Qβ phage particles. The slowest moving product (I) is not a structural component of the phage. The electrophoretic mobilities of these proteins and their comparative proportions are in agreement with previously reported products of the Qβ RNA-directed system identified as replicase subunit (I), readthrough protein (II), and coat protein (III) (4, 6, 16). In addition to these products, smaller amounts of unidentified, polydisperse material are observed between fractions II and III.

In contrast to the Qβ system, the PP7 RNA-directed incorporating system produces only one distinct product (Fig. 4B). This product has

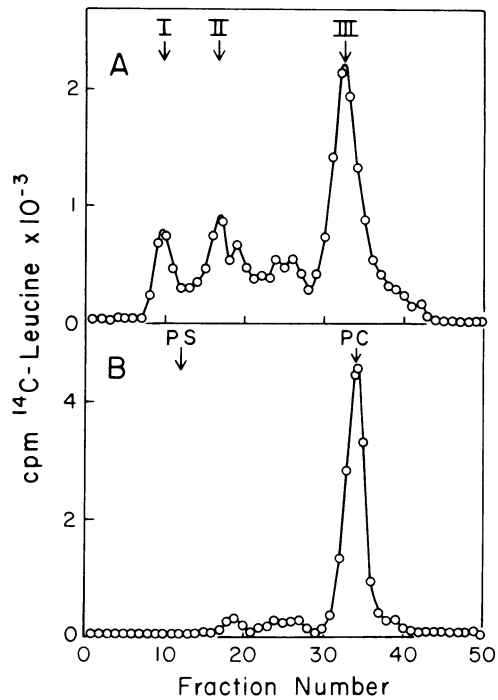

FIG. 4. *Acrylamide gel electrophoresis of the products of cell-free amino acid incorporating systems directed by Qβ RNA (4A) and by PP7 RNA (4B). Complete reaction mixtures containing 500 μg of phage RNA per ml were incubated for 10 min at 37 C, prepared for electrophoresis, and analyzed as described in Materials and Methods. Markers (4B) indicate the positions of phage coat protein (PC) and the second phage structural protein (PS) obtained from mature PP7 phage particles.*

the same electrophoretic mobility as PP7 coat protein from mature phage particles (PC in figure). The mobility of the PP7 coat protein is somewhat greater than that of Qβ coat and approximately the same as that of f2 coat protein. PP7 phage particles contain a second structural protein (PS in figure) with a mobility of about 0.3 relative to PP7 coat. More than 95% of the radioactivity of the gel is found in the coat protein peak. The remainder is polydisperse, and we have detected no peaks other than coat which appear consistently. No material is present in the gel in the region in which the second PP7 structural protein is found (PS in figure).

To test the possibility that non-coat proteins appear only late in the PP7 RNA-directed reaction, we have analyzed the products of a 30-min reaction which contains all of the proteins synthesized in the reaction before it is exhausted. Again, coat protein is the only identifiable product, and the pattern is essentially identical to that obtained with the 10-min reaction shown here.

DISCUSSION

P. aeruginosa phage PP7 RNA is translated actively by a cell-free system derived from *E. coli*, a species only distantly related to the normal host. The PP7 RNA-directed incorporation of leucine and the extent of *E. coli* ribosome-PP7 RNA complex formation exceeds that observed with coliphage RNAs. The only identifiable product of the PP7 RNA-directed reaction has an electrophoretic mobility identical to that of PP7 coat protein isolated from mature phage particles, indicating that at least the coat protein cistron of the PP7 RNA is translated normally by *E. coli* ribosomes.

The high activity in this heterologous host-phage system is unusual. Coliphage RNA translation by ribosomes of other bacterial species is limited (3, 5, 8, 10). *C. crescentus* phage Cb5 is translated only by ribosomes containing the small subunit of the host (7).

In spite of the high incorporation rate and extensive ribosome complex formation with PP7 RNA, our results indicate that only the coat cistron is translated in the *E. coli* system. These results are not conclusive, but the acrylamide gel electrophoresis demonstrates that the translation of noncoat cistrons, if it occurs, is less than 10% of the noncoat cistron translation observed in the systems directed by coliphage RNAs. Studies with extracts of *P. aeruginosa* should provide comparative information about the extent of translation of the noncoat cistrons of PP7 RNA by normal host ribosomes.

The availability of phage RNAs from species other than *E. coli* which have initiation sites with selective specificities provides a capacity to investigate some properties of initiation site-ribosome interactions not possible with the *E. coli*-coliphage RNA systems. These studies may provide new insights into mechanisms of translational level regulation.

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