TRANSLATION OF MS2 RNA BY RIBOSOMES FROM DIFFERENT BACTERIAL SPECIES

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Infection of susceptible strains of E. coli with the closely related RNA phages f2, R17, or MS2, induces the synthesis of three virus-specific polypeptides: coat, RNA replicase, and the maturation or attachment factor (A protein), ref. 1-3. The first and second products are synthesized in relatively large amounts in vitro and are identifiable by polyacrylamide gel electrophoresis (4). At least three protein factors, F_1, F_2, F_3, loosely associated with the 30 S ribosomal subunit (5, 6) are required for initiation of polypeptide chain synthesis in E. coli (7). AUG is the initiator codon of the three viral proteins (8); however, due to the ambiguity of the formylmethionine codons, AUG and GUG, which in internal positions are read as Met and Val codons, respectively, additional information is required for the ribosome to start translation at the proper site. It became clear from recent investigations (8, 9) that the initiation trinucleotides are preceded by presumably non-coding nucleotides which may contain this information. Initiation factor F_3 appears to be specifically involved in initiation of translation of natural messenger (10) and may direct the binding of the ribosome to a region of the messenger preceding an initiation codon (8, 9). The question then arises whether initiation sites of a host or host-specific viral messenger can be recognized only by the

initiation factors of this particular host. A recent report by Lodish (11) that translation of f2 RNA by Bacillus stearothermophilus ribosomes (at 49°) yielded only A protein, regardless of the source of tRNA and supernatant enzymes, suggested species-specific recognition of some of the f2 RNA genes.

We wish to report that ribosomes from two psychrophilic organisms, Pseudomonas sp. 421 and Micrococcus cryophilus, translate MS2 RNA at 37° yielding RNA replicase and coat protein of the same electrophoretic mobility and in the same relative amounts as E. coli ribosomes. However, irrespective of the source of the ribosomes, the amount of MS2 RNA required for optimal rate of translation (as judged by incorporation of labeled amino acids) was much higher with crude initiation factors from either psychrophile than with their E. coli counterpart. No differences were observed in saturation levels of the trinucleotide diphosphate ApUpG (AUG) in the AUG-dependent ribosomal binding of formylmethionyl-tRNA (fMet-tRNA) with E. coli or Ps. sp. 412 ribosomes regardless of the source of initiation factors. Since this reaction appears to involve only the initiation factors F_1 and F_2 (10), our results suggest that the F_3 component of Ps. sp. 412 and M. cryophilus initiation factors has less affinity for MS2 RNA initiation sites than the corresponding component of the E. coli initiation system.

Experimental. Ps. sp. 412 (laboratory strain) was grown on medium No. 187 (ATCC catalogue, 1968), M. cryophilus (ATCC 15174) according to Malcolm (12), and E. coli Q13 according to Haruna and Spiegelman (13). Crude ribosomes were isolated from an S 30 extract (14) in a buffer containing 20 mM Tris, pH 7.8, 10 mM magnesium acetate, 50 mM NH_4Cl, and 5 mM mercaptoethanol. Initiation factor-free ribosomes were prepared by washing with 1.0 M NH_4Cl according to Iwasaki et al. (7) and crude initiation factors by ammonium sulfate precipitation from the NH_4Cl wash (7). The isolation of labeled

proteins, polyacrylamide gel electrophoresis after treatment with sodium dodecyl-sulfate and radioactivity determinations were as described by Vinuela et al. (2). MS2 RNA was prepared according to Weissmann and Feix (15).

Results and Discussion. Experiments on translation of MS2 RNA by crude, i.e., initiation factor-containing ribosomes, measuring the incorporation of ^{14}C-lysine into acid-insoluble material, showed that with appropriate amounts of messenger, using E. coli Q13 150,000 g supernatant and E. coli tRNA throughout, E. coli and Ps. sp. 412 ribosomes were about equally active and M. cryophilus ribosomes somewhat less active. However, the concentrations of MS2 RNA needed for optimal incorporation were, respectively, 0.32, 2.4, and 1.6 mg/ml. The

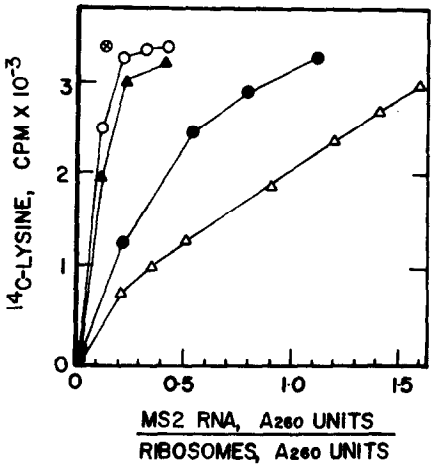

Fig. 1. MS2 RNA dependence of amino acid incorporation with combinations of washed ribosomes and initiation factors from different bacterial sources. Assay according to Iwasaki et al. (ref. 7) with 10 μg of crude initiation factor protein per A_{260} unit of ribosomes. Mg^{2+} concentrations (optimal) were as follows: 14 and 8 mM with homologous E. coli and Ps. sp. 412 ribosomes, respectively, and 12 mM with the "mixed" ribosomes; homologous ribosomes exhibited well-defined Mg^{2+} optima, mixed ribosomes exhibited a plateau between 8 and 14 mM Mg^{2+}. High speed (150,000 g) E. coli Q13 supernatant and E. coli tRNA were used throughout. Incubation, 20 min at 37°. ^{14}C-Lys specific activity 2.5 C/mole. Blanks without initiation factors or without MS2 RNA were 30-80 cpm. -o-o-o-, E. coli ribosomes and initiation factors; -●-●-●-, E. coli ribosomes and Ps. sp. 412 initiation factors; -Δ-Δ-Δ-, Ps. sp. 412 ribosomes and initiation factors; -▲-▲-▲-, Ps. sp. 412 ribosomes and E. coli initiation factors. ⊛, Crude Ps. sp. 412 ribosomes were incubated in the assay mixture for 5 min at 37° followed by addition of crude E. coli ribosomes.

requirement for larger amounts of mRNA with the psychrophilic ribosomes does not seem to be due to messenger degradation by nucleases because the nuclease activity of crude Ps. sp. 412 and M. cryophilus ribosomes was significantly lower than that of their E. coli counterparts. Moreover, preincubation of crude Ps. sp. 412 ribosomes with a subsaturating amount of MS2 RNA, followed by addition of E. coli ribosomes, resulted in a level of incorporation normally observed with that amount of MS2 RNA and E. coli ribosomes (Fig. 1).

As shown in Fig. 1, whereas E. coli and Ps. sp. 412 initiation factors were interchangeable in MS2 RNA translation, the affinity of the ribosomes for the messenger depended on the source of the initiation factors and not on the source of the ribosomes. Thus, high affinity (saturation at low MS2 RNA concentration) was observed with E. coli initiation factors, whether with E. coli or Ps. sp. 412 ribosomes, and conversely. On the other hand, as shown in Table 1

Table 1. AUG-dependent fMet-tRNA binding with combinations of washed ribosomes and initiation factors from E. coli and Ps. sp. 412 (μμmoles ^{14}C-fMet/sample).

	Ps. sp. 412 ribosomes		E. coli ribosomes	
AUG (mμmoles/sample)	Ps. sp. 412 factors	E. coli factors	E. coli factors	Ps. sp. 412 factors
0	0.9 (15)	0.2 (5)	0.15 (3)	0.6 (15)
0.2	3.7 (64)	2.8 (70)	3.1 (65)	2.6 (68)
0.5	4.2 (72)	3.0 (76)	3.4 (71)	2.9 (75)
1.0	4.8 (83)	3.4 (86)	4.1 (85)	3.2 (85)
2.0	5.7 (98)	4.0 (100)	4.8 (100)	3.8 (100)
4.0	5.8 (100)	4.0 (100)	4.75 (99)	3.8 (100)

Samples contained (in μmoles) in a volume of 0.05 ml, Tris-acetate buffer, pH 7.2, 2.5; NH_4Cl, 5; Mg^{2+}, 0.15; GTP, 0.01; dithiothreitol, 0.25; ribosomes, 2.5 A_{260} units; formylmet-tRNA, 22 μμmoles (^{14}C-methionine specific activity 218 C/mole); initiation factors: crude Ps. sp. 412, 20 μg or purified E. coli F_1, 1 μg and F_2, 0.3 μg, where indicated. Incubation for 7 min at 24°. Values in parentheses indicate percent of maximal binding. Blanks without factors were 0.2-0.3 μμmoles.

the ribosome affinity of AUG in the AUG-dependent ribosomal binding of fMet-tRNA was the same throughout.

Fig. 2A shows the virtual identity of electrophoretic patterns of the proteins synthesized by E. coli ribosomes (^{3}H-lysine label) and Ps. sp. 412 ribosomes (^{14}C-lysine label) with MS2 RNA as messenger. Similar results (not shown) were obtained with crude M. cryophilus ribosomes. The positions and relative intensities of the protein peaks were the same in each case, indicating that initiation at cistrons corresponding to RNA replicase (peak I) and coat protein (peak II) was correct and occurred with the same frequency. As observed previously (4), synthesis of the A protein (less than 2% of the total) was not detectable by the method employed. Fig. 2B shows the absence of peak II in both cases when the labeled amino acid was histidine. This is in

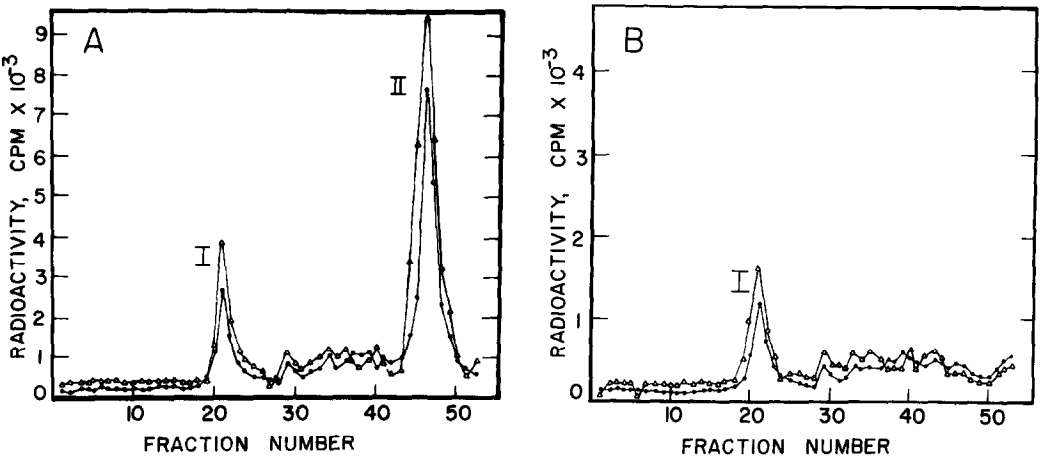

Fig. 2. Coelectrophoresis of labeled polypeptides synthesized in vitro upon translation of MS2 RNA by E. coli (^{3}H-lysine or ^{3}H-histidine label) and Ps. sp. 412 (^{14}C-lysine or ^{14}C-histidine label) ribosomes. The Ps. sp. 412 ribosomes were crude and no initiation factors were added. The E. coli ribosomes were NH_4Cl-washed and purified initiation factors F_1 (1.0 μg), F_2 (12.0 μg), and F_3 (1.5 μg) were added (7). Purified ribosomes and factors were used in the E. coli system in order to reduce the amount of partially degraded proteins observed with crude E. coli ribosomes. A, labeled lysine; B, labeled histidine. -Δ-Δ-Δ-, E. coli ribosomes; -●-●-●-, Ps. sp. 412 ribosomes. The specific radioactivities (C/mole) of the amino acids used were lysine, ^{14}C, 271; ^{3}H, 4380; histidine, ^{14}C, 220; ^{3}H, 5150.

agreement with the fact that MS2 coat protein contains no histidine. A more rigorous proof of identity of the protein products would require a trypsin digest analysis.

In conclusion, our experiments show that the initiation factors from such organisms as E. coli and two species of psychrophilic bacteria are interchangeable in translation of an E. coli-specific messenger; they also suggest that there exists a factor-dependent (possibly F_3) difference in affinity for MS2 RNA binding. In this respect, our observations are in line with Lodish's result (11) indicating species specificity of polypeptide chain initiation, and further point to the initiation factors rather than the ribosomes as the source of this specificity. This fact, as well as differences in the mechanism of initiation, would tend to restrict and control the scope of messenger translation by different cells. It would clearly be desirable to supplement these observations with similar studies on other messengers. It is fortuitous and due to our interest in protein synthesis near 0° that both our systems were derived from psychrophiles, while Lodish worked with a thermophile. The ribosomes and initiation factors of Ps. sp. 412 and M. cryophilus are stable at 37°, i.e., above the maximum growth temperature of the organisms.

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