Initiation of Protein Synthesis in Vitro by a Clostridial System

I. SPECIFICITY IN THE TRANSLATION OF NATURAL MESSENGER RIBONUCLEIC ACIDS*

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SUMMARY

The homologous ribosomal systems from Escherichia coli and Clostridium pasteurianum were compared for their ability to translate f2 RNA, formaldehyde-treated f2 RNA, T4 early messenger RNA, E. coli messenger RNA, and C. pasteurianum messenger RNA in protein synthesis assays in vitro. The E. coli ribosomal system translated all five of these messengers, while the C. pasteurianum ribosomal system translated only the C. pasteurianum messenger RNA. The two ribosomal systems also had different characteristic magnesium profiles and exhibited different levels of endogenous activity in the protein synthesis assays. The messenger RNA responsible for the C. pasteurianum endogenous activity was shown to occur in the salt-washed ribosomes and not in the initiation factor fraction. The formaldehyde-treated f2 RNA and the C. pasteurianum messenger RNA exhibited different characteristics from the other types of messenger RNAs in their behavior with the E. coli ribosomal system in the protein synthesis assay.

The two homologous ribosomal systems were also tested for their ability to bind [14C]formyl-Met-tRNA in response to different types of mRNA. The E. coli system bound fMet-tRNA in response to synthetic poly(A,G,U), f2 RNA, T4 early mRNA, and C. pasteurianum mRNA. The C. pasteurianum ribosomal system bound fMet-tRNA in response to poly(A,G,U) and C. pasteurianum mRNA but not f2 RNA or T4 early mRNA.

Initiation of protein synthesis by bacterial ribosomal systems has been a subject of intensive investigation in the past few years. There have been numerous recent advances in the purification of initiation factors (1-5) and in the study of the mechanism of the initiation process in vitro (4-13). However, the mechanism by which ribosomes recognize the proper initiation sites on messenger RNA is not well understood. The RNA triplet AUG now appears to be the initiation codon on mRNA (14, 15), but it has not yet been determined whether or not the behavior of these viral messengers can be taken as representative of the behavior of bacterial cellular mRNA.

The bulk of the research on the bacterial initiation process has been carried out with Escherichia coli. Investigation in vitro of protein synthesizing systems from other procaryotic organisms has already revealed some evidence for species-specific differences in the ability of ribosomal systems to recognize initiation sites on the RNA from RNA-bacteriophages (19, 20), but further investigations of species other than E. coli are necessary to determine which aspects of the mechanism for initiation of protein synthesis by bacterial ribosomal systems are common to all species and which are species specific.

An amino acid incorporating system from Clostridium pasteurianum has been described previously (21). This paper describes additional properties of a modified ribosomal system derived from C. pasteurianum and presents evidence for species specificity in the translation of several types of natural mRNA by homologous ribosomal systems from E. coli and C. pasteurianum. The ribosomal system from E. coli translates f2 RNA, formaldehyde-treated f2 RNA, T4 early mRNA, E. coli mRNA, and C. pasteurianum mRNA; the ribosomal system from C. pasteurianum translates the C. pasteurianum mRNA but none of the other four types of RNA tested.

[14C]Formyl-Met-tRNA binding assays were also performed using the two homologous ribosomal systems and different types of mRNA. The specificity exhibited in the binding assay by the two ribosomal systems toward the different types of mRNA was analogous to the specificity exhibited by the two systems in the protein synthesis assays.

The roles of the salt-washed ribosomes and the initiation factors in determining the specificity of mRNA recognition were investigated by testing the heterologous combinations of components derived from E. coli and C. pasteurianum cells and will be described in the following paper (22).

EXPERIMENTAL PROCEDURE

Materials

The bacterial strains used were Escherichia coli MRF 600, kindly provided by Dr. M. Grunberg-Manago (Institut de Bio-
logic Physico-chimique, Paris, France), and Clostridium pasteurianum (American Type Culture Collection No. 6013). Bacteriophage T4D+ and E. coli B5 strain were obtained from Dr. Edward Brody (Institut de Biologie Physico-chimique, Paris, France). E. coli strain Q13 was obtained from Dr. R. C. Valentine (University of California, San Diego, California).

(l)-Formyltetrahydrofolate and (l)-[14C]formyl-tetrahydrofolate were prepared by the method of Samuel et al. (23). L-[2,3,4,5-3H]Valine (19 Ci per mmole) was purchased from Amersham-Searle; phosphoenolpyruvate (mno sodium salt) and pyruvate kinase, from Boehringer-Mannheim; ATP, from Miles; GTP, from Sigma; E. coli B stripped tRNA, from General Biochemicals. Synthetic poly(A, G, U: 2, 1, 2) was also provided by Dr. Grunberg-Manago.

Methods

Growth of Cells and Preparation of Ribosomes—E. coli MRE 600 cells were grown in a 200-liter New Brunswick Fermentor at 37° under forced aeration. The growth medium contained, per liter: 13.6 g of KH2PO4, 2.9 g of (NH4)2SO4, 5.5 g of KOH, 0.2 g of MgSO4.7 H2O, 4.0 g of Difco Casamino Acids, 1.0 g of Difco Yeast Extract, and 10.0 g of dextrose (anhydrous). A 1% inoculum from an overnight culture was used. When the A680 reached 2 (late log phase), the fermentor was chilled, and the cells were harvested on an electric Sharples Centrifuge. The yield was 3 g per liter, wet weight. The cells were stored frozen at -90°.

The salt-washed ribosomes were prepared by a modification of the method of Lelong et al. (24). All operations were carried out at 4°. Two-hundred grams of frozen cells were thawed and ground with two weights of alumina (Alcoa, A305). The paste was extracted by stirring on a magnetic stirrer with 350 ml of Buffer A (10 mM Tris-chloride buffer, pH 7.8, 10 mM NH4Cl, 10 mM magnesium acetate, 7 mM 2-mercaptoethanol). An S30 fraction was prepared by centrifuging the suspension for 20 min at 20,000 x g and then recentrifuging the supernatant solution twice for 30 min at 30,000 x g; the pellet was discarded each time. The S30 fraction (about 200 ml) was dialyzed overnight against two 4-liter volumes of Buffer A and then recenterfuged for 30 min at 30,000 x g. The dried S30 fraction was then centrifuged in two portions for 4 hours at 45,000 rpm in a Spinco No. 50 fixed-angle rotor (125,000 x g). The supernatant solution was poured off, and the pellets were resuspended in a total volume of 100 ml of Buffer B (10 mM Tris-chloride buffer, 1 mM NH4Cl, 40 mM magnesium acetate, 2 mM EDTA, 10 mM 2-mercaptoethanol, adjusted to pH 7.5 with NH4OH) and stirred slowly overnight. The suspension was centrifuged 10 min at 30,000 x g, and the pellet was discarded. The salt-washed ribosomes were pelleted by centrifuging 6 hours at 125,000 x g. The supernatant solution was poured off and stored frozen as the source of initiation factors. The pellet was resuspended in 100 ml of Buffer B, stirred overnight, and centrifuged as before. The pellets were resuspended in 50 ml of Buffer C (10 mM Tris-chloride buffer, pH 7.8, 50 mM NH4Cl, 10 mM magnesium acetate, 7 mM 2-mercaptoethanol), and centrifuged 3 hours at 125,000 x g. This final pellet was taken up in Buffer C to a final volume of 20 ml, clarified by a low speed spin, and frozen in small portions at -90°. The A260 of an aliquot of the final preparation was 1200 for 1 cm lightpath.

C. pasteurianum cells for culture were stored lyophilized on sterile filter paper discs under vacuum. Cultures were started by inoculating a tube of "potato medium" with a disc (25) and were subsequently transferred to synthetic medium (25). For preparative cultures (10 to 200 liters), 0.005% CaCl2 was substituted for the CaCO3 in the synthetic medium. To grow a 200-liter culture, the necessary 10 liters of log phase inoculum was prepared as follows. Transfers were made every 12 hours, using a 5 to 10% inoculum, to successively larger volumes of medium; growth was carried out without shaking or aeration at 25-30° in 0.1- to 1-liter Florence flasks which were filled to the neck with medium and plugged with cotton. Each transfer was made when the culture reached an A680 of 2 to 4. For growth of the 10-liter inoculum, a 15-liter New Brunswick fermentor unit was used, and the sterile medium was bubbled with purified nitrogen gas continuously before inoculation and during growth, which was at 30°. The 200-liter culture was grown in a New Brunswick Fermentor at 30° with automatic pH control, set at 6.5. Cells grown without pH control were found to be unsuitable for preparation of a system for protein synthesis in vitro.3 The cells were harvested at an A680 of 5 to 6 (midlog phase) on an electric Sharples Centrifuge. The yield was 6 g per liter, wet weight. The cells were stored at -90°.

C. pasteurianum salt-washed ribosomes and washed, preincubated ribosomes were prepared as follows. Thirty grams of frozen cells were thawed and suspended in 60 ml of Buffer D (10 mM Tris-chloride buffer, pH 7.6, 60 mM KCl, 14 mM magnesium acetate, 20 mM 2-mercaptoethanol). They were disrupted by subjecting them to sonic oscillation from a Branson Sonifier Cell Disrupter for 3 min in a beaker kept in an ice bath. The resulting suspension was then centrifuged twice for 30 min at 30,000 x g, the pellet was discarded each time. The ribosome pelleting and NH4Cl washing procedures were identical to those described above for E. coli ribosomes, except that for C. pasteurianum ribosomes Buffer B contained 2 mM NH4Cl and 20 mM 2-mercaptoethanol.

To prepare salt-washed (nonpreincubated) ribosomes, the pellet from the second NH4Cl washing was taken up in 40 ml of Buffer D and repelleted by centrifuging 4 hours at 125,000 x g. The final pellet was resuspended in Buffer D to a final volume of 4 ml; the final suspension was clarified by a low speed spin and frozen in small portions at -90°. The A260 was 1800.

To prepare salt-washed, preincubated ribosomes, the pellet from the second NH4Cl washing was taken up in Buffer D to a final volume of 6.0 ml. The A260 was 1740. The suspension was dialyzed against 3 liters of Buffer D for 3 hours to remove residual NH4Cl. These ribosomes were then used directly in the preincubation reaction. The composition of the preincubation reaction, using the above 5.0 ml of salt-washed ribosomes, was the same as for a normal protein synthesis assay (described later in the "Methods" section), with the following modifications. The total volume of the reaction was 25 ml; the Mg++ concentration in the reaction was 15 mM; unlabeled valine was substituted for the L-[2,3,4,5-3H]valine; 10-formyltetrahydrofolate, initiation factors, and exogenous mRNA were omitted. The reaction mixture was brought quickly to 37°, incubated at that temperature for 25 min, then cooled on ice. The mixture was then centrifuged for 10 min at 30,000 x g. The supernatant solution was layered over 20% sucrose containing Buffer D (2.5 ml in the bottom of each of four 10-ml nitrocellulose tubes) and centrifuged overnight (10 hours) at 125,000 x g. The supernatant solution was removed carefully. The pellets were then taken up in Buffer D to a final volume of 6 ml; the final suspension was clarified by a low speed spin and frozen in small portions at -90°. The A260 was 1100.

3 J. C. Rabinowitz and C. L. Murray, unpublished results.
Preparation of Initiation Factors—The method used was the same for both E. coli and C. pasteurianum. To 10 ml of supernatant solution from the first NH₄SO₄ washing, 0.2 g of finely ground (NH₄)₂SO₄ were added slowly with stirring. The solution was stirred for 1 hour at 4°C. After being decanted from any residual (NH₄)₂SO₄, the solution was centrifuged 15 min at 20,000 × g. The pellet was taken up in Buffer E (20 mM Tris-chloride buffer, pH 7.25, 20 mM NH₄Cl, 2 mM magnesium acetate, 7 mM 2-mercaptoethanol, 5% (v/v) glycerol) to a final volume of about 3 ml with a final protein concentration of about 17 mg per ml. The solution was dialyzed against Buffer E and then frozen at −90°C in small portions.

Preparations of Messenger RNA—For preparation of f₂ RNA, f₂ phage were grown on E. coli strain Q13. The bacteria were grown in 5-liter New Brunswick fermentor units at 37°C in R broth (26) to an A₆₀₀ of 0.4. Phage f₂ was then added at a multiplicity of infection of about 30. Incubation and aeration were continued for 4 hours, after which the cultures were cooled. Purification of the phage from the crude lysate was accomplished by the method of Gesteland and Boedtker (27) except that the methanol precipitation step was omitted. The procedure included ammonium sulfate precipitation, pelleting of phage by ultracentrifugation, and finally CsCl banding. The f₂ RNA was obtained from the concentrated phage suspension by extracting twice with phenol at 4°C and then precipitating the RNA from the aqueous phase and washing it with ethanol.

For preparation of T₄ early mRNA, phage T₄D⁺ was grown on E. coli strain B². The bacteria were grown and infected at 30°C by Method III as described by Bolle et al. (28). Incubation was continued for 5 min after infection, and then the culture was rapidly chilled by pouring it over crushed, frozen M9 medium. The T₄ early mRNA was prepared from the cells by the method of Salser et al. (29).

E. coli mRNA was prepared from uninfected E. coli B² cells, grown to an A₆₀₀ of 1.0 by the same method used for preparation of T₄ mRNA above.

C. pasteurianum mRNA was obtained from a freshly prepared C. pasteurianum crude polysomal lysate by phenol extraction, using the same extracting procedure as for T₄ mRNA above. The C. pasteurianum lysate was prepared by the method of Brodick and Rabinowitz.

C. pasteurianum ribosomal RNA was obtained by phenol extraction of salt-washed ribosomes, using the same method of extraction as for T₄ mRNA above.

All RNA preparations were stored in small portions at −90°C.

Preparation of High Speed Supernatant Fraction—The high speed supernatant fraction (S₁₅₀) was prepared from E. coli A₁₉ cells by sonic oscillation. The resulting solution was centrifuged at 150,000 × g for 3 hours. The supernatant solution was dialyzed against Buffer D and stored in small portions at −90°C.

Assay for Protein Synthesis in Vitro—The assay for protein synthesis in vitro, used for measuring the incorporation of L-[2,3-³H]valine into acid-insoluble material, was based on the assay described by Nirenberg (30). The standard assay contained the following ingredients in a total volume of 0.125 ml: 64 mM Tris-chloride buffer, pH 7.6; 5.5 to 6.0 mM magnesium acetate (5.5 mM for E. coli ribosomes, 6.0 mM for C. pasteurianum ribosomes); 80 mM NH₄Cl; 1.0 mM GTP; 1.0 mM dithiothreitol; 3 A₆₅₀ units of ribosomes; 50 μg of crude initiation factors; 1.0 A₆₅₀ unit of formyl-methionyl-tRNA in the filter discs was determined by comparing the counts per min obtained from equal portions of L-[2,3-³H]valyl-tRNA (a) precipitated into a filter disc and counted in the toluene scintillation fluid; and (b) dissolved in water and counted in Bray's solution. The counting efficiency of an aqueous tritium sample counted in Bray's solution was determined by using a 15-H2O-water standard.

* J. W. Brodick and J. C. Rabinowitz, manuscript in preparation.

* M. R. Stallcup and J. C. Rabinowitz, unpublished results.
formyl-Met-tRNA was added last, and the incubation was immediately started in a 37°C water bath. After incubation for 15 min, the assay was diluted with about 2.9 ml of cold assay buffer (50 mM Tris-chloride buffer, pH 7.4; 5 mM magnesium acetate; 80 mM NH₄Cl) and filtered immediately through a nitrocellulose filter (Millipore, type HA, 0.45 μ pore size). The filter was washed three times with cold assay buffer, dried, and counted in the toluene scintillation fluid described above. The specific activity of the [14C]formyl-Met-tRNA on the Millipore filter, corrected for a counting efficiency of 80%, was 88 cpm per pmole. The assay results are expressed as picomoles of fMet-tRNA bound without subtraction of control blank values. The background for the scintillation counter used was 12 cpm. Time studies of fMet-tRNA binding to ribosomes at 37°C indicated that the reaction was 80 to 90% complete after 5 min and was complete after 10 to 12 min.

RESULTS AND DISCUSSION

Magnesium Dependence of Protein Synthesizing Systems—Fig. 1, a and b, shows the magnesium dependence of valine incorporation into protein by the E. coli and C. pasteurianum ribosomal systems, respectively, in response to their endogenous messengers and to four exogenous messengers, f2 RNA, T4 early mRNA, E. coli mRNA, and C. pasteurianum mRNA. All four exogenous messengers stimulate valine incorporation by the E. coli ribosomal system far above the endogenous level (Fig. 1a). However, with the C. pasteurianum system, only the C. pasteurianum mRNA stimulates protein synthesis above the endogenous level; the addition of any of the other three messengers to the assay results in no increase in valine incorporation above the endogenous level (Fig. 1b). Greater stimulation of amino acid incorporation by the C. pasteurianum mRNA is observed when larger amounts of the mRNA are used. Table I shows that omitting salt-washed ribosomes or supernatant fraction from the assay reduces amino acid incorporation to a very low level.

The concentration of Mg++ for optimal amino acid incorporation with the E. coli ribosomal system varies with the different messengers. The magnesium profiles also indicate that the C. pasteurianum ribosomal system has a somewhat higher Mg++ optimum for protein synthesis than does the E. coli ribosomal system. For this reason, different Mg++ concentrations, 11 mM and 15 mM, were used in further experiments to assay the ribosomal systems of E. coli and C. pasteurianum, respectively.

Reduction of Level of Endogenous Activity in C. pasteurianum Ribosomes—Another difference in the results obtained with ribosomes from the two species is in the level of endogenous activity. As shown in Fig. 1, a and b, C. pasteurianum ribosomes exhibit more than twice the level of endogenous activity as an equivalent amount of E. coli ribosomes. This relation-

![Diagram](image-url)

**Fig. 1.** L-[2,3-3H]Valine incorporation into protein by the two ribosomal systems in the presence of various mRNA preparations as a function of the concentration of Mg++ added to the assay. The assay conditions were the same as in Fig. 1. The Mg++ concentration was 11 mM for E. coli ribosomes and 15 mM for C. pasteurianum ribosomes.

**Table I**

**Dependence of protein synthesis on ribosomes, supernatant fraction, and initiation factors**

| mRNA                  | Omissions | L-[2,3-3H]Valine incorporated |
|-----------------------|-----------|-------------------------------|
|                       | E. coli ribosomal system | C. pasteurianum ribosomal system |
| Endogenous            | None      | 121 272                       |
|                       | Initiation factors | 14 77                         |
| C. pasteurianum mRNA  | None      | 605 493                       |
|                       | Salt-washed ribosomes | 5 3                           |
|                       | Supernatant fraction | 5190                          |

The assay conditions were the same as in Fig. 1. The Mg++ concentration was 11 mM for E. coli ribosomes and 15 mM for C. pasteurianum ribosomes.
The conditions for the assay were those given under "Methods," except that 5.2 A260 units of ribosomes were used. The Mg++ concentration was 15 mM. C. pasteurianum mRNA, 6.6 A260 units, was used.

| Additions | \( \text{L-[2,3-3H]} \text{Valine incorporated} \) |
|-----------|----------------------------------|
|           | C. pasteurianum salt-washed ribosomes | C. pasteurianum salt-washed and preincubated ribosomes |
| a. None (endogenous activity) | 292 | 112 |
| b. C. pasteurianum mRNA | 506 | 316 |
| Net synthesis (b - a) | 214 | 205 |

The endogenous activity could be reduced by about two-thirds, without any significant reduction in the response to added C. pasteurianum mRNA, by preincubating the salt-washed ribosomes in a protein synthesis assay under conditions unfavorable for reinitiation (by omitting formyltetrahydrofolate and initiation factors). The details of the preincubation and recovery of the ribosomes are given under "Methods." Table II shows a comparison of the protein synthesis activities of the salt-washed ribosomes and the salt-washed, preincubated ribosomes.

Sucrose gradient profiles of the salt-washed C. pasteurianum ribosomes indicated the presence of a relatively small amount of material sedimenting faster than 70 S ribosomes; the salt-washed ribosome preparation consists of about half 70 S ribosomes and half subunits, while the salt-washed, preincubated ribosomes consist of 80 to 90% subunits. Therefore, the RNA responsible for the endogenous activity probably consists of small fragments of mRNA retained by the salt-washed 70 S ribosomes and released during the preincubation treatment. Although the endogenous messenger fragments do not come from the initiation factor fraction, the endogenous protein synthesis activity is dependent upon the presence of initiation factors (Table I), which suggests that reinitiation by the ribosomes on these small messenger fragments is occurring.
Protein Synthesis in Response to Varied Amounts of Messenger RNA Preparations—Fig. 2, a and b, shows the response of E. coli salt-washed ribosomes and initiation factors (2a) and of C. pasteurianum salt-washed, preincubated ribosomes and initiation factors (2b) to four different types of mRNA and to C. pasteurianum ribosomal RNA. These curves show, more strikingly than the magnesium-dependence curves in Fig. 1, that the E. coli ribosomal system synthesizes protein in response to all four mRNA preparations, while the C. pasteurianum system responds only to C. pasteurianum mRNA and not at all to the other three mRNA preparations. C. pasteurianum rRNA serves as a control for both ribosomal systems. Its failure to stimulate amino acid incorporation indicates that rRNA is not the active component of the C. pasteurianum mRNA preparation, which is a polysomal extract and therefore contains rRNA. The points on the ordinates of Fig. 2, a and b represent the levels of endogenous activity. The f2 RNA preparation does not contain contaminating ribosomal RNA as do the other mRNA preparations, and therefore it has a much higher specific activity than the other preparations in the assay with the E. coli ribosomal system.

In these mRNA curves, the C. pasteurianum mRNA exhibits some properties different from the other three messengers. In the assay with the E. coli ribosomal system, the C. pasteurianum mRNA is saturating at a lower level of RNA than the other two cellular RNA preparations, T4 early mRNA and E. coli mRNA. Even more striking is the fact that C. pasteurianum mRNA stimulates protein synthesis by E. coli and C. pasteurianum salt-washed ribosomes in the absence of any added initiation factors, whereas all of the other messengers exhibit absolute initiation factor dependence.

Fig. 2 shows that C. pasteurianum mRNA is more active in protein synthesis with the E. coli ribosomal system than with the C. pasteurianum ribosomal system. This result could be attributed to (i) more efficient (faster) translation by the E. coli ribosomal system; or (ii) initiation by the E. coli system at a larger number of sites on the C. pasteurianum mRNA.

Formaldehyde-treated f2 RNA—f2 RNA was treated with formaldehyde by the method of Lodish (17), who showed that this treatment unfolds the secondary structure of the f2 RNA molecule, with the result that initiation of protein synthesis can occur at several new positions, not accessible on the native f2 RNA. The formaldehyde-treated f2 RNA (HCHO-f2 RNA) was still found to be inactive in assay with the C. pasteurianum ribosomal system, as shown in Fig. 3, which shows valine incorporation measured as a function of mRNA concentration. The HCHO-f2 RNA was active with the E. coli ribosomal system, but as Fig. 3 shows, its behavior with E. coli ribosomes is different from that of the untreated f2 RNA. The HCHO-f2 RNA is saturating at a much lower level than the untreated f2 RNA; and the treated RNA stimulates some protein synthesis by E. coli salt-washed ribosomes in the absence of initiation factors, while untreated f2 RNA elicits no activity without initiation factors. In these

![Graph showing incorporation of L-[2,3-3H]valine into protein as a function of the amount of mRNA added to the assay](http://www.jbc.org/)

![Graph showing binding of [3H]formyl-Met-tRNA by the E. coli ribosomal system (a) and by the C. pasteurianum preincubated ribosomal system (b) as a function of the amount of mRNA added to the assay](http://www.jbc.org/)
two characteristics, the HCHO-f2 RNA interacts with E. coli ribosomes in a manner similar to C. pasteurianum mRNA. Poly (A, G, U) and C. pasteurianum [14C]formyl-Met-tRNA binding assays carried out for the E. coli ribosomal system (4a) and for the C. pasteurianum ribosomal system (4b) with five different types of mRNA: synthetic Poly(A, G, U), f2 RNA, T4 early mRNA, E. coli mRNA, and C. pasteurianum mRNA. Poly(A, G, U) and C. pasteurianum mRNA stimulate fMet-tRNA binding by both the E. coli and the C. pasteurianum ribosomal systems; f2 RNA and T4 early mRNA stimulate binding by the E. coli ribosomal system, but not by the C. pasteurianum ribosomal system; and E. coli mRNA does not give a significant stimulation of fMet-tRNA binding by either ribosomal system (although it is active as a messenger for protein synthesis with the E. coli ribosomal system). The results with f2 RNA, T4 early mRNA, and C. pasteurianum mRNA reflect the same messenger specificity for the two ribosomal systems that they exhibited in the protein synthesis assays. Therefore, the ability or inability of each ribosomal system to translate each of these three types of mRNA in protein synthesis assays is reflected by a corresponding ability or inability to form a protein synthesis initiation complex involving fMet-tRNA in the binding assays.

Although the amount of fMet-tRNA binding stimulated by the C. pasteurianum mRNA with the C. pasteurianum ribosomal system is small (about 150 cpm over an endogenous level of 400 cpm), it is entirely reproducible, and it becomes significant by consideration of the fact that f2 RNA, T4 early mRNA, and E. coli mRNA give no stimulation of fMet-tRNA binding over the endogenous level with the C. pasteurianum ribosomal system (Fig. 4b).

Conclusions—The difference in specificities exhibited by the homologous ribosomal systems from E. coli and C. pasteurianum indicates some difference in their manner of recognition of initiation sites on messenger RNA. The fact that the C. pasteurianum system does not respond at all to several messengers which the E. coli system can translate, makes this a good system with which to study certain aspects of the mechanism of initiation of protein synthesis. Further investigations into the nature and cause of the type of species specificity reported in this paper should provide more information about the interactions responsible for the specific nature of protein synthesis initiation.

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