RIBOSOME AND MESSENGER SPECIFICITY IN PROTEIN SYNTHESIS BY BACTERIA

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Received March 19,1974

SUMMARY: Ribosomes from *Escherichia coli* and *Pseudomonas fluorescens*, two Gram-negative bacteria, translated messenger RNA preparations from all bacteria tested (3 Gram-negative species, and 6 Gram-positive species) as well as f2 RNA and T4 early messenger RNA. Ribosomes from *Clostridium pasteurianum*, *Streptococcus faecalis*, and *Bacillus subtilis*, three Gram-positive organisms, did not translate messenger RNA preparations derived from any of the Gram-negative organisms, the f2 RNA, or the T4 early messenger RNA but did translate messenger preparations from all 6 Gram-positive bacterial species tested.

Several recent reports have documented the fact that ribosomes from different species of bacteria have different specificities for the translation of natural messengers. Lodish found that ribosomes from *Escherichia coli* could translate all three cistrons of the f2 RNA molecule, while the ribosomes from *Bacillus stearothermophilus* could translate only the cistron coding for the A-protein (1). Leffler and Szer found that *E. coli* ribosomes could translate RNA from MS2 bacteriophage, an *E. coli*-specific RNA phage, but not RNA from Cb5 bacteriophage, a *Caulobacter crescentus*-specific RNA phage; while ribosomes from *C. crescentus* could translate Cb5 RNA but not MS2 RNA (2). Stallcup and Rabinowitz demonstrated differences in the abilities of ribosomes from *E. coli* and *Clostridium pasteurianum* to translate several natural messengers (3,4). In all of these studies the salt-washed ribosomes, and not the initiation factors, determined the absolute specificity. Initiation factors have also been shown to discriminate between different messengers, but their specificity only influences the translation in a quantitative manner and is subordinate to the absolute specificity determined by the ribosomes (4-9).

We have shown previously that ribosomes from *E. coli* could translate f2 RNA, T4 early messenger RNA (mRNA), *E. coli* mRNA, and *C. pasteurianum* mRNA,

while ribosomes from *C. pasteurianum* could translate *C. pasteurianum* mRNA but not the other three messengers (3,4). We have now extended our studies on translational specificity among bacterial ribosomes to include ribosomes, initiation factors and mRNA from several more species of bacteria.

EXPERIMENTAL PROCEDURE:

All materials were the same as those given previously (3), unless otherwise stated. The procedures for the preparations of *E. coli* MRE 600 salt-washed ribosomes and crude initiation factors, *C. pasteurianum* salt-washed, preincubated ribosomes and crude initiation factors, f2 RNA, T4 early mRNA, *E. coli* mRNA, and *C. pasteurianum* mRNA have been given previously, as were the details of the assay for protein synthesis *in vitro* (3).

Culture conditions for *Escherichia coli*, MRE 600 (for ribosomes), *E. coli*, B^E (for mRNA), and *Clostridium pasteurianum* (ATCC 6013) were as previously described (3,4). Conditions for the culture of *Clostridium acidi-urici* 9a (ATCC 7906) (10), *Clostridium tetanomorphum* H1 (ATCC 15920) (11), *Streptococcus faecalis* R (ATCC 8043) (12), *Peptococcus aerogenes* (ATCC 14963) (13), *Bacillus subtilis* (UC Strain 4) (14), *Pseudomonas fluorescens*, C-18 (phosphate-lactate-yeast extract medium), and *Azotobacter vinelandii*, OP (15) were as indicated.

Salt-washed ribosomes and crude initiation factors were prepared from *S. faecalis* cells by the method used for *E. coli* (3); *B. subtilis* and *P. fluorescens* cells were broken by sonic oscillation, and the salt-washed ribosomes and crude initiation factors were prepared from these extracts by the procedure used for *E. coli* (3).

Messenger RNA preparations from bacteria other than *C. pasteurianum* were prepared from rapidly harvested growing cells essentially by the procedure used to prepare T4 early mRNA (3). However, the Gram-positive bacteria required additional lysozyme and 2-3 min of incubation at 37 C with the lysozyme before they were rendered susceptible to lysis by sodium dodecyl sulfate.

The high-speed supernatant fraction (S-150) used in the protein synthesis assays was prepared from *E. coli* MRE 600. The post-ribosomal supernatant was

TABLE I. TRANSLATION OF ELEVEN DIFFERENT NATURAL MESSENGERS BY RIBOSOMES AND INITIATION FACTORS FROM FIVE DIFFERENT SPECIES OF BACTERIA.

mRNA	Gram Stain	Amount of mRNA	Source of Salt Washed Ribosomes and Initiation Factors				
			Escherichia coli	*Pseudomonas fluorescens**	*Clostridium pasteurianum*	*Streptococcus faecalis*	*Bacillus subtilis*
		A_{260} units	picomoles of valine incorporated into protein				
None			31	5	61	15	13
f_2RNA	(-)	2	749	41	60	16	14
T_4Early	(-)	5	260	87	60	25	23
E. coli	negative	7	170	64	61	20	17
P. fluorescens	negative	6	149	28	63	19	15
A. vinelandii	negative	5.5	89	12	64	24	11
C. pasteurianum	positive	6	258	113	272	53	127
C. acidi-urici	positive	4	218		169	70	
C. tetanomorphum	positive	2.5	207		125	56	
S. faecalis	positive	5	440	64	120	89	145
B. subtilis	positive	5	453	97	152	110	208
P. aerogenes	positive	2.5	196	36	99	30	50

Each assay contained approximately 3 A_{260} units of salt-washed ribosomes and 0.1-0.17 mg of crude initiation factors. The results are expressed in picomoles of L-[2,3-$^3H_2$]valine incorporated into protein, without subtraction of control blank values. The magnesium concentrations used were close to the optimal values for each type of ribosomes: *E. coli*, 11 mM; *C. pasteurianum*, 15 mM; *S. faecalis*, 13 mM; *P. fluorescens*, 11 mM; *B. subtilis*, 9 mM. The data in Table I was compiled from several separate sets of assays. Since the relative amount of activity varied from one set of assays to the next, all the data have been normalized with respect to the average endogenous activity of each type of ribosome. The ratios of the activity with and without added exogenous mRNA (that is, the ratios of total protein synthesis activity to endogenous activity) were not altered by the normalization.

*With *P. fluorescens* salt-washed ribosomes, *E. coli* crude initiation factors were used instead of *P. fluorescens* initiation factors.

concentrated by ammonium sulfate precipitation; amino acids and other small molecules were removed by desalting on "Sephadex" G25, and nucleic acids were removed by passing the S-150 over a DEAE-cellulose column.

RESULTS:

Table I shows the protein synthesizing activity of the ribosomes and initiation factors from five different species of bacteria in response to mRNA preparations from nine different species of bacteria and from two bacteriophages. Protein synthesizing activity varied greatly among the five different types of ribosomes examined. In interpreting the data, we have considered 1) the absolute amount of protein synthesizing activity stimulated by a particular messenger after subtracting the endogenous activity; and 2) the ratio of total protein synthesizing activity stimulated by a particular messenger to the endogenous activity.

E. coli ribosomes showed an intermediate level of endogenous activity, and they translated all eleven exogenous messengers. Even with *A. vinelandii* mRNA, the least active of all the messengers tested, the addition of mRNA stimulated the total protein synthesis activity to approximately 3 times the endogenous level.

P. fluorescens ribosomes, like *E. coli* ribosomes, were stimulated by all eleven messengers. The absolute amount of stimulation by *P. fluorescens* and *A. vinelandii* messengers was small, but it should be noted that these two mRNAs were the least active of the eleven messengers with *E. coli* ribosomes; the ratios of total protein synthesis to endogenous activity for these two messengers with *P. fluorescens* ribosomes were comparable to the ratios for these messengers with *E. coli* ribosomes. The results shown for *P. fluorescens* ribosomes with the various messengers were obtained in the presence of *E. coli* initiation factors rather than *P. fluorescens* initiation factors. When *P. fluorescens* initiation factors were used, the results were qualitatively the same (that is, all eleven messengers were translated), but the activities were lower. Since the *P. fluorescens* ribosomes themselves were already considerably

less active than the other types of ribosomes, the *E. coli* initiation factors were used to help increase the sensitivity of the assays.

C. pasteurianum ribosomes had the highest level of endogenous activity of all five types examined. Unlike the ribosomes from *E. coli* and *P. fluorescens*, *C. pasteurianum* ribosomes were completely inactive with f2 RNA, T4 early mRNA, and the mRNAs from *E. coli*, *P. fluorescens*, and *A. vinelandii*. The *C. pasteurianum* ribosomes were active with the other six messengers.

S. faecalis and *B. subtilis* ribosomes behaved very much like *C. pasteurianum* ribosomes. They gave very little or no activity in response to f2 RNA, T4 early mRNA, and the mRNAs from *E. coli*, *P. fluorescens*, and *A. vinelandii* but they were active with the other other types of mRNA tested. The inability of *B. subtilis* ribosomes to translate f2 RNA in the studies reported here is unexpected in view of the previous report of Lodish (1) that f2 RNA did stimulate synthesis of A protein by *B. stearothermophilus*. Additional studies may resolve this point. However, it should be noted that the methods used for detection of protein synthesis in the two studies differ.

For all five species of bacteria whose ribosomes and initiation factors were examined in these studies, it was the salt-washed ribosomes, and not the initiation factors, which determined the translation specificity. The same qualitative results were obtained for each type of ribosomes no matter which type of initiation factors was used.

The products from the translation of each of the eleven messengers by *E. coli*, *C. pasteurianum*, and *S. faecalis* ribosomes were examined by slab-SDS-polyacrylamide gel electrophoresis and autoradiography. The results from the autoradiograms of the gels agreed with the quantitative data from the protein synthesis assays; that is, whenever the protein synthesis assays indicated that the addition of a particular mRNA stimulated additional protein synthesis activity by ribosomes, the autoradiograms showed the appearance of new product bands in response to the addition of the messenger; however, when the quantitative assays indicated that a messenger was not translated by a particular

type of ribosomes, the autoradiograms showed that the product bands formed in the presence of the messenger were not new bands but were merely due to the endogenous activity of the ribosomes. The autoradiograms showed that in cases where translation did occur, a broad spectrum of protein products of both high and low molecular weights was produced; each messenger produced a unique, characteristic pattern of products.

DISCUSSION:

From Table I it is apparent that the five different types of ribosomes may be classified in two different categories relative to their ability to translate the different mRNA preparations. Moreover, the messengers can also be divided into two different classes relative to their ability to stimulate protein synthesis by ribosomes derived of different bacteria.

When all the members within each of the two classes are examined for other points of similarity, a very striking correlation can be drawn: all the messengers and ribosomes in the class with *E. coli* were prepared from Gram-negative bacteria, while all the messengers and ribosomes in the class with *C. pasteurianum* came from Gram-positive bacteria (16,17). Notice that f2 and T4 bacteriophages infect *E. coli*, a Gram-negative bacterium. Thus, the results in Table I can be summarized as follows: the ribosomes from Gram-negative bacteria translated mRNAs from both Gram-negative and Gram-positive species or from *E. coli*-specific phages; the ribosomes from Gram-positive bacteria translated the mRNAs from Gram-positive bacteria but did not translate the mRNAs from the Gram-negative bacteria, or from *E. coli*-specific phages.

The difference in activity of mRNAs in stimulating protein synthesis suggests that at least two different types of protein synthesis initiation sites may exist among the eleven different mRNAs discussed here. The difference in specificities exhibited by the ribosomes from the two different classes indicates that they do not recognize initiation sites on mRNA in the same way. The ribosomes from Gram-positive bacteria appear to have a more restricted specificity

for the type of initiation site which they can recognize. The ribosomes from Gram-negative bacteria appear to have a more general specificity which tolerates both types of initiation sites.

ACKNOWLEDGMENTS:

This investigation was supported by NIH research grant number AM02109 of the National Institute of Arthritis, Metabolism and Digestive Diseases.

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