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Functional Correspondence Between 30S Ribosomal Proteins of *Escherichia coli* and *Bacillus stearothermophilus**

(ribosome reconstitution/immunochemical crossreaction/protein purification/protein structure conservation)

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ABSTRACT **30S ribosomal proteins from *Bacillus stearothermophilus* (*B.* proteins) have been fractionated and characterized with respect to their ability to replace various *E. coli* 30S proteins (*E.* proteins) in the *E. coli* 30S ribosome reconstitution system. The functional counterparts of all the *E.* proteins, except S1, S6, S9, and S13, have been tested. In all cases, *B.* proteins can substitute for *E.* proteins. Several purified *B.* proteins are chemically different from their functionally homologous *E.* proteins. Five *B.* proteins are immunochemically related to *E.* proteins; this set includes two proteins that could not be tested in the reconstitution system (S9 and S13). Thus most, if not all, of the *E.* proteins have functionally equivalent counterparts among *B.* proteins, even though properties of the two ribosomes are different in several respects. These results suggest that the fundamental structural organization of ribosomes may be the same throughout prokaryotic organisms.**

Since all ribosomes from various organisms carry out essentially the same protein synthetic reactions, we would expect that ribosomes from different organisms might have common structural features. The sizes of the ribosomal subunits and ribosomal RNAs are about the same in various prokaryotic organisms (1, 2). However, similarity in size does not mean that different ribosomes utilize the same numbers and kinds of proteins.

Our previous experiments have demonstrated evolutionary conservation of ribosomal structure pertinent to RNA–protein interaction. We have shown that functionally active 30S ribosomal subunits can be reconstituted from 16S RNA of one species of bacteria and the 30S ribosomal-protein mixture from a distantly related species (3). This observation suggests that certain specific regions of 16S RNA that interact with ribosomal proteins have the same or similar structures in several different bacterial species. Conversely, among the various bacterial species examined, those ribosomal proteins that interact with specific regions on the ribosomal RNA should have structural features in common.

We have now extended our previous work and asked whether ribosomal proteins from distantly related bacterial species can be shown to be functionally equivalent on a one-to-one basis. For this purpose, we fractionated 30S ribosomal proteins from *Bacillus stearothermophilus* (*B.* proteins) and looked for functional correspondence between these proteins and *Escherichia coli* 30S ribosomal proteins (*E.* proteins) using the reconstitution technique. These bacteria were chosen because of known differences. There are distinct chemical differences between the 16S RNAs of the two species (ref. 3, and papers cited therein). Moreover, the proteins from 30S subunits differ with respect to their column chromatographic or gel electrophoretic patterns (refs. 3, 4, and our unpublished experiments), their ability to confer heat resistance upon the ribosomal subunit (3), and their immunochemical properties (5). In addition, it is known that *B. stearothermophilus* 30S subunits cannot translate the coat and the replicase cistrons of RNA messenger from f2 and related RNA phages, whereas *E. coli* 30S can (6).

In this paper, we show that most, if not all, of the *E.* proteins have functionally equivalent counterparts among *B.* proteins. The results are consistent with the concept that the fundamental structural organization of ribosomes is the same throughout prokaryotic organisms.

MATERIALS AND METHODS

E. coli strain Q13 and *B. stearothermophilus* strain 799 were used, as in previous work (3). The methods of preparation of ribosomal subunits, ribosomal RNA, and total ribosomal protein mixtures from *E. coli* and *B. stearothermophilus* are described elsewhere (7, 8). Reconstitution of 30S subunits and the assay of their activity were done as described (3, 7). The 21 pure *E. coli* 30S ribosomal proteins used for reconstitution were obtained by the methods described elsewhere (refs. 9, 10, and manuscript in preparation). Purity of the proteins was confirmed by the two-dimensional gel electrophoretic technique of Kaltschmidt and Wittmann (11). Antisera against the following purified ribosomal proteins were obtained from New Zealand white rabbits as described in a separate paper (manuscript in preparation): S4, S5, S7, S8, S9, S10, S11, S13, S14, S16, S18, S19, S20, and S21. The antisera obtained reacted only with the protein used for injection and did not react with any other purified *E.* proteins.

RESULTS

Fractionation of *B.* proteins

Ribosomal proteins were isolated from 30S subunits of *B. stearothermophilus* and fractionated by phosphocellulose column chromatography (Fig. 1). The protein content of each fraction was examined by polyacrylamide gel electrophoresis (7). Several fractions showed more than one major protein band and some of them were subjected to further purification. For

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example, proteins in fraction XII were separated on Sephadex G-100 (*insert* in Fig. 1) and four proteins, tentatively called XII-1, XII-2, XII-3, and XII-4, were obtained. Although XII-4 was still not completely pure, the other three were found to be essentially pure as judged by 2-dimensional polyacrylamide gel analysis.

Functional correspondence between purified *B.* and *E.* proteins

We found that some of our antisera against particular *E.* proteins crossreact with certain fractionated *B.* proteins. In particular, anti-S11 serum and anti-S19 serum reacted with some proteins in fraction XII. As shown in Fig. 2, purified *B.* protein XII-3 and purified *E.* protein S11 showed a pattern of partial fusion (12) in immunodiffusion with anti-S11 serum, indicating substantial sequence homology (13). Similarly, XII-4 and S19 showed a pattern of partial fusion (Fig. 2).

In order to test the functional correspondence suggested by the immunochemical crossreactions, reconstitution of 30S subunits with *E. coli* 16S RNA and a mixture of 21 purified *E.* proteins was performed. Single *E.* proteins were omitted and replaced by purified *B.* proteins to see which *B.* proteins might substitute in reconstituting functional 30S particles. Poly (U)-dependent polyphenylalanine synthesis was used to assay the activity of the reconstituted particles. As shown in Table 1, each of the four purified *B.* proteins in fraction XII in fact specifically replaced one of the *E.* proteins tested. Thus, XII-1 corresponds to S4, XII-2 to S7, XII-3 to S11, and XII-4 to S19. Titration experiments showed that about as much XII-1 as S4 is required to get the maximum activity in the reconstitution system (data not shown). We conclude that

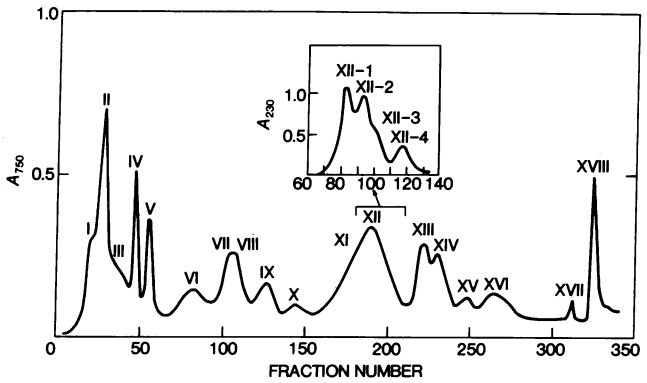

FIG. 1. Phosphocellulose column chromatography of *B. stearothermophilus* 30S ribosomal proteins. Total *B.* proteins [in 0.15 M LiCl–"urea-phosphate buffer" (6 M urea, 0.02 M H_3PO_4 neutralized with methylamine to pH 7.8, 3 mM 2-mercaptoethanol)] were applied on a phosphocellulose column (Mannex-P, Mann Research Lab., 1.9 × 90 cm) equilibrated with 0.15 M LiCl–urea-phosphate buffer. After washing of the column with 150 ml of the same buffer, proteins were eluted from the column with a 3-liter linear 0.2–0.6 M LiCl gradient in the same buffer. At the end of the gradient, fraction 281, 250 ml of 0.6 M LiCl followed by 1 M LiCl in urea–phosphate buffer were added to elute the remaining proteins. Protein concentration was determined by the method of Lowry *et al.* (25). Fractions were pooled and named as indicated. Fraction XII was concentrated to about 7 ml, and fractionated by Sephadex G-100 chromatography with 0.15 M LiCl–urea-phosphate buffer. Protein concentration was monitored by absorbance at 230 nm (see *insert*).

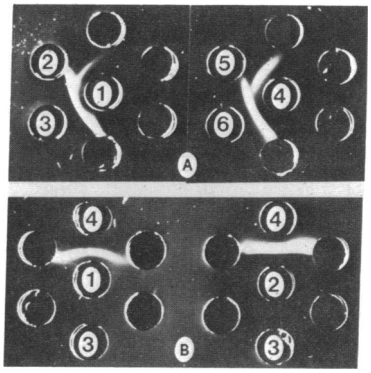

FIG. 2. Immunochemical reactivity of *B. stearothermophilus* proteins that correspond to *E. coli* proteins S11, S19, and S4. Immunodiffusion in 1% agar gels made in 0.03 M Tris·HCl (pH 7.4)–1 M KCl–20 mM $MgCl_2$–6 mM 2-mercaptoethanol. Each 3-mm well contained 25 μl of the indicated solution. Diffusion was carried out for 36 hr at 22°. ***A.*** (1) Anti-S11 (5-fold concentrated). (2) *B.* protein XII-3 40 μg/ml. (3) S11 75 μg/ml. (4) anti-S19 (5-fold concentrated). (5) *B.* protein XII-4 40 μg/ml. (6) S19 38 μg/ml. ***B.*** (1) Anti-S4 (2-fold concentrated). (2) Anti-S4 (10-fold concentrated). (3) *B.* protein XII-1 80 μg/ml. (4) S4 50 μg/ml. Although not shown here, several appropriate control experiments were done. For example, anti-S11 adsorbed with pure *E.* protein S11 did not show any crossreaction with *B.* protein XII-3.

the four *B.* proteins studied are functionally equivalent to *E.* proteins S4, S7, S11, and S19, respectively.

Chemical and immunochemical difference between the functionally corresponding proteins

Although *B.* proteins XII-1, XII-2, XII-3, and XII-4 can functionally substitute for corresponding *E.* proteins, their chemical structures are clearly different from the *E.* proteins. Our anti-S4 serum failed to show any crossreaction with XII-1 (Fig. 2). The pair S11 and XII-3 and the pair S19 and XII-4 are immunochemically related but distinguishable, as noted above (Fig. 2). Polyacrylamide gel electrophoretic mobilities of purified XII-1 and XII-3 at pH 4.5 were similar to those of the corresponding *E.* proteins S4 and S11, respectively. However, XII-2 moved faster than S7, and XII-4 moved slower than the corresponding S19 (data not shown).

The amino-acid compositions of *E.* protein S11 and the corresponding *B.* protein (XII-3) indicated a minimum 7% difference in their amino-acid sequences. Similarly, S4 and S7 showed 10 and 12% minimum sequence differences, respectively, from their corresponding *B.* proteins (XII-1 and XII-2) (our unpublished data)†.

Other proteins

Using the reconstitution technique described above, we looked for *B.* proteins that substitute for the remaining *E.* proteins that are required for reconstitution of functional 30S subunits. As shown in Table 2, in all the cases tested, we have found *B.* proteins that can substitute for *E.* proteins

† Ansley, Campbell, and Sypherd (23) isolated several proteins from 30S subunits of *B. stearothermophilus* and compared their amino-acid compositions with *E. coli* 30S proteins having similar gel electrophoretic mobilities. Since some of the proteins analyzed were clearly mixtures of several proteins and since correspondence between *E.* proteins and *B.* proteins was unknown, the significance of their data remains unclear.

TABLE 1. *Identification of B. stearothermophilus proteins functionally equivalent to E. coli S4, S7, S11, and S19*

Protein mixture (*E. coli*)	Proteins added (origin*)	Activity cpm	Activity %
ΣSi-S4	–	1208	29
	S4 (*E*)	4110	100
	XII-1 (*B*)	3792	92
	XII-4 (*B*)	1069	26
	XII-2 (*B*)	1356	33
ΣSi-S7	–	718	16
	S7 (*E*)	4630	100
	XII-2 (*B*)	3802	82
	XII-1 (*B*)	1307	28
ΣSi-S11	–	686	13
	S11 (*E*)	5148	100
	XII-3 (*B*)	4503	88
	XII-1 (*B*)	605	12
	XII-4 (*B*)	801	16
ΣSi-S19	–	662	17
	S19 (*E*)	3835	100
	XII-4 (*B*)	3794	99
	XII-1 (*B*)	594	16
	XII-3 (*B*)	807	21

Protein mixtures were made from 21 proteins purified from *E. coli* strain Q13 (see Table 2) with one protein omitted, as indicated in the Table. To these protein mixtures, either the omitted *E.* protein or one of the purified *B.* proteins was added. The amount of 16S RNA used for the reconstitution was 0.9 A_{260} units and all the *E.* proteins were added to a 2-fold molar excess (that is, 1.8 A_{260} equivalents). Approximately equivalent amounts (i.e., about 1.8 A_{260} equivalents) of *B.* proteins were added as calculated on the assumption that they have the same molecular weight and extinction coefficients in Lowry's reaction (25) as the corresponding (identified) *E.* proteins. The total volume of the reaction mixture for reconstitution was 240 μl and the final ionic compositions were those described (7). After incubation at 40° for 1 hr, the samples were cooled and 40-μl aliquots were taken to assay directly the poly(U)-dependent polyphenylalanine synthetic activity of the reconstituted particles (7). Blank values obtained by omitting reconstituted 30S particles were subtracted.

* Proteins from *E. coli* and *B. stearothermophilus* are indicated as (*E*) and (*B*), respectively.

in the reconstitution. Unfortunately, *E.* proteins S1, S6, and S13 (and also S20) could not be tested unambiguously because the omission of these proteins from the reconstitution mixture causes at most only a weak decrease in the poly(U)-dependent polyphenylalanine synthesizing activity of the reconstituted particles (9). Similarly, with the present "micro-reconstitution" technique used (see the legend to Table 1), the omission of S9 gave variable results, and therefore, S9 was not tested by the reconstitution technique. (The reason for this variability is currently under study.) However, using immunochemical methods, we have identified three additional *B.* proteins that crossreact with S9, S13, and S20 (Table 2). Thus, all the *E.* proteins, except S1 and S6, were found to have counterparts among *B.* proteins‡.

‡ *E. coli* protein S1 may not be a genuine ribosomal protein, but a ribosome-associated protein that can be removed by a high-salt washing (24). Thus, as surmised by Traut (4), *B. stearothermophilus* 30S subunits may not have a protein equivalent to S1.

Some of the reconstitution experiments shown in Table 2 were done with completely purified *B.* proteins. Other experiments were done with partially purified proteins or fractions from the first phosphocellulose column step. So far, we have purified *B.* proteins corresponding to *E.* proteins S2, S4, S7, S8, S10, S11, S16, and S20 to a nearly homogeneous state. We cannot exclude the possibility that a *B.* protein replaces more than one *E.* protein; rigorous proof of *one-to-one* correspondence between *E.* and *B.* proteins must await purification of all the *B.* proteins. In addition, in our *B.* protein preparations, there appear to be some minor proteins that do not correspond to any of the known *E. coli* 30S proteins. We feel that these are either nonribosomal proteins or 50S proteins contaminating our *B.* protein preparations; further studies are necessary to clarify this problem.

DISCUSSION

As we have disscussed above, ribosomes from *E. coli* and those from *B. stearothermophilus* are significantly different. Taxonomically, the two bacterial species studied are very different. One is mesophilic and non-spore-forming, and belongs to the family *Enterobacteriaceae*. The other is thermophilic and spore-forming, and belongs to the family *Bacillaceae*. The proteins of such different organisms may be expected to be very different from each other, although the amino-acid sequences around the "active centers" of enzymes might be conserved (14–18). Thus, there is no obvious reason why ribosomes from such unrelated organisms must have the same structural organization.

According to the present results, most, if not all, of the 20 *E.* proteins‡ have functionally equivalent counterparts among *B.* proteins. It is known that the *in vitro* assembly of 30S subunits is highly cooperative; binding of some proteins to the particles is strongly dependent on the presence of several other protein components (19). The fact that, in every testable case, a *B.* protein can replace a specific *E.* protein in reconstitution indicates that 30S subunits from these two distantly related bacterial species, and perhaps from all prokaryotic cells, have essentially the same structural organization. This conclusion is consistent with our previous observations on RNA–protein interactions in ribosome assembly (3), as well as recent observations made by Traut and his coworkers (4) that the size distributions of ribosomal proteins from various bacterial species are similar.

As shown in this paper, most of the *E. coli* 30S proteins are functionally replaceable by corresponding proteins from *B. stearothermophilus*, even though they are chemically different. Thus, the regions of these proteins that interact with 16S RNA or other proteins must have common structural features. Comparison of amino-acid sequences among functionally equivalent proteins from such unrelated bacterial species may help to identify the important structural features required for the interactions.

Although we believe that an essential ribosome structural organization is common to diverse bacterial species, the ribosomes of different origins certainly show different properties. Thus, *B. stearothermophilus* 30S subunits are more heat stable than *E. coli* 30S subunits (3, 20). In addition, as we have noted above, *B. stearothermophilus* 30S subunits are unable to translate the coat and replicase cistrons of RNA messenger from f2 and related RNA *coli* phages. The present

TABLE 2. *Functional and immunochemical identification of the B. stearothermophilus proteins corresponding to each E. coli 30S protein*

Si* (*E. coli*)	Activity of [−Si] (%)†	*B.* protein fractions or pure *B.* proteins tested‡															
		II	III	IV	V	VI	VII	VIII	IX	X	XI	XII§	XIII	XIV	XV	XVI	XVIII
S1	−																
S2	33		±	[62]	±												
S3	10					[84]	±	−	−								
S4	15											[88]					
S5	34			[80]	±	−	−										
S6	−																
S7	13											[88]					
S8	2	±	[106]	±	±	−	−										
S9	−								[I]	I							
S10	9	−	−	±	[96]	−	−	−	−								
S11	13											[88, I]					
S12	51								−	−	−		[125]	+	−	−	
S13	−															[I]	
S14	23					−	−	−	−	−	−		−	−	−	−	[81]
S15	31	−	−	−	−	−	−	−	−	−	+	+	[114]				
S16	47			−	−	−	±	[97]	−									
S17	46	−	−	−	−	−	−	−	−	−	−		[122]	+	−		
S18	31									−	−	−	−	−	±	[124]	
S19	32										I	[103, I]					
S20	79													[107, I]	I		
S21	62								−				−			−	[101]

* Nomenclature of *E.* proteins (Si) is according to Wittmann *et al.* (26). The correlation with the nomenclature (Pi) used in our previous publications (9, 10, 19) is described (26).

† Activity of particles reconstituted from 16S RNA and purified *E.* proteins with the indicated *E.* protein omitted.

‡ Several *B.* protein fractions from phosphocellulose columns or purified *B.* proteins were examined to see if they could replace individually omitted *E.* proteins (*E.* Si) in reconstituting active 30S subunits. (Fractions I and XVII were not tested.) The method used is essentially that described in Table 1. The following symbols are used: −, no significant stimulation; ±, 50–80% activity compared to complete reconstituted particles with *E.* proteins; +, greater than 80% activity. The fractions (or pure proteins) that gave the highest activity in this test are indicated by showing in a *box* the actual % activity relative to complete reconstituted particles with *E.* proteins. When pure proteins were used for the test, a *box* with a *bold line* is used. When proteins were identified by immunochemical methods (see Fig. 2), the symbol *I* is given and boxed to indicate greatest concentration as above. Fractions VI to XVI and XVIII were tested for S9, S11, S13, S19, and S20 with negative results, except as indicated.

§ As shown in Table 1, four proteins purified from this fraction were tested and specific correspondence to *E.* proteins S4, S7, S11, and S19 was demonstrated. In other cases, fraction XII was used.

heterologous reconstitution system may be useful in identifying the proteins that are responsible for such special properties.

It is known that the size of rRNAs and the number of protein components of ribosomes from eukaryotic cells are different from those of prokaryotic ribosomes (refs. 21, 22, and papers cited therein). Thus, the two types of ribosomes appear to have somewhat different structural organization. It may be interesting to find out whether some parts of eukaryotic ribosomes have the same basic structural organization as prokaryotic ribosomes.

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