HETEROLOGOUS PROTEIN–RNA INTERACTIONS IN BACTERIAL RIBOSOMES*

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1. Introduction

A number of *E.coli* ribosomal proteins have been identified which can bind specifically to 16 S, 23 S and 5 S RNA's of *E.coli* [1–9]. Knowledge of these proteins is necessary for determining the structural organisation of the ribosome: firstly, to establish the position of the proteins along the RNA and secondly, to determine the three-dimensional structure of complexes of proteins and their RNA-binding sites. Aside from the importance for the ribosome structure, however, this system may be a very important one for investigating the basis of protein–nucleic acid structural specificity. Not least because sequencing studies of the binding proteins ([10]; B. Wittmann-Liebold, unpublished work; R.R. Crichton, unpublished work; H. Stadler, unpublished work), and of the RNA [11, 12] are so well-advanced. Recently, small *E.coli* rRNA fragments (40–350 nucleotides) have been isolated which are the binding sites of the proteins, and their sequences are either known or are currently being determined [7, 8, 13–15).

The problem remains, however, to establish which regions of the nucleic acid are actually interacting with the protein. Whilst some single amino acids can be modified in the protein, it is very difficult, for example, to specifically modify only one or two bases at a time in a given section of double-stranded RNA. Heterologous binding of *E.coli* ribosomal proteins to RNA's of closely related bacteria which is known to occur at least for *E.coli* 30 S subunit proteins and *Bacillus stearothermophilus* 16 S RNA [4, 16], could, however, help to solve this problem if a few changes, only, in the base-sequence occur.

In the following experiments we have explored the feasibility of this approach. Two *E.coli* proteins were chosen, namely S4 and L24, which bind to 16 S and 23 S RNA's respectively [1, 5]. Their RNA binding sites are at the 5′end of the RNA's and can readily be isolated by controlled nuclease digestion [7, 14, 15, 17]. They were bound to RNA's from bacteria of the same family as *E.coli (Enterobacteriaceae)* and to RNA's of bacteria from other families including *Bacillaceae* and *Pseudomonaceae*. Strong heterologous binding occurred to some RNA's, weak- or non-binding occurred to others. On the basis of these results, it is proposed that sequence analysis of the RNA binding sites should yield useful information on the specificity of the interaction.

A secondary reason for this approach was to explore the use of protein–RNA interactions as a criterion for taxonomical classification of bacteria. Differences of interaction should reflect, to some extent, the degree of homologous structure in the ribosomal RNA. A fairly good correlation was obtained with existing taxonomic data.

2. Materials and methods

2.1. *Preparation of RNA's*

16 S and 23 S RNA's were prepared from the bacteria by the method of Robinson and Wade [18]. The RNA was checked for degraded material, and for a 1:2 molar ratio of 16 S and 23 S RNA, by electrophoresing in polyacrylamide gels and staining with pyronin G.

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2.2. *Preparation of proteins and antisera*

S4 and L24 were prepared according to the methods of Hindennach et al. [19, 20]. The purity of the protein batches was checked by electrophoresis on two-dimensional polyacrylamide gels [21]; no contaminants were detected. One batch of S4 and two batches of L24 were used throughout this study; they were kindly provided by Dr. H.G. Wittmann. Antisera specific for proteins S4 and L24 were prepared and characterised as described earlier [22–24].

2.3. *Protein–RNA binding methods*

Complexes were prepared in TMK buffer (0.03 M Tris–HCl, pH 7,4; 0.35 KCl; 0.02 M $MgCl_2$; 0.006 M 2-mercaptoethanol) as described earlier [4]. All complexes were precipitated with 1.5 vol ethanol for 36 hr after separating non-bound protein on agarose columns. Two methods were used for the detection of binding. Both methods have been described in detail [4]. The first is an electrophoretic method in which the complex is electrophoresed in polyacrylamide gels and the protein stained quantitatively with Coomassie Brilliant Blue. The second is an immumological method in which the amount of protein is estimated by the extent of immunoprecipitation at three points approaching and reaching maximum immunoprecipitation. Immunoprecipitation curves were prepared for anti S4 and S4 protein, and for anti L24 and L24 protein. Three samples of complex, each containing 3 A_{260} units of complex, were mixed with increasing amounts of antisera, such that for two points the degree of immunoprecipitation was at the plateau. Each time a heterologous complex was prepared the corresponding homologous complex was also prepared and used as a control for both the electrophoretic and immunological methods. The S4 and L24 proteins were checked for specific binding first by exclusive binding to 16 S and 23 S RNA's respectively, and second by saturation at a 1:1 molar ratio of binding with the RNA's [4, 6].

3. Results and discussion

The extent of heterologous protein–RNA binding was estimated as a percentage of the homologous binding observed for proteins S4 and L24 with *E.coli* ribosomal RNA's. The results given in table 1 reveal a range of binding strengths, within error limits of ± 20% for different bacterial RNA's, which correlates approxi-

Table 1

Bacteria		Percent S4–16 S RNA binding		Percent L24–23 S RNA binding	
Family	Genus	Electrophoretic method	Immunological method	Electrophoretic method	Immunological method
Enterobacteriaceae	*Escherichia coli*	100	100	100	100
	Proteus vulgaris	100	100	100	100
	Serratia marcescens	100	80	50	60
	Erwinia carotovora	85	80	20	80
Bacillaceae	*Bacillus stearothermophilus*	100	100	100	100
	Bacillus pumilis	70	50	100	100
	Bacillus circulans	35	30	80	70
	Bacillus coagulans	20	25	0	0
	Clostridium perfringens	0	0	0	0
Pseudomonadaceae	*Pseudomonas fluorescens*	20	100	20	100
	Pseudomonas morsprunorum	70	80	70	100
	Aeromonas punctata	45	50	0	0
	Plesiomonas shigelloides	40	0	0	0
Rhizobiaceae	*Rhizobium leguminosarum*	25	20	20	20
Spirillaceae	*Vibrio cuneatus*	40	40	0	0

ximately with the taxonomic classification of the bacteria.

The strongest heterologous binding occurred for RNA's of bacteria in the *Enterobacteriaceae* family, RNA's from *Proteus vulgaris* and *Serratia marcescens* all produced maximum binding and binding for *Erwinia carotovora* was only slightly less. Maximum binding was also observed for two members of the *Bacillaceae* family, namely *B. stearothermophilus* and *B. pumilis* rRNA. The former confirmed the earlier heterologous binding result for S4 [4]. Given the complexities of the RNA binding sites for S4 [7, 13, 17] and L24 [14, 15], these results probably reflect a high degree of conservation of RNA sequence, and, indeed for 16 S RNA of *Proteus vulgaris* this has been indicated [25]. Undoubtedly, therefore, these bacterial RNA's are the best candidates for further structural investigations of protein–RNA interactions. If the heterologous RNA-binding sites are isolated and their nucleotide sequences compared with the *E.coli* RNA-binding sites, useful information should emerge concerning possible protein interaction sites.

Of some interest was the result that for a given bacterium the extent of S4-16 S RNA binding was generally close to that of L24-23 S RNA. This suggests that the level of evolutionary change is similar for both RNA's. The main exceptions to this were *B. circulans* for which the L24-23 S RNA binding was higher, and *Serratia marcescens*, *B. coagulans*, *Aeromonas punctata* and *Vibrio cuneatus* for which the S4-16 S RNA binding was higher.

Only rarely were there marked differences between the gel electrophoretic and the immunological methods. Whenever this occurred, the experiments were repeated two or three times with freshly made complexes to test for reproducibility, and this was always attained. The main discrepancy was for *Pseudomonas fluorescens* where the gel electrophoresis method gave a low result for both proteins. This may be due to weak binding of S4 such that dissociation of the protein occurred during the gel-electrophoresis run.

The reason for the low-binding or non-binding results is unclear. Although the RNA nucleotide sequences and structures are likely to differ markedly from that of *E.coli*, the possibility cannot be eliminated that the S4 and L24 proteins might bind heterologously more strongly under slightly different ionic conditions [26], or in the presence of other *E.coli* binding proteins [1, 3, 4, 27] such that, for example, heterologous 30 S subunit reconstitution might occur [16]. Indeed, other *E.coli* ribosomal proteins may yield different results from those in table 1. One highly conserved and functionally important protein L7/L12, for example, from a number of the bacteria studied can be reconstituted in the *E.coli* system to yield functionally active ribosomes (Geisser, M., Hasenbank, R., Bodley, J., Highland, J. and Stöffler, G., unpublished work).

The results presented show a fairly good agreement with the current taxonomical classification of bacteria. For example, Osawa et al. [27], who compared chromatographic elution profiles of the total ribosomal proteins, concluded that members of the *Enterobacteriaceae* family are more closely related to one another than members of *Bacillaceae*. Similar conclusions have been drawn from electrophoretic and immunological studies on the proteins [28, 29]. For both S4-16 S RNA and for L24-23 S RNA binding, our results correlate well with this, although the reverse heterologous binding experiments with *E.coli* 16 S RNA are not yet completed. Indeed, protein–RNA interactions have one advantage over either protein or RNA studies, in that they should reflect the sum of the degree of variation in both ribosomal protein and rRNA genes, both of which, owing to the mutal interdependence of their gene products, are subjected to a strong evolutionary pressure.

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