A COMPARISON OF THE PRIMARY STRUCTURES OF THE 16 S RIBOSOMAL RNAs FROM *ESCHERICHIA COLI* AND *PROTEUS VULGARIS*

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1. Introduction

About 70% of the ribosomal proteins from the closely related species *E. coli* and *P. vulgaris* exhibit immunological cross-reactivity (G. Stoeffler and H.G. Wittman, personal communication). This suggests that the ribosomal RNAs from these species might also display considerable homologies, and this has been confirmed by hybridisation studies which have shown that rRNA sequences are extensively conserved in members of the Enterobacteriaceae [1–3]. A detailed study of the similarities and differences between rRNAs from *E. coli* and *P. vulgaris* might be valuable as an approach to ribosomal sub-structure problems. The sequences of those parts of the rRNAs involved in interactions with proteins of similar primary structures might be expected to differ less than some other parts of the molecules.

We have previously carried out extensive studies of the nucleotide sequence of the 16 S ribosomal RNA from *E. coli* (MRE 600) [4–6] and we are now undertaking some preliminary work on the 16 S RNA from *P. vulgaris*. In this paper we report analyses of some of the products arising from T_1 ribonuclease digestion of this molecule. Our results indicate that the nucleotide sequence of most of this molecule is the same as that of the 16 S RNA from *E. coli*, but that some characteristic changes are discernable. Our preliminary findings indicate that some regions of the molecule may be more susceptible to change than others.

2. Methods

Labelled 16 S RNA was prepared from *P. vulgaris* (using cultures grown in the presence of 10 mCi of ^{32}P-orthophosphate) in the way previously described for the case of *E. coli* [7]. However, instead of the ribosomes being isolated as an intermediate step, the cells were digested with lysozyme to remove their walls, and the total RNA was extracted with sodium dodecyl sulphate in the presence of diethyl pyrocarbonate, as described in [8] in an attempt to reduce the action of endogenous nucleases on the RNA in the course of its isolation. The RNA was digested with T_1 ribonuclease and bacterial alkaline phosphatase, and the products were fingerprinted according to Brownlee and Sanger [9]. Subsequent hydrolysis of oligonucleotides with pancreatic RNase or venom phosphodiesterase was carried out in the way described by Sanger et al. [10].

3. Results and discussion

The fingerprints of the 16 S RNAs from *E. coli* and *P. vulgaris* are fairly similar (fig. 1). However, in the case of *P. vulgaris*, a background of products present in low amounts is apparent. We believe this arises because of hidden breaks in the RNA, caused by nuclease action during its isolation. These products could also be derived from fragments of the 23 S RNA contaminating

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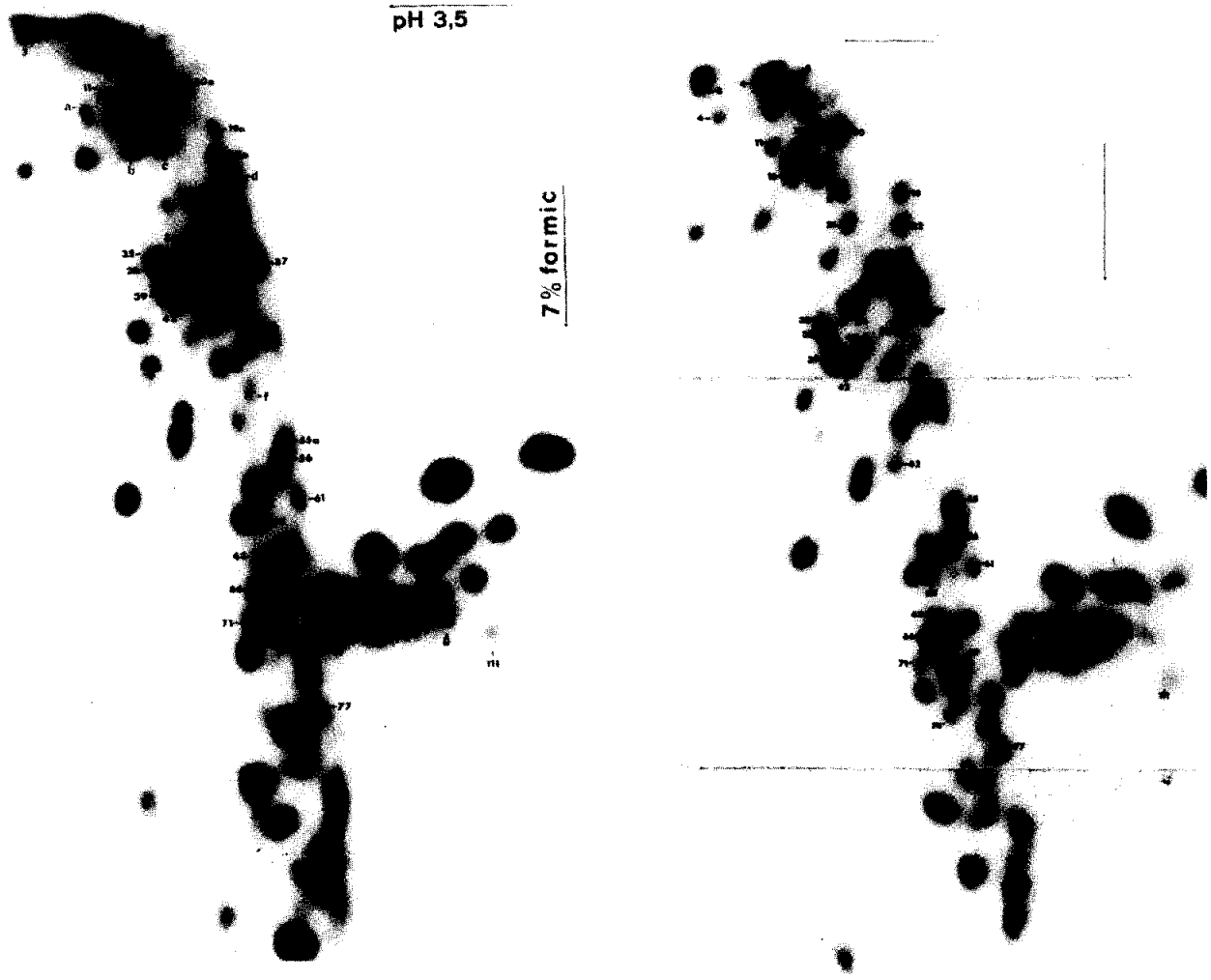

Fig. 1. Fingerprints of the products from T_1 ribonuclease digestion of the 16 S RNA from *P. vulgaris* (a) and *E. coli* (b), carried out according to [9, 10].

the 16 S RNA preparation. Some spots are found outside the "graticule" system on the fingerprint of *P. vulgaris* RNA, and are probably products which in this instance have not been dephosphorylated completely during digestion.

Many of the products appeared to fractionate in the same positions on both fingerprints. We examined 28 of the oligonucleotides from the *P. vulgaris* RNA, to see if they also gave rise to the same products on pancreatic RNase digestion as the corresponding oligonucleotides from the *E. coli* 16 S RNA. The results are listed in table 1. In 24 cases indentical products were found. On the basis of identical position and pancreatic RNase products, we conclude that these oligonucleotides are likely to be the same as those from the 16 S RNA of *E.coli*. In *E.coli* RNA, 18 of these oligonucleotides are present only once within the molecule, and the remaining 5 are present twice each. We have no information about their frequencies within the 16 S RNA from *P. vulgaris*. It is interesting that two of the methylated oligonucleotides in *E. coli* RNA are probably present unchanged in the RNA from *P. vulgaris*.

Table 1
Comparison of T_1 ribonuclease products from the 16 S ribosomal RNAs of *E. coli* and *P. vulgaris*.

No.	Sequence (*E. coli*)	Panc. RNase products * (*P. vulgaris*)	Possibly identical
3	UAAUCUUUG	AAU, C, U_4	+
4	CUUACCACUUUG	AC, C, U_5	+
5	UUAAAACUAAAAUCG	AAAU, AAAAC, C, U_3	+
8	UCUAACCUUG	AAC, C, U_4	+
10a	AACCUUACCUG	AC, AU, C, U_2	–
10b	(AAAU, AU_2, AC_2, C_3, U) A		–
11	AAUAUUG	AAU, AU, U	+
12	AAUUACUG	AAU, AC, U_2	+
15	UUAAUCG	AAU, C, U_2	+
19	ACCCUCAUAAG	AU, AAC, C	–
22	C (C, U) ACAACAUG	AU, AAC, C	–
34	AUCAUG	AU, C	+
35	AAUUG	AAU, U	+
37	CCCCUUACG	AC, C, U_2	+
38	AUAUG	AU	+
39a	UUAAG	AAU, U	–
39b	UAAUG		+
40	UAAUCG	AAU, C, U	+
42	AUCUG	AU, C, U	+
55	AAAUCCCCG	AC, C, U	–
56a	CAAUACG	AAU, AC, C, U	+
56b	ACACACUG		+
61	ACCCACUG	AC, C, U	+
62	AAUACG	AAU, AC	+
63	UAAACG	AAAC, U	+
66	UAAAG	AAAG, U	+
69	CUAACG	AAC, C, U	+
71	m_2^6 Am_2^6 ACCUG	"fast" AAC, C, U	+
77	CUCAG	AG, C, U	+
111	CCm^7 GCG	VPDE products: C, m^7 G, G	+

Products of pancreatic RNase digestion were fractionated by electrophoresis at pH 1.9 on DEAE-paper. They were identified by comparing their mobilities with those of products arising from *E. coli* 16 S RNA which had known sequences. The numbers of U residues were deduced from the positions of the oligonucleotides on the original fingerprints. The oligonucleotides used in the analyses were dephosphorylated. The pancreatic RNase products all possess 3'-phosphates except AG and AAAG. Spot 111 occurs in a singular position on the fingerprint. Venom phophodiesterase digestion of this spot yields a product which is neutral at pH 3.5, and remains close to the origin upon electrophoresis on No. 52 paper at this pH. This is almost certainly pm'G, which is present in the corresponding spot from the *E. coli* RNA. The other VPDE products indicate that the oligonucleotides are probably entirely identical. The spot corresponding to m_2^6 Am_2^6 ACCUG yields a product of slightly greater mobility than AAC, which we think is m_2^6 Am_2^6 AC [7].

* The products were estimated by visual inspection of the films [see 9, 10], and the relative amounts are indicated by underlining, e.g. (AC, C, U) G represents (AC_2C_2U) G. The numbering system used corresponds to that used in [4] for the T_1 ribonuclease products of the *E. coli* 16 S RNA.

This supports the notion that the methylated areas have an important function within the ribosome. Possibly they are widely conserved, in the way that the sequence G-T-Ψ-C-$\frac{G}{A}$ is among the tRNAs, for example.

Oligonucleotides 19a, 22a and 55a from the *P. vulgaris* RNA did not give rise to the same pancreatic products as the spots in identical positions on the fingerprint of *E. coli* RNA. The difference between 22 and 22a might be explained by an inversion of the order of two nucleotides, but the other differences cannot be simply explained, and probably involve oligonucleotides derived from different parts of the molecule which coincidentally migrate in the same positions on the fingerprints. Spot "d" from the *P. vulgaris* RNA yields pancreatic products identical with those of spot 22, but since the positions of these spots on the fingerprints are not the same, they must be different isomers. In the case of spot 39, the pancreatic RNase products of the *P. vulgaris* RNA spot indicate that only one of the components (39b) is present. Spot 10 from the *E. coli* RNA is mixture of two components, one of which (10b) is the 3′-terminal fragment. The pancreatic RNase products of the analogous spot from the *P. vulgaris* RNA did not correspond with either of these oligonucleotides. Therefore it seems likely that a sequence alteration in the neighbourhood of the 3′-terminus has occurred. The situation with regard to the 5′-terminus is unclear. In the 16 S RNA from *E. coli*, the 5′-terminal T_1 ribonuclease product is pAAAUUG, present without either phosphate terminus in digests carried out with alkaline phosphatase. A spot occurs in an analogous position on the fingerprint of *P. vulgaris* RNA, but yields AAAU, AAU, AU, AC and some U on pancreatic RNase digestion, indicating a mixture of oligonucleotides, even though it appears to be present in rather low yields in any event.

The differences discussed above have been included in table 2, listed together with some other oligonucleotides which are absent in *E. coli* RNA and present in *P. vulgaris* RNA, or vice versa. We have recently determined the nucleotide sequences of several large sections of the 16 S RNA from *E. coli* [5, 6 and unpublished work] and we wondered whether any changes between the two 16 S RNAs occurred more frequently in some parts than in others. Five of the oligonucleotides in table 1 (4, 8, 39b, 56b, 62) are each present

Table 2
Some differences between T_1 ribonuclease products from the 16 S ribosomal RNAs of *E. coli* and *P. vulgaris*

Products only found in RNA from *E. coli*	* Products only found in RNA from *P. vulgaris*
(6) UAUUCUG	(10a) (AC_2, AU, C_2, U_2) G
(10a) AACCUUACCUG	(19a) (AU_2, AAC, C_3) G
(10b) (AAAU, AU_2, AC_2, C_3, U) A	(22a) (AU_2, AAC, C_3) G
(17) CCUCUUG	(55a) (AC_3, C_2, U) G
(19) ACCCUCAUAAG	(a) (AU_2, U) G
(22) C (C, U) ACAACAUG	(b) (C_2U_3) G
(33) AUACUG	(c) (C_3U_3) G
(36) ACCUUCG	(d) (AU, AAC, AC, C_2, U) G
(39a) UUAAG	(e) (AC, C_2, U_2) G
(52) UCUCG	(f) (AU, AAAC) G
(55) AAAUCCCCG	(g) CCCCG
(68) AACUG	
(74) UAACG	

* The partial sequences of the products from *P. vulgaris* are suggested on the basis of their pancreatic RNase digestion products.

once in section A (174 nucleotides), and in fact only occur once each in the molecule. These all appear likely to be present in the *P. vulgaris* RNA, and none of the other products from this section can definitely be said to be absent, although not all can be distinguished from other material on the fingerprint of the *P. vulgaris* RNA. However, in section C (72 nucleotides), the oligonucleotides 33, 39a, 55 and 68 appear to be absent in the RNA from *P. vulgaris*, indicating a greater frequency of changes in this section. These results are very fragmentary, and it will only be possible to study such differences fully by working with the analogous large fragments from the *P. vulgaris* RNA. Equally, any conclusions about oligonucleotides which are present in fingerprints of *P. vulgaris* RNA, but absent from *E. coli* RNA, must be very tentative, since it cannot be ruled out that they result either from degradation of larger products or from contamination of the 16 S RNA with material derived from the 23 S RNA. However, we think it unlikely that certain of the more prominent oligonucleotides such as "b", 19a or 22a arise in this way.

It is readily apparent that there are further differences between the fingerprints of the RNAs which have not been studied. However, we think it will be more useful in the future to work directly with large, specific fragments of the 16 S RNA from *P. vulgaris*, corresponding to those studied in the case of *E. coli* 16 S RNA.

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