THE NUCLEOTIDE SEQUENCE OF DOLPHIN AND BOVINE 5S RIBOSOMAL RIBONUCLEIC ACID

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1. Introduction

Insight into the selective pressures incurred by 5S ribosomal RNA (rRNA) genes during evolution can be provided by determining the nucleotide sequence of 5S rRNA isolated from various species that span the evolutionary scale. The sequence of 5S rRNA from three representative mammalian species, mouse [1], marsupial [2] and human [3], have been determined and are identical. In the interest of assessing genetic drift among the 5S rRNA genes of mammals, we wish to report that the sequence of 5S rRNA derived from dolphin and steer are very likely identical to each other and to the other mammalian 5S rRNAs sequenced to date.

2. Materials and methods

Cultures of embryonic bovine trachea (*Bos taurus*, American Type Culture Collection Cell Respository designation CCL 44) and dolphin kidney (*Stenella plagiodon*, CCL 78) were obtained from the American Type Culture Collection, Bethesda, Maryland, and were cultured in Eagle's minimal essential medium (Earle's salts) supplemented with 10% fetal calf serum and three times the normal concentration of essential and non-essential amino acids (Gibco). The isotopic labeling of cells, purification and nuclease digestions of the RNA, and the methods involved in the analysis of the resulting oligonucleotides have all been described [2].

3. Results and discussion

Fig.1 illustrates the distribution of bovine 5S rRNA and tRNA following electrophoresis through an 8% polyacrylamide gel; that of dolphin 5S rRNA and

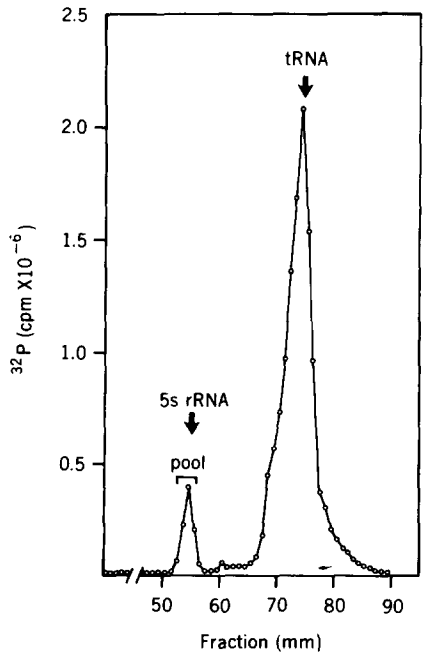

Fig.1. Preparative gel electrophoresis of *Bos* 5S rRNA. Low mol. wt RNA from *Bos taurus* was prepared as described [2] and purified by electrophoresis through and elution from an 8% polyacrylamide gel. The gel was fractionated into 1 mm slices, and each slice was monitored for its radioactive content by Cerenkov emission.

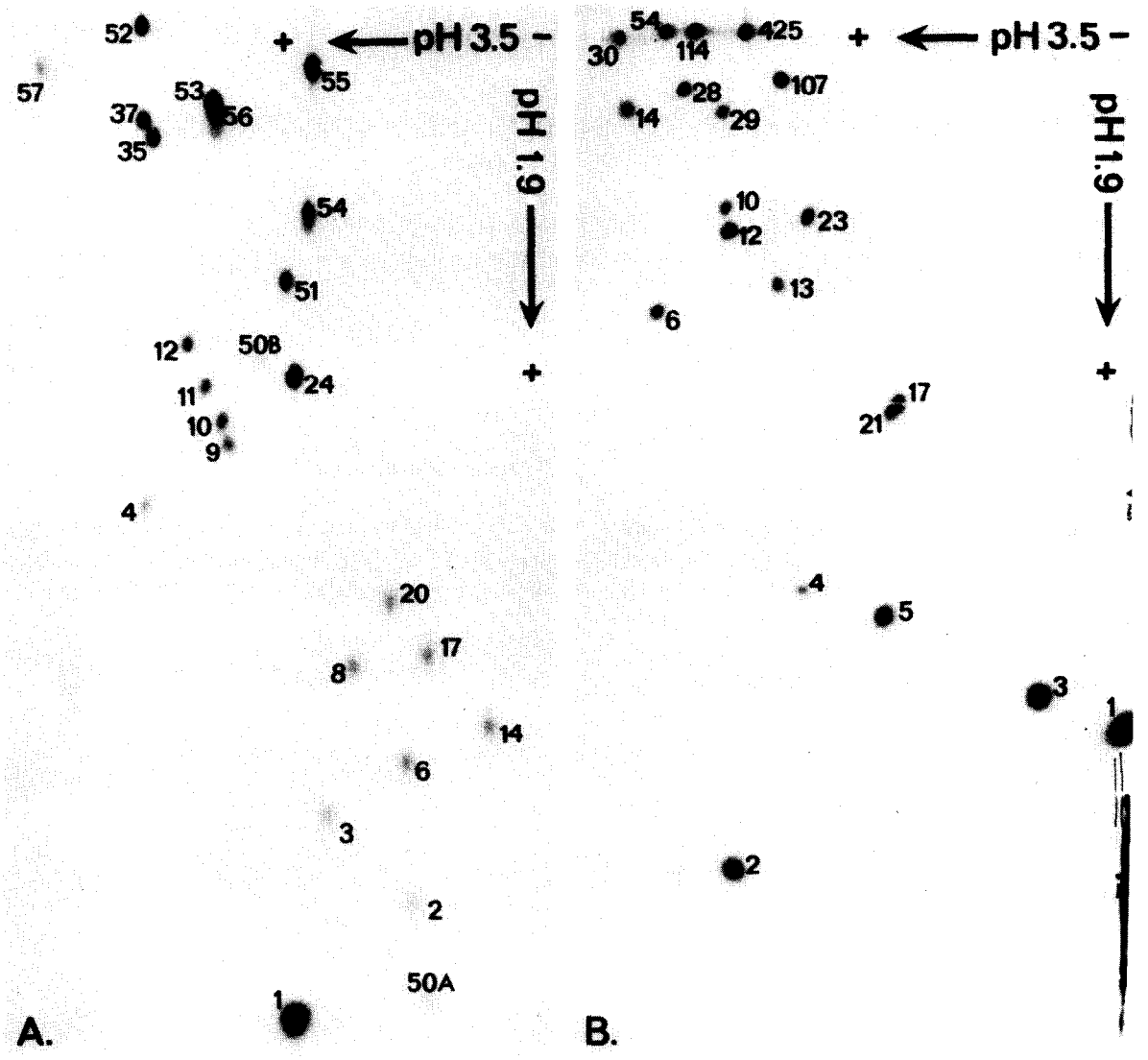

Fig.2. Two-dimensional electrophoresis of (A) RNase T_1 and (B) pancreatic RNase digestion products of *Bos* 5S rRNA. Purified *Bos* 5S rRNA was digested with the appropriate enzyme and the digestion products resolved by two-dimensional electrophoresis as described by Sanger et al. [4].

tRNA is identical. After elution from the gels, the 5S rRNA populations were digested completely with either RNase T1 or pancreatic RNase, and the products were resolved by two-dimensional electrophoresis [4]. Autoradiographs presented in fig.2 show the resulting distributions of oligonucleotides. The autoradiographs of fingerprints recovered from dolphin 5S rRNA are identical in all respects to those of bovine 5S rRNA, and so are not shown.

The molar yields and sequences or compositions of individual oligonucleotides obtained from dolphin and bovine 5S rRNA were assembled in table 1. These all are identical to homologous fragments from human [3], mouse [1] or marsupial [2] 5S rRNA. Assuming

Table 1
Digestion products of *Stenella* and *Bos* 5S rRNA

A. T_1 RNase digestion products					B. Pancreatic RNase digestion products				
1 Spot No.	2 Sequence or Composition	3 Theor.[a]	4 Exper. Dolphin	5 Exper. Bovine	1 Spot. No.	2 Sequence or Composition	3 Theor.	4 Exper. Dolphin	5 Exper. Bovine
1	G	15	15.3	14.2	1	C	17	14.2	15.75
2	CG	1	0.91	0.90	2	U	14–15	12.6	15.4
3	AG	1	1.07	1.04	3	AC	5	5.0	4.89
4	UG	1	1.04	1.02	4	AU	1	0.93	1.16
6	CAG	1	0.92	0.88	5	GC	4	4.0	4.15
8	AAG	1	0.93	0.86	6	GU	2	1.6	2.22
9	UCG	1	0.98	0.98	10	AGU	1	1.2	0.99
10	CUG	1	1.14	1.12	12	GAU	2	1.9	2.22
11	UAG	1	1.16	0.99	13	GGC	1	1.0	1.02
12	AUG	1	1.21	1.16	14	GGU	1	1.1	0.97
14	CCCG	1	0.81	0.79	17	AAGC	1	1.0	0.75
17	AC,C,G	1	0.82	0.74	21	GAAC	1	1.0	1.03
20	AACG	1	0.81	0.77	23	AGCG	1	1.1	1.06
24	C_2,U,G	2	1.98	2.05	28	GGAU	1	1.2	0.99
35	C,U_2,G	1	1.33	1.16	29	GGGC	1	1.1	1.07
37	UUAG	1	0.96	1.17	30	GGGU	1	1.3	1.06
51	C,U,AAG	1	1.03	1.0	54	AG,G_2,U	1	1.1	1.14
52	U_3,AC,G	1	0.97	1.15	107	A_2G,G_2,C	1	1.1	0.97
53	U,AU,C_2,G	2	2.0	1.78	114	GGGAAU	1	1.2	1.05
54	AAU,AC,C,G	1	0.95	0.95	425	AG,G_3,AC	1	1.2	0.82
55	U,C_5,AU,$(AC)_2$,G	1	0.88	0.85	6′	pGU	1	ND	ND
56	U_2,C,AC,G	1	1.0	1.28					
50a	CUU_{OH}	1	0.66	0.48					
50b	$CUUU_{OH}$	1	0.3	0.24					
1′	pG	1	ND[b]	1.07					

[a] Theor., theoretical; Exper., experimental.
[b] ND, not determined

RNase T_1 (A) and pancreatic RNase (B) digestion products were resolved by two-dimensional electrophoresis as shown in fig.2. Each oligonucleotide was analyzed by conventional techniques (4). In column 1 the oligonucleotides are numbered according to Forget and Weissman [3]; column 2 gives the composition or sequence of the oligonucleotide as inferred from secondary digestions; column 3 gives the theoretical yield of oligonucleotides as derived from the sequence of KB cell 5S rRNA (37); columns 4 and 5 give, respectively, the yields of oligonucleotides from *Stenella* and *Bos* 5S rRNA.

that no sequence displacements exist in these molecules, we conclude that their nucleotide sequences are identical. We believe that the assumption regarding sequence displacements is a valid one since Sankoff et al. [5] have recently provided calculations suggesting that evolutionary mutations in 5S rRNA follow a pattern of nonrandomness, and that the most frequently observed transitions are C ⟷ U and G ⟷ A. If such mutations had occurred during the evolution of *mammalian* 5S rRNA, then they would have profound consequences with respect to the nuclease fingerprint patterns. A C ⟷ U conversion would result in the involved oligonucleotide fractionating in a completely different graticule and/or isoplith; G ⟷ A transitions would generate additional RNase T1 cleavage sites or eliminate preexisting ones. It is, therefore, highly improbable that such changes could escape detection in both RNase T1 and pancreatic RNase catalogues.

The observation that diverse mammals (including

human, mouse, cow and dolphin as well as their progenitors, the marsupials), produce apparently identical 5S rRNA molecules suggests that the 5S genes are highly conserved through evolution. Among these same organisms considerable divergence has occurred in the structures of their genes specifying the cytochromes *c* [6]. One possible explanation for the conservativity of the 5S structure is that the molecule is sufficiently specialized in its biological function(s) that permissible mutational drift is a rare event. Drift in the primary structure of 5S rRNA is evident in other vertebrates, however. The nucleotide sequences of 5S rRNA from chicken [7] and *Xenopus* [8,9] differ from each other and from the 5S rRNA of mammals.

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