THE NUCLEOTIDE SEQUENCE OF SOMATIC 5 S RNA FROM *XENOPUS LAEVIS*

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1. Introduction

The 5 S ribosomal RNA of human, mouse, rabbit and rat cells have the same nucleotide sequence [1–3], although this differs considerably from that of *E. coli* [4]. In order to determine the extent of homology among eukaryotes we have studied the 5 S RNA of *Xenopus laevis*, the South African toad. T_1 and pancreatic ribonuclease digests of uniformly ^{32}P-labelled 5 S RNA were studied and the results allow a sequence to be derived which differs only in eight positions from the mammalian sequence.

2. Methods

Uniformly ^{32}P-labelled 5 S RNA was prepared by growing a *X. laevis* cell line (originally derived from kidney, and a gift from Dr. D.D. Brown) in [^{32}P]phosphate. Cells were grown for 48 hr at 25° on the surface of plastic bottles in Eagle's medium with 80% Hank's salts and supplemented with 10% heated foetal calf serum and 50 μCi/ml [^{32}P]phosphate. After harvesting by trypsinization, the 5 S RNA was purified from ribosomes as before [2]. In one experiment cells were lysed in 0.5% Nonidet P40 in 0.01 M KCl, 0.001 M magnesium acetate, 0.01 M Tris-chloride, pH 7.5 for 10 min at 0°. SDS was added to 0.2% and the RNA extracted by shaking with an equal volume of phenol for 1 hr at room temperature. 5 S RNA was purified by 10% acrylamide gel electrophoresis on slab gels [5].

3. Results

A fingerprint of a complete T_1-ribonuclease digest of the 5 S RNA, with the spots identified by number, is shown in fig. 1. Oligonucleotides were eluted and analysed by further digestion with enzymes using standard radiochemical sequence methods [5]. The following methods were used: alkaline hydrolysis, digestion with pancreatic ribonuclease, U_2-ribonuclease partial digestion with spleen phosphodiesterase, and pancreatic ribonuclease digestion after chemical blocking with a water-soluble carbodiimide. These methods allowed an unambiguous identification of the sequence of all the oligonucleotides t1–t24 which are listed in table 1 except for t15, t23 and t24 where only a partial sequence was obtained. Relative molar yields of the oligonucleotide of fig. 1were determined [5] and are also given in table 1.

A similar, although less extensive, analysis was made of the oligonucleotides in the complete pancreatic ribonuclease digests of 5 S RNA, as shown in fig. 1 and table 1. Oligonucleotides were sequenced by further digestion with alkali, T_1-ribonuclease and partial digestion with spleen phosphodiesterase. Sufficient evidence was accumulated to sequence all except three of the oligonucleotides. Yields of some of these were determined and the results given in table 1 are the average of two separate experiments.

3.1. *Derivation of sequence*

A comparison of the oligonucleotides present in *Xenopus* but absent in human 5 S RNA [1] indicates

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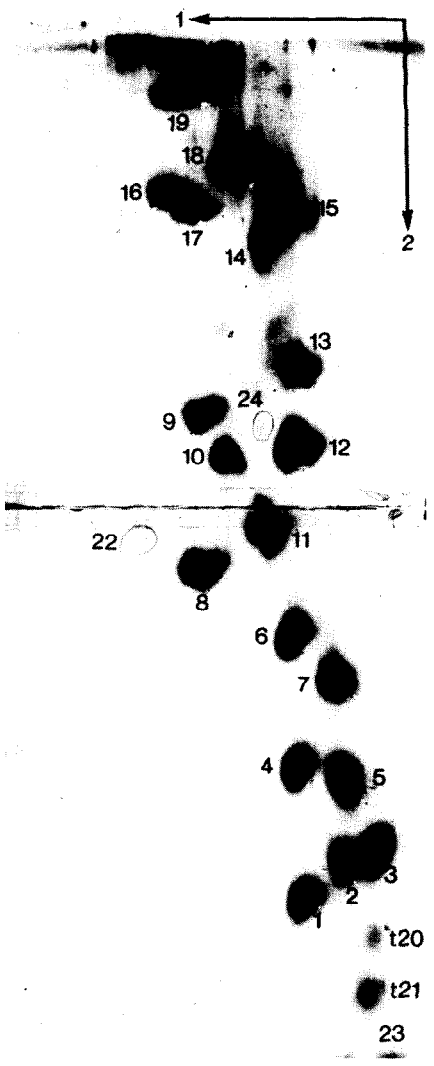

Fig. 1. Radiograph of a two-dimensional fractionation of a T_1-ribonuclease digest of 5 S RNA of *X. laevis*. Fractionation was by electrophoresis on cellulose acetate at pH 3.5 (direction 1) and on DEAE-cellulose using 7% formic acid (direction 2). Oligonucleotides are identified in table 1. The positions of oligonucleotides t22 and t24 are shown although they are not present in this experiment. Gp has run off in the second dimension.

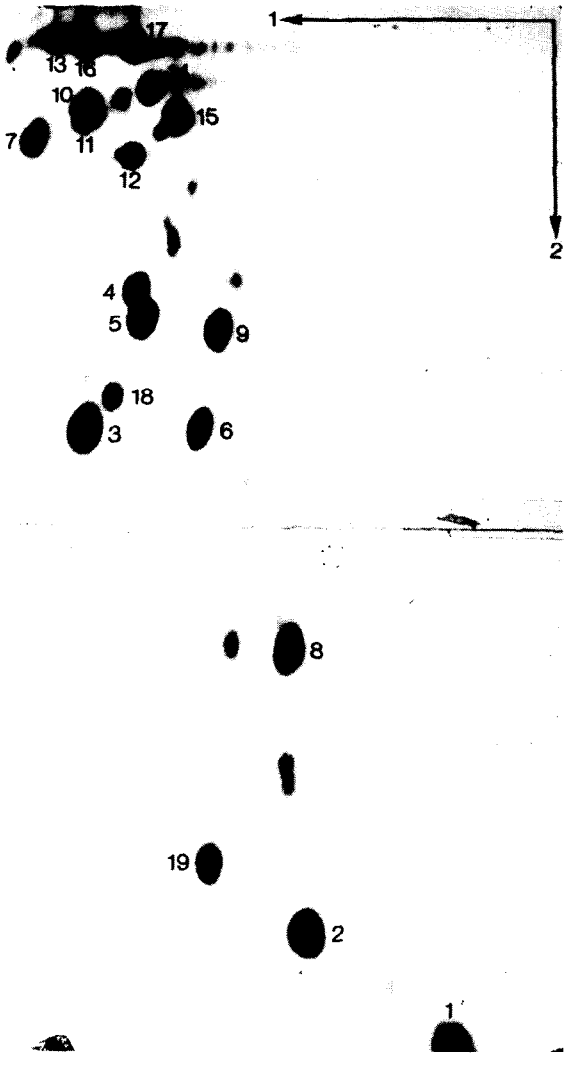

Fig. 2. Radiograph of a two-dimensional fractionation of a pancreatic ribonuclease digest of 5 S RNA of *X. laevis* fractionated as in fig. 1. Cp and Up have run off in the second dimension. Spots 10 and 11 are only partially separated.

that *Xenopus* has the following seven new oligonucleotides: t7, t8 (1 mole), t11, t13, t14 and t15. These oligonucleotides can be paired off with oligonucleotides in human but absent in *Xenopus* 5 S RNA as shown in table 2. The base changes that occur are clear except in the case of t15. Here, even with a partial sequence, it is apparent that the A–U of human 5 S RNA has changed to an A–C sequence i *Xenopus*. Fortunately each of the T_1-end products of the human 5 S RNA, which are altered in *Xenop* occurs only once in the molecule. Thus the new sequence can be derived by replacing the relevant fragments of human 5 S RNA as shown in fig. 3.

The results of the pancreatic ribonuclease digest can be used to cross-check the sequence. Thus the

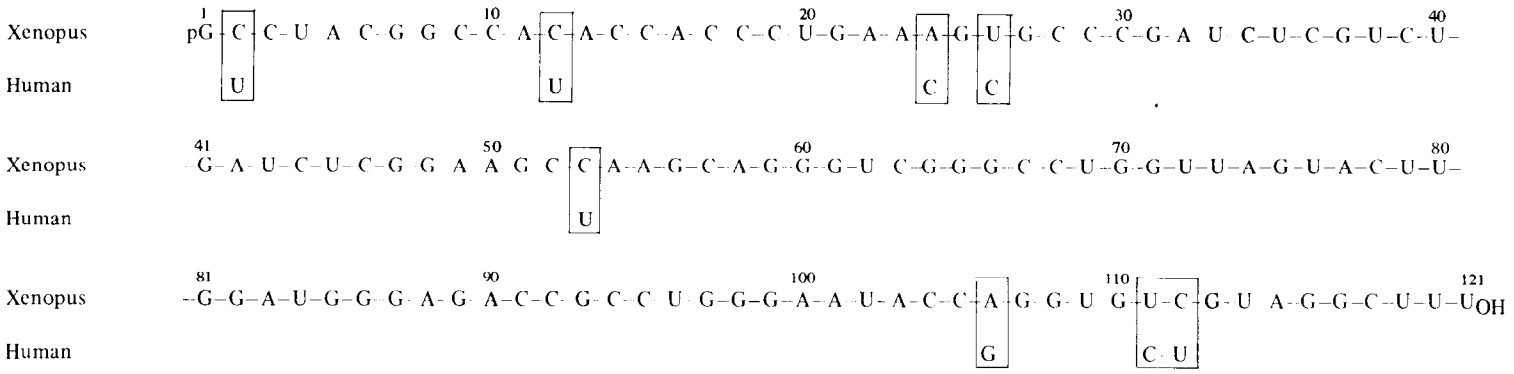

Fig. 3. Sequence of *X. laevis* 5 S RNA, showing the eight positions where the sequence differs from human 5 S RNA.

Table 1**

T_1-ribonuclease products				P-ribonuclease products			
Spot (fig. 1)	Sequence	Yield		Spot (fig. 2)	Sequence	Yield	
		Experimental	Theoretical			Experimental	Theoretical
t1	A–G	1.3	1	p1	A C	–	6
t2	C–A–G	0.8	1	p2	G–C	2.4	2
t3	C–C–C–G	0.8	1	p3	G–U	3.5	3
t4	A–A–G	1.3	1	p4	A–G–U	1.0	1
t5	A–C–C–G	1.3	1	p5	G–A–U	2.0	2
t6	A–A–A G	0.9	1	p6	G–G–C	1.1	1
t7	C–C–A–A–G	0.6	1	p7	G–G–U	–	1
t8	U–G	2.1	2	p8	A–A G C	–	1
t9	A–U–G	1.1	1	p9	A–G G C	0.6	1
t10	U–A–G	1.1	1	p10	(A–G,G)U	–	1
t11	U–C–G	1.7	2	p11	G–G–A–U	–	1
t12	C–C–U–G	2.6	2	p12	G–G–G–C	0.5	1
t13	C C U A–C G	0.4 (0.9*)	1	p13	(A–G,G)G–U		1
t14	A–A–U–A–C–C–A–G	0.8	1	p14	G–A A–A–G U	–	1

(table 1 cont.)

Spot (fig. 1)	Sequence	T_1-ribonuclease products Yield Experimental	Theoretical	Spot (fig. 2)	Sequence	P-ribonuclease products Yield Experimental	Theoretical
t15	$(C_{2-3},A-C_2)A-C-C-U-G$	0.4	1	p15	G–G–A–A G C	–	1
t16	U–U–A–G	0.7	1	p16	G–G–G–A–A–U	–	1
t17	U–C–U–G	1.1	1	p17	$(A-G,G_3)A-C$	–	1
t18	A–U–C–U–C–G	2.5	2	p18	pG–C		1
t19	U–A–C–U–U–G	1.2	1	p19	A–U	1.0	0
t20	C–C–G	0.1	0				
t21	C–G	0.4	0				
t22	pGp	0.4	1				
t23	$(C,U)N^+$	0.6	–				
t24	$(C,U_2)N_{OH}$	–	–				

* Yield estimated in another experiment.
\+ N is any nucleotide.
** No estimation was made of the yield of the mononucleotides Gp, Cp and Up.

Table 2

Paired T_1-end-products	Species+
U–C–U–A–C–G	H
C–C–U–A–C–G	X (t13)
C–C–A–U–A–C–C–A–C–C–C–U–G	H
$(C_{2-3},A-C_2)A-C-C-C-U-G$	X (t15)
C–U–A–A–G	H
C–C–A–A–G	X (t7)
A–A–U–A–C–C–G	H
A–A–U–A–C–C–A–G	X (t14)
A–A–C–G	H
A–A–A–G	X (t6)
C–U–G	H
U–C–G	X (t11)
C–G	H
U–G	X (t8)

+ H is human and X is *Xenopus laevis* 5 S RNA. Oligonucleotides are identified in table 1.

following are the new pancreatic products: G–U (p3, 1 mole), (A–G,G)U(p10), G–A–A–A–G–U(p1 and pG–C(p18). THe oligonucleotides G–G–G–U, pG–U and G–A–A–C, present in human, are absent in *Xenopus* 5 S RNA. These oligonucleotides are entirely consistent with fig. 3 and confirm the postulated sequence.

The quantitation of most oligonucleotides (table 1) was consistent with the postulated sequence. Oligonucleotide t13 was in particularly low yield, 0.4, but was obtained in higher yield, 0.9, in another experiment. t15 is in low yield, but this can be expected as it is large and may be overdigested with T_1-ribonuclease or incompletely transferred in the fractionation procedure [6]. Other small products are present in less than molar yields, e.g. C–G (0.4) and C–C–G (0.1) and are most likely to be contaminants, or possibly to derive from larger products by 'overdigestion' with T_1-ribonuclease. A–U is apparently a major contaminant among the pancreatic ribonuclease products. Nevertheless, becase of the presence of thes minor oligonucleotides in the fingerprint and because of the incomplete nature of the sequence studies, we cannot entirely exclude some form of microheterogeneity in the postulated sequence, such as occurs in the 5 S RNA of *E. coli* [4]. Only a complete and in-

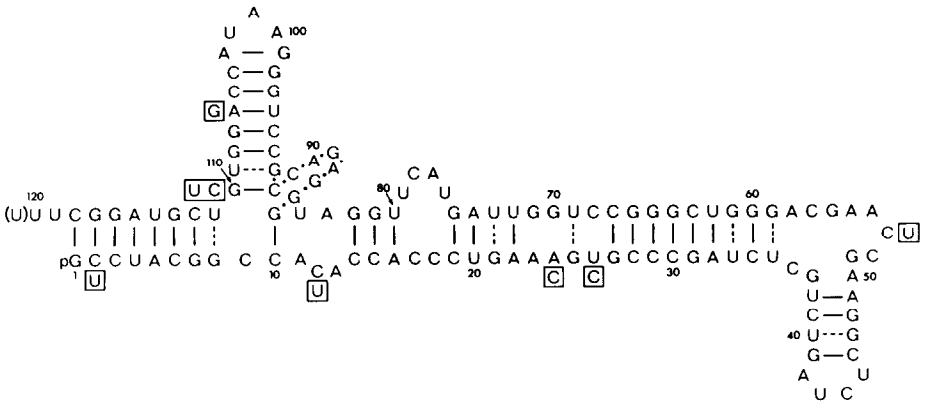

Fig. 4. A possible secondary structure model of *Xenopus* 5 S RNA compared with human (KB) 5 S RNA. The residues in boxes are those of human 5 S RNA.

dependent sequence study of the 5 S RNA of *Xenopus* would be sufficient to establish this point.

The 5′ terminal residue pGp is also present in less than molar yields suggesting that the 5′ terminal phosphate was absent from some of the molecules. No pppG was observed as has been reported for HeLa 5 S RNA [7]. The presumed 3′ terminal residues t23 and t24, corresponding to C–U–U_{OH} and C–U–U–U_{OH} of human 5 S RNA, were both present although the longer sequence was always in very low yields or absent as in the experiment of fig. 1.

4. Discussion

Forget and Weissman [8] have suggested a possible model for the secondary structure of human 5 S RNA which is asymmetric. The eight base changes in *Xenopus* are, on the whole, consistent with a similar model for *Xenopus* 5 S RNA as shown in fig. 4. Four base changes occur within the two extensively hydrogen-bonded region at one end of the molecule and are such as to conserve this basepairing. For example U–G between positions 2 and 117 become C–G in *Xenopus* 5 S RNA. Two other base changes occur at residues which are not base-paired whilst the remaining two destroy postulated base pairs. Thus the comparison between human and *Xenopus* secondary structure supports extensive base-pairing near the two ends of the molecule while throwing doubt on the need for base-pairing in human 5 S RNA between positions 12 and 84, and between 24 and 70 (see fig. 4).

The 5 S RNA studied in this work was synthesized by a cell-line derived from a mature kidney. This 'somatic' 5 S RNA should be of interest for comparison with the 5 S RNA molecules synthesized by immature oocytes [9]. Unpublished results of Drs. Ford, Southern and Denis indicate that the sequence of the oocyte material differs in several positions from that of somatic 5 S RNA.

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