SEQUENCE HETEROGENEITY OF 5 S RNA IN *XENOPUS LAEVIS*

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1. Introduction

Young *Xenopus laevis* oocytes produce large amounts of 5 S RNA, less than 1% being incorporated into ribosomes. 5 S RNA accumulates in equal proportions in the cell sap and in 42 S particles [1, 2]. Oocyte 5 S RNA can be clearly distinguished from 5 S RNA extracted from somatic cells on the basis of its rate of elution from MAK columns. On the other hand, upon thermal denaturation the hyperchromicity of oocyte 5 S RNA (O-5S RNA) is somewhat higher than that of somatic 5 S RNA (S-5 S RNA) [3]. In order to characterize further the two types of RNA preparations, we have determined their primary structure. Clear sequence differences have been demonstrated. They are certainly responsible for the chromatographic differences previously observed.

2. Methods

2.1. *Culture conditions*

2.1.1. Kidney cells

S-5 S RNA was obtained from kidney cells cultivated as already described [4], except that the medium was depleted of phosphate. The cells were labelled 2 or 3 days with 25 to 50 μCi[^{32}P]orthophosphate (carrier-free) per ml.

2.1.2. Ovaries

O-5 S RNA, probably contaminated with some S-5 S RNA, was obtained from young female ovaries (2 cm long) incubated in 2 ml of the same medium, to which 2 mCi [^{32}P] phosphate were added. Incubation was carried out for 2 or 3 days.

2.2. *5 S RNA purification*

Total RNA was extracted according to the cold phenol technique of Brown and Littna [5]. RNA was fractionated on 12.5% acrylamide gel slabs. The 5 S RNA band was located by autoradiography and eluted as described by Jordan [6].

2.3. *5 S RNA partial hydrolysis*

The conditions for T_1 RNAase partial degradation and the bidimensional gel electrophoresis technique of Vigne and Jordan [7] were used.

2.4. *Fingerprinting and sequencing*

The techniques were essentially those described by Brownlee et al. [8]. Oligonucleotide compositions were determined after complete hydrolysis with T_2 RNAase [9].

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Fig. 1. Fingerprints of S- and O-5 S RNA's. a) T_1 RNAase digest of S-5 S RNA. b) T_1 RNAase digest of O-5 S RNA. In order to display the differences between S- and O-5 S RNA digests more clearly, we show here a finger print the second dimension of which was run for twice as long as the corresponding one in a. c). RNAase A digest of S-5 S RNA. d) RNAase A digest of O-5 S RNA.

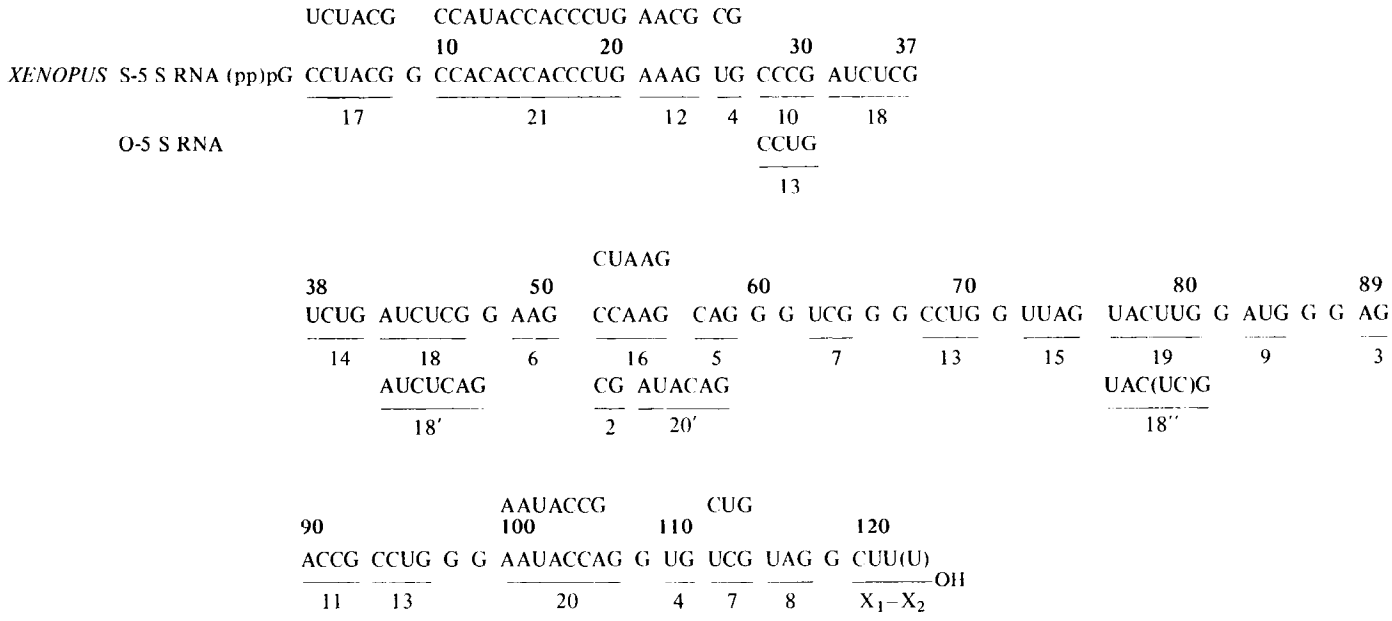

Fig. 2. Oligonucleitide sequence of *Xenopus* 5 S RNA's. The complete sequence of *Xenopus* S-5 S RNA is shown. KB-specific T_1 RNAase oligonucleotides are written above while O-5 S RNA-specific oligonucleotides are written below the S-5 S RNA sequence.

Table 1

Oligo-nucleo-tide	T_2 RNAase products				RNAase A products	Venom diesterase digests					Spleen diesterase partial digests	Sequence
						Total				Partial		
	C	A	G	U		C	A	G	U			
7	1.17		1.00	1.12		0.88		1.00				UCG
12		3.18	1.00									AAAG
16	1.82	2.18	1.00		C : 2.40 AAG: 0.86							CCAAG
17	3.21	1.09	1.00	1.20	C : 2.19 AC : 0.97 G : 1.07 U : 1.07	1.74	1.08	1.00	1.03		CCU–	CCUACG
20	2.10	3.93	1.00	0.99	C : 1.25 AC : 0.98 AG : 0.90 AAU: 1.00	1.88	2.94	1.00	1.11	CAG	AAUA–	AAUACCAG
21	8.42	3.34	1.00	1.11	C : 5.86 AC : 2.35 G : 1.17 U : 0.86							CCACACCACCCUG*
18′	1.35	2.89	1.00	1.11	AC : 0.78 AG : 0.79 AU : 1.06					AG	AU–	AUACAG
20′	2.43	1.91	1.00	1.53	C : 2.49 AG : 0.64 AU : 0.74 U : 0.91					AG	AUCUC–	AUCUCAG

* Deduced from a comparison with oligonucleotide 55 of KB 5 S RNA.

3. Results

3.1. *Sequencing of 5 S RNA from kidney cells*

T_1 RNAase-fingerprints of *Xenopus* S-5 S RNA (fig. 1a) are very similar to the KB 5 S RNA fingerprint of Forget and Weissman [10]. Sequencing of all the oligonucleotides showed that only 7 differed from the corresponding KB oligonucleotides. The sequencing data for 6 of them are presented in table 1, the seventh being UG. Because of lack of material it was not possible to determine the sequence of oligonucleotide 21 completely by conventional techniques. The proposed structure is nevertheless fully compatible with the results of the analyses of both T_2 RNAase and RNAase A digests. Assuming that the locations of the 7 *Xenopus*-specific oligonucleotides are the same as those of the corresponding ones in KB 5 S RNA the sequence presented in fig. 2 was deduced. The results of the analysis of RNAase A digests were in complete agreement with the proposed sequence (fig. 1, c and table 3).

The sequence in fig. 2 was further confirmed by partial T_1 RNAase degradation and analysis of the specific fragments thus obtained. After bidimensional electrophoresis, the material which moved at the same rate as intact 5 S RNA in the first dimension, was resolved into 7 bands (fig. 3,a). Fingerprinting analysis of the material in each band was made after complete T_1 or RNAase A digestion. The results obtained for the most interesting bands are shown in table 2. The data clearly establish that band 4 corresponds to the medial fragment of S-5 S RNA (positions 38 to 89). Bands 6 and 7 contain material originating from the 5′-end (positions 1 to 37) and from the 3′-end (positions 90 to 120), respectively. Our conclusions about the positioning of the *Xenopus*-specific oligonucleotides are thus perfectly confirmed.

3.2. *Sequencing of 5 S RNA from ovaries*

3.2.1. Analysis of total RNA

T_1 RNAase-fingerprinting of O-5 S RNA shows clear-cut differences relative to S-5 S RNA. Two additional spots appear (18′ and 20′, in fig. 1,b). Sequencing of these oligonucleotides gave the sequences AUCUCAG for spot 18′ and AUACAG for spot 20′ (table 1). Positioning of these oligonucleotides in the sequence will be discussed later. The amount of the dinucleotide CG, which is present only as a trace

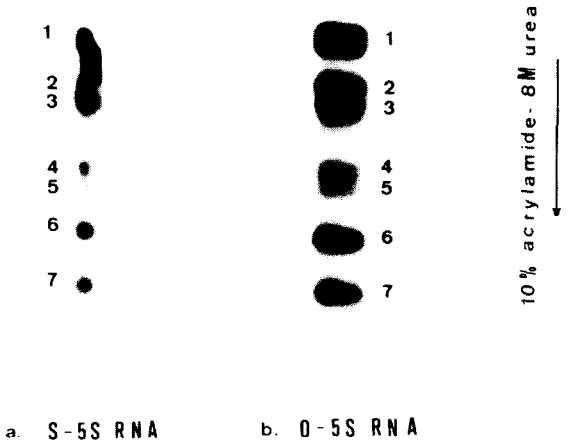

Fig. 3. Bidimensional electrophoreses of partial T_1 RNAase digests of *Xenopus* 5 S RNA's. a) S-5 S RNA; b) O-5 S RNA. The second dimension analyses on 8 M urea-containing polyacrylamide gels of the material which ran in the first dimension without urea at the position of intact 5 S RNA are shown.

in S-5 S RNA, is clearly increased. On the other hand, the yield of 4 spots (5, 10, 16 and 19), is about 35% of that found in S-5 S RNA digests (table 2).

Differences are not so apparent between RNAase A fingerprints of S- and O-5 S RNA's (fig. 1,c and d). One oligonucleotide (15′), whose sequence is AGAAGC, is specific of O-5 S RNA. Oligonucleotides 10 and 15 appear in low yields whereas the amount of oligonucleotide 7 is increased (table 3).

3.2.2. Localization of the sequence differences betwee S- and O-5 S RNA's

The partial T_1 RNAase degradation pattern of O-5 S RNA shows only slight differences relative to that of S-5 S RNA (fig. 3,b).

3.3. *Analysis of band 6*

T_1 RNAase fingerprint analysis of the material recovered from band 6 shows that it corresponds to the 5′-end of the molecule (table 2). One oligonucleotide, which was not found in the T_1 RNAase fingerprint of the corresponding fragment from S-5 S RNA, was identified as CCUG. Since the yield of CCCG is correlatively low, we conclude that a fraction of the molecules in our O-5 S RNA preparations differs at

Table 2
Molar yields of oligonucleotides in T_1 RNAase digests.

Oligonucleotide		S-5 S RNA		O-5 S RNA	5 S RNA T_1 RNAase fragments					
					band 6 from		band 4 from		band 7 from	
No.	Sequence	Theor.	Exper.	Exper.	S-5 S RNA	O-5 S RNA	S-5 S RNA	O-5 S RNA	S-5 S RNA	O-5 S RNA
1	G	14	12.06	10.51	1.42	1.34	9.16	7.68	3.85	3.20
1′	pG*	1	0.24	0.06	0.42	0.19	–	–	–	–
2	CG	0	0.18	0.54	–	0.15	–	0.53	–	–
3	AG	1	1.04	0.85	–	–	1.02	0.87	–	–
4	UG	2	1.95	1.77	1.31	0.83	–	–	1.09	1.00
5	CAG	1	0.95	0.36	–	–	0.88	0.49	–	–
6	AAG	1	0.84	0.74	–	–	1.02	0.84	–	–
7	UCG	2	1.94	1.27	–	–	0.94	0.80	0.87	0.86
8	UAG	1	1.00	0.91	–	–	–	–	0.85	0.86
9	AUG	1	1.01	1.00	–	–	0.94	0.99	–	–
10	CCCG	1	0.99	0.33	1.01	0.51	–	–	–	–
11	ACCG	1	0.88	0.66	–	–	–	–	0.74	0.65
12	AAAG	1	0.79	0.87	1.03	1.02	–	–	–	–
13	CCUG	2	1.95	2.23	–	0.57	1.11	1.12	1.10	0.92
14	UCUG	1	1.08	1.05	–	–	1.20	1.13	–	–
15	UUAG	1	0.91	1.04	–	–	0.87	0.99	–	–
16	CCAAG	1	0.82	0.29	–	–	0.89	0.47	–	–
17	CCUACG	1	0.74	0.72	1.03	0.86	–	–	–	–
18	AUCUCG	2	1.98	2.04	1.44	1.05	1.33	1.00	–	–
18′	AUCUCAG	0	–**	0.50	–	–	–	0.43	–	–
19	UACUUG	1	0.92	0.38	–	–	0.96	0.52	–	0.12
20	AAUACCAG	1	0.97	0.84	–	–	–	–	0.88	0.83
20′	AUACAG	0	–	0.50	–	–	–	0.36	–	–
21	CCACACCACCCUG	1	0.64	0.50	0.31	0.67	–	–	–	–
X_1	CUU_{OH})	1	0.69	0.98	–	–	–	–	0.57	0.82
X_2	$CUUU_{OH}$)		0.09	0.33	–	–	–	–	–	0.43
–	CCG	0	0.08	0.16	–	–	–	–	–	0.19
–	UUG	0	0.05	0.11	–	–	–	0.06	–	–

* The low yield of pG is due to the presence of di- and triphosphate groups at the 5′-end [3].
** Means that the corresponding oligonucleotide has not been detected.

position 30 from S-5 S RNA. This interpretation is confirmed by the fact that no CCUG was found in a slightly shorter fragment extending from 1 to 25, which was isolated from 0-5 S RNA in another experiment.

3.4. *Analysis of band 4*

It is clear from the T_1 RNAase fingerprint that material in band 4 corresponds to the medial section 38–89 of the molecule (table 2).

The two spots 18′ and 20′, which were previously considered as specific of O-5 S RNA, are present in the digests of this fragment. Oligonucleotide 18′ was identified as AUCUCAG (table 1). Its sequence derives from that of oligonucleotide 18 by a single G–A substitution (fig. 2). This can be correlated with the low yield of oligonucleotide GGAAGC in the RNAase A digest and the simultaneous appearance of oligonucleotide AGAAGC (table 3). The localization is confirmed by the fact that spot 18′ is not present in the digest of a fragment extending from 54 to 85–87 isolated from a different partial digest.

Although the G–A transition at position 47 should

Table 3
Molar yields of oligonucleotides in RNAase A digests.

Oligonucleotide		S-5 S RNA		O-5 S RNA Experimental	O-5 S RNA T_1 RNAase fragments		
No.	Sequence	Theor.	Exper.		Band 6	Band 4	Band 7
1	C	19	10.08	9.98	5.65	4.94	2.53
2	U	12	7.61	10.80	2.66	4.53	3.60
3	AC	6	4.52	5.09	3.17	1.96	1.55
4	GC	2	1.76	1.46	0.67	–	0.72
5	GU	3	2.83	3.36	–	–	2.43
6	GGC	1	1.05	0.79	0.79	–	–
7	GAU	2	1.83	2.60	1.38	1.90	–
8	AGU	1	1.01	1.47	–	0.75	–
9	GGU	1	1.09	1.37	–	0.82	–
10	AAGC	1	0.79	0.41	–	0.38	–
11	AGGC	1	0.93	0.89	–	–	0.89
12	GGGC	1	0.42	0.39	–	0.29	–
13	AGGU	1	0.94	0.91	–	–	0.95
14	GGAU	1	0.75	1.06	–	0.79	–
15	GGAAGC	1	0.82	0.42	–	0.40	–
15′	AGAAGC	0	–*	0.53	–	0.28	–
16	GAAAGU	1	0.96	1.08	1.07	–	–
17	GGGAGAC	1	0.38	0.40	–	–	–
18	AGGGU	1	0.48	0.66	–	0.75	–
19	GGGAAU	1			–	–	0.80
Y	pGC**	1	0.43	0.23	0.30	–	–
–	GAC	0	0.13	0.16	–	–	–
–	AU	0	0.17	0.30	–	–	–
–	G	0	–	–	1.04	–	–
–	GGGAG	0	–	–	–	0.48	–

* Means that this oligonucleotide has not been detected.
** The low yield of pGC is due to the presence of di- and tri-phosphate groups at the 5′-end [3].

reduce the yield of oligonucleotide 18 in the T_1 RNAase fingerprint of band 4, the expected decrease was apparently not observed (see table 2). In fact a single C–U transition also occurs between positions 76 and 81, producing an O-5 S RNA-specific oligonucleotide, which is an isomer of oligonucleotide 18, and therefore migrates to the same position. This oligonucleotide (18″) could be obtained pure from the fragment 54 to 85–87, already mentioned. The complete sequence of 18″ could not be unequivocally identified because of lack of material. The location of the C–U transition is therefore either at position 79 or position 80. It cannot be at position 76, since the yield of oligonucleotide AGU in RNAase digests of band 4 is close to the theoretical value (table 3).

The sequence of oligonucleotide 20′ was identified as AUACAG (table 1). This oligonucleotide corresponds to position 54 to 59 in some of the O-5 S RNA molecules. Actually three nucleotide substitutions exist in sequence 52–59. C_{53} of S-5 S RNA is sometimes replaced by a G and its substitution explains the presence of the dinucleotide CG in the T_1 RNAase digest of the band 4 fragment (table 2). A_{55} and G_{56} are also changed to U and A, respectively, thus producing the oligonucleotide 20′: AUACAG (fig. 2). Oligonucleotide 20′ was also recovered from the T_1 RNAase digest of fragment 54 to 85–87. RNase A fingerprinting of the material from band 4 confirms our interpretation. The yields of oligonucleotides AC and GAU, as expected, are high, whereas the yield of oligonucleotide 10 is low (table 3).

3.5. *Analysis of band 7*

As shown in table 2, the T_1 RNAase digest of the material from band 7, which contains the 3′-end of the molecule, is almost identical with that derived from the 3′-end of S-5 S RNA (table 2). The only difference pertains to the higher yields of the 3′-terminal oligonucleotides X_1 and X_2 in T_1 RNAase digests of the 3′-terminus from O-5 S RNA. This difference is unexplained at the moment. RNAase A fingerprinting of the band 7 fragment confirms the sequence of the trinucleotide UCG, since two GU and one GC are indeed found (table 3).

4. Discussion

The heterogeneity of 5 S RNA in bacteria is well substantiated [8, 11, 12]. It appears that the multiple copies of the 5 S RNA genes in one particular bacterial strain are not identical. They differ by discrete nucleotide substitutions. Moreover, strain differences with respect either to the nature of the nucleotide substitution or to the frequency of various molecular types in the total 5 S RNA population have been observed [8, 11].

Although the number of 5 S RNA gene copies is much higher in eukaryotes, only limited heterogeneity has yet been reported in 5 S RNA isolated from human cell lines [13]. Consideration of the data of table 2 and table 3 shows that a few oligonucleotides (CG, CCG and UUG in T_1 RNAase digests, GAC and AU in RNAase A digests), which are not expected from the *Xenopus* S-5 S RNA sequence proposed in fig. 2, were nevertheless found in low amounts in S-5 S RNA digests. Although we cannot completely exclude that they originate from contaminations by other RNA species, the fact that they were repeatedly observed suggests that they actually correspond to nucleotide substitutions which could occur in ca. 10% of the molecules in S-5 S RNA prepared from kidney cells.

Evidence for heterogeneity however is much better in the case of 5 S RNA prepared from ovaries. Several nucleotide substitutions have been clearly identified and localized. Analyses of [^{32}P]5 S RNA extracted from liver, spleen and testis have always failed to detected these substitutions, which can therefore be considered as specific of 5 S RNA prepared from ovaries, i.e. of ocyte 5 S RNA, since it is known that oocyte 5 S RNA makes up about 99% of the total 5 S RNA in young ovaries [1]. Nevertheless, the contamination by [^{32}P]S-5 S RNA synthesized in follicle cells in preparations extracted from ^{32}P-labelled oavrie is certainly higher. We cannot therefore make any definite evaluation of the frequencies of the occyte-specific substitutions. But we can definitely state that oocyte 5 S RNA is more heterogeneous than somatic 5 S RNA and contains sequences which are never detected in somatic 5 S RNA.

We are thus led to the conclusion that some 5 S RNA genes, which are not transcribed in somatic cells, are transcribed in young oocytes. It should be mentioned that the number of 5 S RNA gene copies is particularly high in *Xenopus laevis* [14]. No gene amplification has been accordingly observed in the case of 5 S RNA [15, 16], and it can be proposed that the rate of synthesis of 5 S RNA in oocytes is increased through derepression of some of the 5 S RNA genes.

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