AN ANALYSIS OF THE DEGREE OF HOMOLOGY BETWEEN 28S rRNA FROM XENOPUS LAEVIS AND XENOPUS MULLERI

Michael D. Griswold[1], Ronald D. Brown[2],
and Glauco P. Tocchini-Valentini

Laboratory of Cell Biology, C N R, Via Romagnosi 18/A,
00196 Rome, Italy.

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SUMMARY

Ribosomal 28S RNA labeled with $^{32}PO_4$ was isolated from Xenopus laevis and Xenopus mulleri. The ribonuclease digests of the RNAs were then analyzed and compared by oligonucleotide homochromatography and fingerprinting. No differences could be detected between these molecules from the two Xenopus species in the fingerprint or oligonucleotide patterns. The 28S rRNA of HeLa cells was shown to contain sequences which differ greatly from the Xenopus.

INTRODUCTION

The ribosomal DNA (rDNA) of Xenopus laevis and Xenopus mulleri have been shown to contain multiple copies of alternating nucleotide sequences (1). Each unit contains the sequences coding for 28S and 18S rRNA, a transcribed spacer region, and a nontranscribed spacer (2,3). The spacer regions from the two species have been

[1]Present address: Department of Pharmacology, Baylor College of Medicine, Houston, Texas 77025, U S A.

[2]Present address: Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland 21210, U S A.

shown to differ greatly in their nucleotide sequence (4). Experiments utilizing hybridization analysis and denaturation mapping indicate that the sequences which code for mature ribosomal RNA might be similar to each other (4). A determination of the degree of this homology between the ribosomal genes of these two species is important for an understanding of the mechanism for the maintenance of homogeneity with multiple genes. The more direct way to answer this question is by oligonucleotide mapping.

The methods available for an analysis of sequences of large RNA molecules include fingerprinting and homochromatography of ribonuclease digests of these molecules (5-8). We have utilized modifications of these methods to obtain more detailed information concerning the degree of homology within the 28S rRNA sequences of the two Xenopus species. We have been unable to detect any differences between these molecules in the fingerprint pattern of methylated bases or in the homochromatography pattern of large oligonucleotide fragments produced by T1 RNase. However, the 28S rRNA of HeLa cells, used as a standard of comparison, has been shown to contain oligonucleotide sequences which differ greatly from the two Xenopus species.

MATERIALS AND METHODS

Mature Xenopus laevis were purchased from supply houses and Xenopus mulleri were the gift of Dr. Marco Crippa. HeLa cells were grown in monolayer cultures in miniumum essential Eagle's medium supplemented with 7% horse serum and Earle's salts. Each Xenopus was labeled by intraperitoneal injection of 5 mC $^{32}PO_4^{=}$. After 48 hours livers were excised and liver ribosomes prepared. HeLa cells were labeled by incubation with 100 μC/ml. $^{32}PO_4^{=}$ for 24 hours followed by a subsequent 24 hour incubation with 10 mM Na-phosphate (pH 7.0).

Xenopus livers were homogenized in 5 volumes of TKM (0.05 M Tris-HCl, pH 7.5; 0.07 M KCl; 0.005 M $MgCl_2$) plus 0.25 M sucrose. The homogenate was centrifuged at 600 xg for 5 minutes. The supernatant was made 0.5% in Na deoxycholate, centrifuged at 15,000 x g for 20 minutes and the resulting supernatant centrifuged in a Spinco SW 50.1 rotor at 49,000 RPM for 90 minutes. The ribosomal pellet was resuspended in 2 ml 0.1 M Na-acetate (pH 5.2), 2% SDS and extracted with an equal volume of phenol. The aqueous phase was precipitated overnight with two volumes of ethanol at -20° C. The precipitate was collected, redissolved in 0.5 ml 0.1 M Na-acetate (pH 5.2), 0.5% SDS and layered on a 15-30% sucrose gradients which contained 0.5% SDS, 0.01 M Tris-HCl (pH 7.5), 0.1 M NaCl and 1 mM EDTA. The gradients were centrifuged in a SW 27.1 rotor at 24,000 RPM for 20 hours at 20° C. The 28S rRNA was collected, precipitated with ethanol, and stored at -20° C.

HeLa cells were collected by trypsinization, washed with PBS (NaCl 0.137 M; KCl 0.0027 M; Na_2HPO_4 0.0081 M; KH_2PO_4 0.00147 M), resuspended in RSB (0.01 M NaCl; 0.01 M Tris-HCl pH 7.4; 0.0015 M $MgCl_2$) plus 0.1% triton X-100 and homogenized after being allowed to swell. The homogenate was centrifuged at 600 x g for 5 minutes and treated with deoxycholate. Ribosomes and 28S rRNA were prepared as described above.

Homochromatography was done essentially according to the procedure of Brownlee and Sanger (7) and Barrell (8) using a modified homomixture B containing 5 mg/ml RNA and 3.5 M urea.

Fingerprinting of combined T1-pancreatic RNase digests was carried out as described by Maden and Forbes (6).

RESULTS AND DISCUSSION

Combined T1 and pancreatic ribonuclease digestion followed

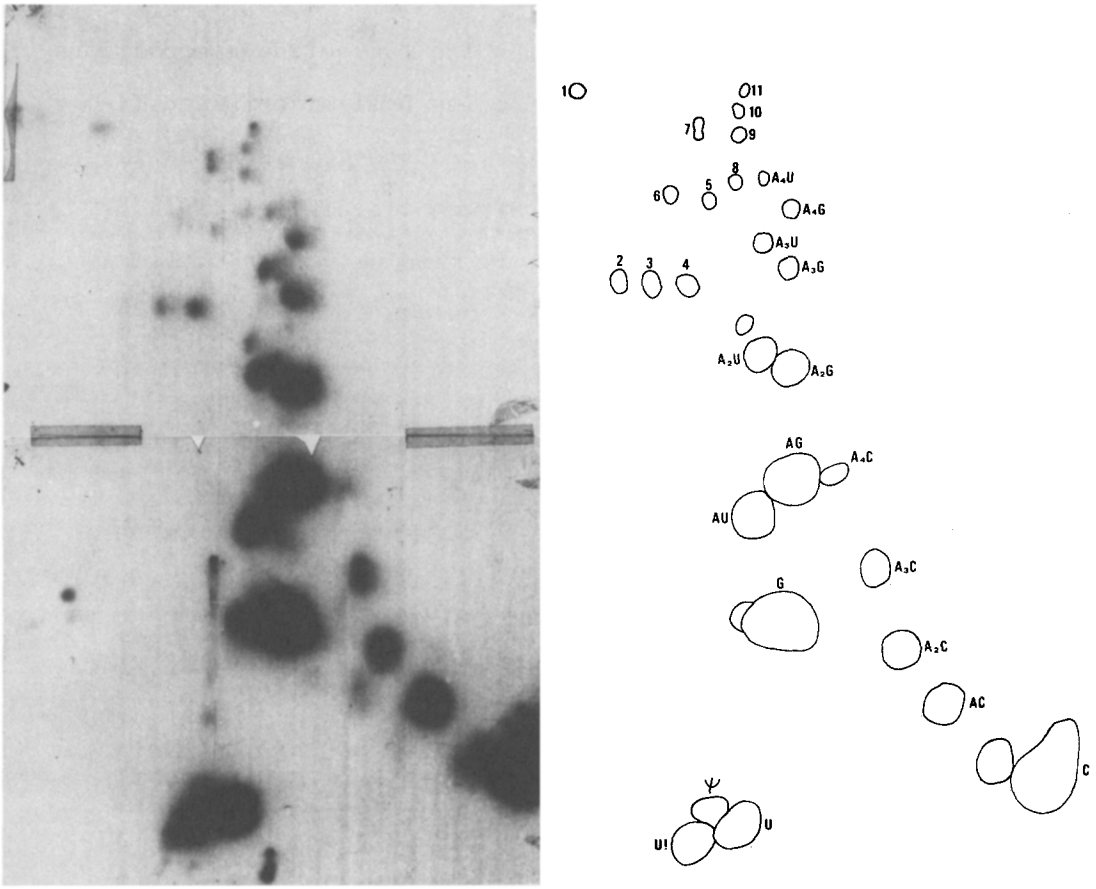

Figure 1 Fingerprint of a combined T1-pancreatic RNase digest of the 28S rRNA of Xenopus laevis. Xenopus laevis 28S rRNA (75 μg, 10,000 cpm/μg) was incubated at 37° for 30 minutes with 7.5 μg (1:10) pancreatic ribonuclease and 4 μg (1:20) T1 ribonuclease (Sankyo). Abbreviation: ψ denotes pseudouracil.

by RNA fingerprinting allows the visualization and analysis of some RNase-resistent methylated sequences and regions containing oligo-A sequences (6,9). Also, from this analysis, quantitative data concerning molecular weight and overall nucleotide composition can be obtained. A fingerprint of a combined digest of Xenopus

laevis 28S rRNA is shown in Figure 1a. Figure 1b is a diagrammatic representation of this fingerprint with the nucleotide compositions denoted. A comparison of the slower moving half of combined T1-pancreatic fingerprints of HeLa and Xenopus laevis 28S rRNAs is presented in Figure 2. This portion of the fingerprint contains

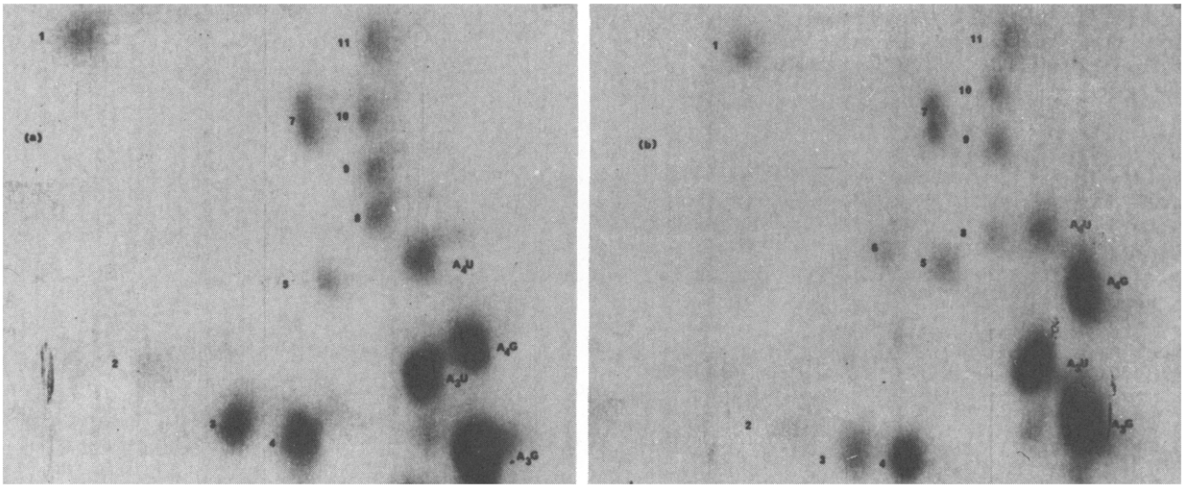

Figure 2 The slow moving half of a fingerprint of a combined T1-pancreatic ribonuclease digest of the 28S rRNA of Xenopus laevis and HeLa cells. Digestion of rRNA was done as described in Figure 1 legend. The specific activity of HeLa cell rRNA was 2 x 10^5 cpm/μg.
(a) HeLa 28S rNA (b) Xenopus 28S rRNA

the best resolved stable methylated sequences as reported by Maden and Forbes, and Maden et al (6,9). It can be seen that spot 8 (using the numbering system of Maden et al.) in the Xenopus fingerprint is shifted from the position observed in the HeLa fingerprint. Also, there is the appearance of an additional spot (denoted as 6) which appears on the Xenopus fingerprint. Position differences may exist in spots 9, 10 and 11 and further analysis may reveal

sequence differences in these oligonucleotides. While these differences are readily visible between the fingerprints of Xenopus laevis and HeLa cells, no differences can be observed between the fingerprints of Xenopus mulleri rRNA and that shown for Xenopus laevis.

From combined T1-pancreatic RNase fingerprints, all spots were cut out for scintillation counting. Molar abundances of the digestion products were derived from a knowledge of the sequences and counts per minute in the individual products and by assuming one sequence per 28S molecule of the least frequent methylated spots. The compositions of the Xenopus methylated spots which correspond in their positions in the fingerprints to HeLa methylated spots have been assumed to contain those sequences as reported by Maden et al (9).

Table I gives sequence data and estimated molecular abundances of the resolved products of T1-pancreatic digests of Xenopus laevis, Xenopus mulleri, and HeLa cell 28S rRNA. Also shown are molecular weights and numbers of nucleotides in each molecule calculated from the molecular abundances. Again, it can be noted that the 28S rRNA molecules of the two Xenopus species are essentially identical while that of HeLa differs greatly. Though each calculation represents the average of several determinations, the low specific activity of the RNA used in these experiments makes it unclear whether small differences which can be noted between Xenopus laevis and Xenopus mulleri in several residues are significant.

Homochromatography, as developed by Brownlee and Sanger (7), can be used to obtain fingerprint patterns which resolve the largest fragments of RNA produced by a T1 RNase digestion. While a homochromatography fingerprint of the oligonucleotides of an RNA molecule is characteristic for that molecule, some alterations in the sequence

TABLE I

MOLAR ABUNDANCES OF OLIGONUCLEOTIDES IN 28S rRNA

Fingerprints were done as described in Figure 1. The sequences of spots 1-11 have been assumed to be the same in Xenopus as have been described by Maden et al (9) for the purposes of these calculations even though differences in some spots are evident, as described in the text.

Product	Xenopus laevis	Xenopus mulleri	HeLa
G	1369	1365	1800
AG	214	208	228
AAG	68	63	67
AAAG	12	15	13
AAAAG	4.4	5.0	3.7
U	616	612	963
AU	92	98	98
AAU	25	24	28
AAAU	6.3	8.5	5.6
AAAAU	1.6	1.6	1.8
C	1110	1164	1143
AC	112	119	168
AAC	33	21	41
AAAC	11	13	16
AAAAC	2.5	2.2	2.1
A2	3.1	4.0	3.1

TABLE I CONTINUED

1	UmGmU	1.5	2.0	1.7
2	UmU	0.7	1.0	1.0
3	UmG	4.1	2.8	5.0
4	GmG	8.2	8.2	7.7
5	AGmG	1.0	1.6	1.1
7	AAUmg	1.4	2.0	2.0
8		--	--	1.0
9	GmAAmAG	0.8	1.0	0.9
10	A(A,GmA)G	0.8	1.0	1.1
11		0.9	1.0	0.9
Mol. wt.		1.58×10^6	1.58×10^6	1.83×10^6
no. of ntds.		4652	4624	5540

within the oligonucleotide would not result in large changes in their position on the fingerprint.

Figure 3 shows an autoradiogram of a typical homochromatography fingerprint of a T1 digest of Xenopus mulleri 28S rRNA. The patterns of those spots which contain oligonucleotides with greater than 8 residues have been used to compare the different 28S molecules. Figure 4 is a diagrammatic representation of these regions of the homochromatography fingerprints. The Xenopus laevis and Xenopus mulleri patterns are similar if not identical while the HeLa cell pattern shows little, if any, similarity. The size of the oligonucleotides was estimated from the specific activity of the rRNA and CPM in each spot.

While the methods we have used are capable of detecting the divergence between the Xenopus species and HeLa cells by examining

Figure 3 Homochromatography fingerprint of a T1 RNase digest of Xenopus mulleri 28S rRNA. Xenopus mulleri 28S rRNA (65 μg, 7000 cpm/μg) was incubated at 37° for 30 minutes with 3 μg (1:20) T1 RNase. Homochromatography was done for 16 hours at 65° C on 20 x 20 cm DEAE-cellulose 300 plates (Macherey-Nagel) to which a 20 cm piece of Whatman 3 MM paper had been sewn so as to allow the solvent front to move 40 cm. The region below the arrow contains the oligonucleotides with 8 or more residues. It is this area which is used for the analysis shown in Figure 4.

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the properties of a small but specific fraction of the nucleotides of each 28S molecule, we have been unable to observe any differences in the 28S rRNA of Xenopus laevis and Xenopus mulleri. These

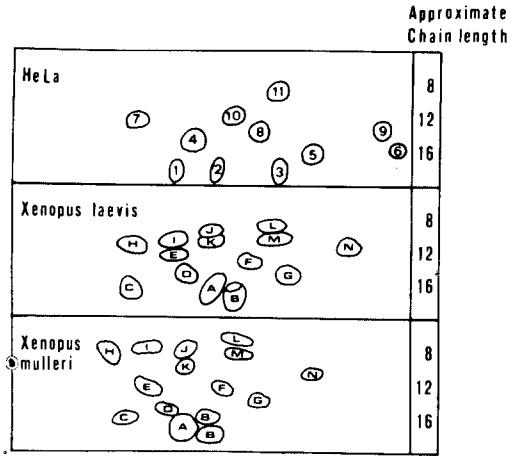

Figure 4 A comparison of the region of the homochromatography fingerprints containing oligonucleotides with 8 or more residues. Homochromatography was done as described in Figure 3 legend on 28S RNA from Xenopus laevis (75 μg, 10,000 cpm/μg), Xenopus mulleri (65 μg, 7000 cpm/μg) and HeLa cells (70 μg, 40,000 cpm/μg).

results confirm the similarity of the rRNA molecules of the two Xenopus species first noted by Brown et al (4) and suggest the possibility that the sequences are highly conserved during the evolution of the two species.

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