Volume 2 number 12 December 1975 Nucleic Acids Research

The nucleotide sequence of the 5S RNA of chicken embryo fibroblasts

G.G.Brownlee and E.M.Cartwright

Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK.

Received 24 October 1975

ABSTRACT

The nucleotide sequence of uniformly ^{32}P-labelled chicken 5S RNA has been determined by analysing the end-products of T_1 and pancreatic ribonuclease digestion. These oligonucleotides can be aligned by homology with the human sequence to give a sequence differing in only seven positions from that of Man. The sequence deduced here differs in two positions from that previously published for chicken 5S RNA.

INTRODUCTION

The nucleotide sequence of many different 5S ribosomal RNAs both of procaryotic and eucaryotic origin have now been described (1-3). Although the precise function of 5S RNA in terms of its molecular structure is still unknown, it is particularly suitable for comparative sequence studies as it is relatively easy to purify. Moreover, at least amongst higher organisms, its structure is remarkably conserved (4). For example, its sequence in mouse and Man is identical and even in amphibia, in Xenopus laevis kidney cells, it differs in only eight nucleotides from Man (5). We have now studied chicken 5S RNA to examine whether its structure is similarly conserved. Its sequence has also been studied by Pace et al. (6), but our sequence differs in two positions from theirs.

METHODS

A primary culture of fibroblasts was prepared from the livers of ten day old chick embryos and cultured in minimal Eagle's medium (7) lacking phosphate, but supplemented with 10% horse serum and 10% chicken embryo extract. After two days growth (inoculum at 1 x 10^5 cells/ml) in plastic flat-bottomed flasks (Falcon) using 5% CO_2, air at 37°C, 1 mCi of [^{32}P] orthophosphate (Radiochemical Centre, Amersham) was added to each of eight flasks and growth continued to confluence for a further 40 hr. After harvesting cells by "trypsinisation" (7), a crude RNA fraction was prepared by phenol extraction of the cells (after washing) in 10 ml of 0.01 M Tris-

chloride, pH 7.5, 0.01 M $MgCl_2$ for 1½ hr at room temperature. After precipitation of RNA by standard methods, 5S RNA was purified by 10% acrylamide gel electrophoresis (8) and appeared as a sharp band slower than the more diffuse tRNA band (see Fig. 1).

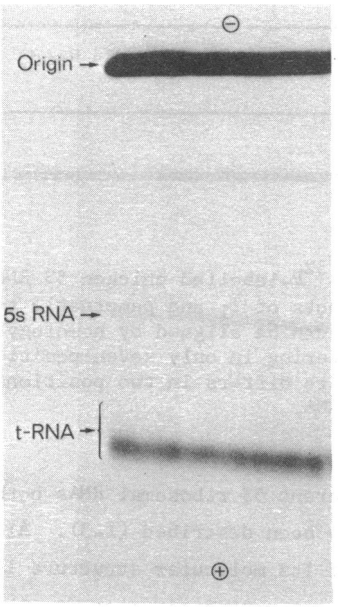
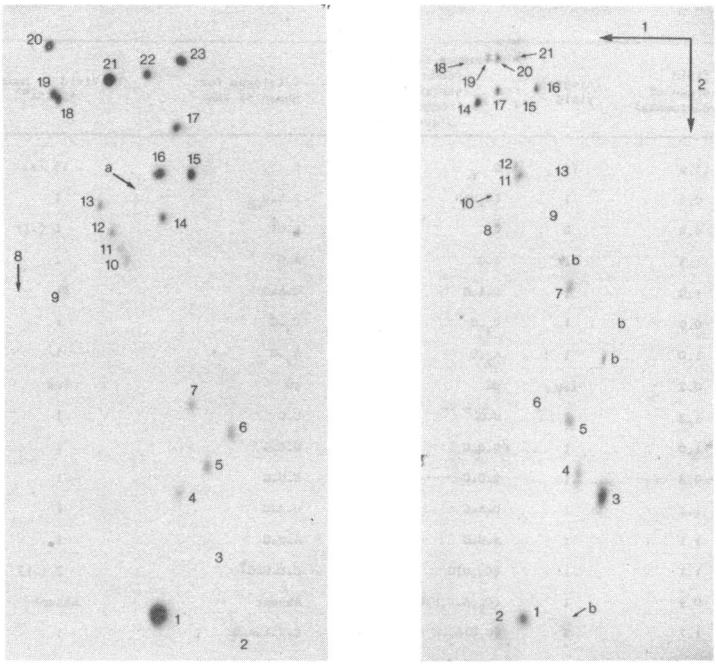


Fig. 1. Radioautograph of a purification of 5S RNA by 10% acrylamide gel electrophoresis in Tris-borate-EDTA buffer pH 8.4 on a 20 x 40 cm gel slab (8).

After elution the 5S RNA was digested separately with pancreatic and T_1-ribonucleases and fingerprinted using standard methods (8). Oligonucleotides were analysed with either pancreatic or T_1-ribonucleases, or in a few cases with U_2-ribonuclease by standard methods (8).

RESULTS

Fig. 2 shows fingerprints of the end-products of T_1 and pancreatic ribonuclease digestion of the 5S RNA. All major spots were analysed for their relative molar yield (8) and sequence (see Tables 1 and 2). A comparison of the T_1-ribonuclease end-products with the catalogue of T_1-oligonucleotides for human 5S RNA (9) shows that most spots are common to both species (see Table 1). Even where the sequence of the oligonucleotide was incomplete (in the case of most of the larger oligonucleotides), the partial sequence agreed with the corresponding oligonucleotide from human 5S RNA. But four spots (15, part of 16, 22 and 23 - as denoted by an asterisk in Table 1) were present in chickens but absent in human 5S RNA. Conversely six oligonucleotides (spots 3, 14 [one of the two moles] and the



(a) (b)

Fig. 2. Radioautographs of (a) the end-products of T_1-ribonuclease digestion and (b) of pancreatic ribonuclease digestion of 5S RNA. Both were fractionated in direction (1) by ionophoresis on cellulose acetate at pH 3.5 in 7 M urea and in direction (2) by ionophoresis on DEAE paper using 7% formic acid. The faint spot marked "a" is probably C-U-U-U_{OH} (see text). Spots identified by "b" are artefacts of radioautography and do not correspond to oligonucleotides.

last four sequences in the catalogue of human oligonucleotides, all marked with † in Table 1) were present in human but absent from chicken 5S RNA.

A similar comparison was made of the end-products of pancreatic ribonuclease digestion (Table 2) with those published for human 5S RNA. Only two products, pG-C (spot 10) and 1 mole of G-G-U (spot 14) were identified as being present in chicken, and absent from human 5S RNA. Conversely, G-A-A-C was absent in chicken (although its isomer in spot 7, A-A-G-C, was still present) as was pG-U and 2 moles of A-C. It will be noted from Table 2 that there are large discrepancies between observed yields and the assumed integral values for some large oligonucleotides (e.g. see spot 18). But such deviations have been observed and their significance discussed before (8) and were not unexpected.

Table 1

Spot (fig. 2a)	Yield (average of 2 experiments)	Integral yield	Sequence deduced from products of pancreatic ribonuclease digestion‡	Catalogue for human 5S RNA	Yield in human 5S RNA**
1	15.9	16	G	G	15 (+1)
2	0.6	1	(C,U)N	C-U-U_{UH}	1
3	0.4	0	C-G	C-G†	1 (-1)
4	1.3	1	A-G	A-G	1
5	1.0	1	C-A-G	C-A-G	1
6	0.9	1	C_3-G	C_3-G	1
7	1.0	1	A_2-G	A_2-G	1
8	0.2	low	pG	pG	low
9	1.3	1	U-G	U-G	1
10	1.0	1	U-C-G	U-C-G	1
11	0.8	1	C-U-G	C-U-G	1
12	1.1	1	U-A-G	U-A-G	1
13	1.1	1	A-U-G	A-U-G	1
14	1.1	1	(C_2,U)G	C-C-U-G†	2 (-1)
15*	0.9	1	(C_2,A-C,U)G	Absent	Absent
16*	1.7	2	(C,U)A_2-G + (A_2C,U)G	C-U-A-A-G	1
17	0.9	1	(A_2-U,A-C,C)G	A-A-U-A-C-C-G	1
18	1.1	1	(U_2,C)G	U-C-U-G	1
19	0.9	1	U_2-A-G	U-U-A-G	1
20	1.1	1	(U_3,A-C)G	U-A-C-U-U-G	1
21	1.7	2	(A-U,C_2,U)G	A-U-C-U-C-G	2
22*	0.8	1	(C_3,A-C,U_2)G	Absent	Absent
23*	0.7	1	(C_{5-6},A-U,A-C,U)G	Absent	Absent
				A-C-C-G†	1
				U-C-U-A-C-G†	1
				A-A-C-G†	1
				C_2-A-U-A-C_2-A-C_3-U-G†	1

Footnotes

* Spots present only in chicken 5S RNA (see text)

† Spots present only in human 5S RNA (see text)

** Differences between chicken and human (cf. columns 3 and 6) are indicated by "Absent" or the figures in brackets

‡ After digestion, products were fractionated by DEAE-paper ionophoresis at pH 3.5 and identified by their position [8]. Yields of mononucleotides were estimated by visual inspection of the radioautographs. Spots 10 and 11 were identified by position alone [10].

The sequence of chicken 5S RNA was derived by pairing off the four characteristic T_1 end products of chicken 5S RNA with the six characteristic and homologous products in Man. Table 3 shows the derivation which involves a point mutation in three cases (A, B and C) but necessitates

Table 2

Spot (fig. 2b)	Experimental yield	Integral yield	Sequence deduced from products of T_1-ribonuclease digestion	Yield in human 5S RNA**
1*, 2*	11.4	11	U + U!	11
3*	13.7	14	C	16 (-2)
4*	3.0	3	A-C	5 (-2)
5*	2.8	3	G-C, A-A-C	Not applicable
6*	1.1	1	A-U	1
7	1.0	1	A-A-G-C	2†(-1)
8	1.6	2	G-U	2
9	0.5	1	G-G-C	1
10	0.2	1	pG-C	0 (+1)
11	1.8	2	G-A-U	2
12	1.1	1	A-G-U	1
13	0.5	1	(A-G,G)C	1
14	2.0	2	G-G-U	1 (+1)
15	0.5	1	G-G-G-C	1
16	0.8	1	$(A_2G,G_2)C$	1
17	1.1	1	G-G-A-U	1
18	0.4	1	G-G-G-U	1
19	0.6	1	$(G_2,A-G)U$	1
20	0.6	1	G_3-A-A-U	1
21	0.5	1	$(G_3,A-G)A-C$	1

Footnotes

* Spots 1-6 were not eluted but were identified from their position on the fingerprint. Spot 5 was assumed to be a mixture of G-C and A-A-C as G-A-A-C was absent (see text)

** The yield of the human oligonucleotides is shown here [9] with the differences from chicken (cf. columns 3 and 5 of the table) shown in brackets

† Human 5S RNA gives 1 mole of A-A-G-C and 1 mole of G-A-A-C whilst chicken only has A-A-G-C. Human 5S RNA also has pG-U replacing pG-C.

mutation and rearrangement in case D. The evidence for each will now be considered in detail.

Table 3

Paired sequences of chicken and human 5S RNAs to show derivation of new chicken sequence

	9 20	
A	C-C-A-U-A-C-C-A-C-C-C-U-G	Human
	(C_{5-6},A-U,A-C,U)G	Chicken (spot 23)
	C-C-A-U-C-C-C-A-C-C-C-U-G	Derived chicken
	90 97	
B	A-C-C-G-C-C-U-G	Human
	(C_3,A-C,U_2)G	Chicken (spot 22)
	A-C-C-U-C-C-U-G	Derived chicken
	2 7	
C	U-C-U-A-C-G	Human
	(C_2,U,A-C)G	Chicken (spot 15)
	C-C-U-A-C-G	Derived chicken
	22 27	
D	G-A-A-C-G-C-G	Human
	G(A-A-C,U)G	Chicken (spot 16, part of)
	G-G-U-A-A-C-G	Derived chicken

(A) Spot 23 in chicken analyses as (C_{5-6},A-U,A-C,U)G and is clearly homologous with the sequence C-C-A-U-A-C-C-A-C-C-C-U-G in Man. Assuming one base change, an A-C in Man must have changed to a C-C sequence in chickens. As there are two A-C sequences and either could have changed there are two possibilities for spot 23 - either C-C-A-U-C-C-C-A-C-C-C-U-G (i) or C-C-A-U-A-C-C-C-C-C-C-U-G (ii). Ribonuclease U_2, which cleaves after A residues in T_1 end-products, was used to distinguish the two possibilities as well as to check that further base changes had not occurred. The products, after enzyme digestion (8), were analysed against marker C-G, C-U-G and C-C-U-G (Fig. 3). Three products - a, b and c - were present in positions compatible with C_3-U-G, U-C_3-A and C_2-A whilst no product was present in the position of C_6-U-G, thus supporting sequence (i)

above. Unfortunately, there was insufficient material for further analysis of the composition of these products.

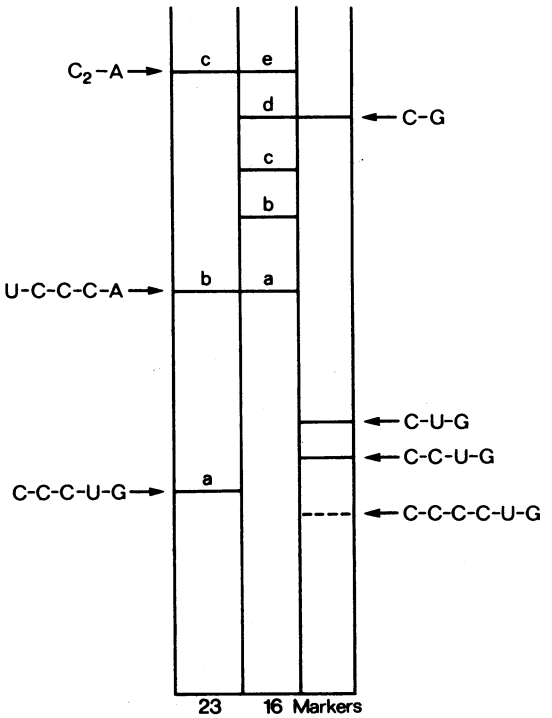


Fig. 3. Ribonuclease U_2 degradation pattern of spots 16 and 23 (from Fig. 2a) fractionated by ionophoresis on DEAE paper using 7% formic acid together with some marker oligonucleotides. The probable identity of 23a, 23b and 23c is shown on the left (see text).

(B) Spot 22 (C_3,A-C,U_2)G has the correct composition, in terms of its pancreatic digestion products, to be A-C-C-U-C-C-U-G, this being a G→U change at position 93 (see Fig. 4). This clearly explains the absence of A-C-C-G and C-C-U-G (1 mole) in chickens. There is also no doubt that spot 22 has two U residues because of its position on the fingerprint (10).
(C) Spot 15 (C_2,A-C,U)G is homologous to U-C-U-A-C-G, if one of the U residues is changed to a C residue. There are two possibilities, either C-C-U-A-C-G (i) or U-C-C-A-C-G (ii). Sequence (i) is supported by the presence of pG-C (spot 10 of Table 2) - the unique product overlapping spot 15, in the pancreatic ribonuclease digest (see also Fig. 4).
(D) Spot 16 could be either A-A-C-U-G (i) or U-A-A-C-G (ii). The analysis of this spot was complicated because it was mixed with C-U-A-A-G which is

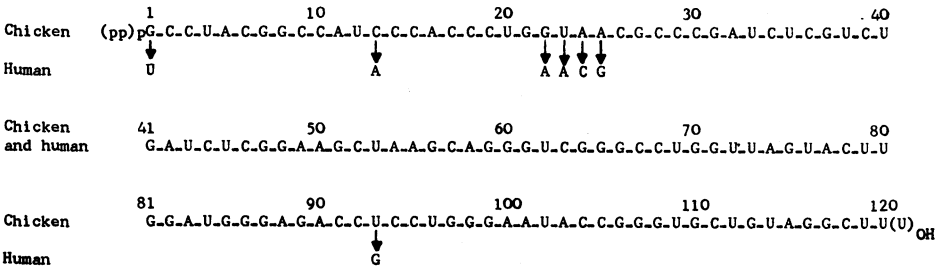


Fig. 4. Sequence of chicken 5S RNA

present in both chicken and human 5S RNA. Ribonuclease U_2 digestion, followed by ionophoresis with markers (Fig. 3) gave five bands (a-e). Product d is likely to be C-G, and c to be U-A thus supporting sequence (ii) above. (Neither U-A nor C-G should be obtained from C-U-A-A-G.) The absence of (i) was also clear as there was no product in the position of C-U-G (see Fig. 3). There was insufficient material for further analysis of a-e. It is therefore clear (Table 3) that the A-A-C sequences in chickens and Man are no longer aligned. The only product not so far accounted for is the additional G-G-U in the pancreatic ribonuclease digest which may thus be overlapped with U-A-A-C-G giving the sequence G-G-U-A-A-C-G. Strong supporting evidence for this alignment comes from the absence of G-A-A-C in the pancreatic ribonuclease digest and of C-G (spot 3) in the T_1-ribonuclease digest. (In fact, C-G was present in the low yield of 0.4 moles, but this result was interpreted as indicating the absence of C-G. The origin in 0.4 moles of C-G may be from contaminants in the 5S RNA or it might conceivably be due to some form of heterogeneity with the sequence. A similar low yield of C-G was found in the 5S RNA of Xenopus laevis and may represent microheterogeneity as position 53 in that molecule (11).

The results are summarised in Fig. 4 showing that chicken 5S RNA differs from human 5S RNA in seven positions. Three base changes occur at positions 2, 13 and 93 and a further four bases differ between residues 22-25. Within this region the sequence A-A-C-G is retained, although it is displaced two positions to the right in chickens.

It may be noted here that the chicken sequence is shown in Fig. 4 starting with a triphosphate 5' end group and ending with three U residues, as occurs in Xenopus laevis and Man (11,12). Although we did not detect

pppGp, ppGp and C-U-U-U_{OH} here, this was almost certainly for technical reasons and they have been found by others (6). Thus spot a on Fig. 1(a) is likely to be C-U-U-U_{OH}, and ppGp and pppGp were probably run off in the first dimension of the fingerprint of Fig. 1(a). This hypothesis explains the low yield of pGp and C-U-U_{OH} in Table 1.

DISCUSSION

We were able to deduce the sequence of chicken 5S RNA from the limited sequence analysis presented here, principally because of the few differences between chicken and the known sequence in Man. Fortunately all the differences occurred in oligonucleotides which could be uniquely positioned in the homologous structure so that it was not necessary to investigate partial enzymatic digests to position any of the new oligonucleotides. Nevertheless this approach, relying on homology, does not have the safeguards of a fully independent sequence analysis, partly because of the shorter time and effort required, and partly because supporting evidence derived from partial enzymatic digests of the RNA is not obtained. Thus one cannot be absolutely sure that all base changes have been detected. In particular, microheterogeneity, involving a base change in only a fraction of the molecules, may well be overlooked (see above). Also, it is possible to imagine base changes of a 'compensating' nature which would remain undetected by the methods used here - for example, the insertion of a G at position 8 and a corresponding deletion of a G at position 65. However, in view of the small number of base changes between the two species such compensating changes would be very unlikely, and would in most cases still be detected.

The sequence deduced by Pace et al. (6) by similar methods to ours differs by a U→C change (position 20) and a G→U change (position 21). The origin of the difference at position 20 derives from a difference in the analysis of spot 23 (our nomenclature) using ribonuclease U_2. Pace et al. (6) report this as C-C-A-U-C-C-C-A-C-C-C-C-U-G which should give the sequence C_4-U-G after ribonuclease U_2. As may be seen in Fig. 3, our product is clearly faster than the position of C_4-U-G, which was derived by measurement of its mobility relative to C-U-G and C-C-U-G on a similar fractionation system in other studies (13). The second difference (position 21) appears in part to derive from a difference in the quantitation of G-U in the pancreatic ribonuclease end-products. Pace et al. (6) obtain an additional mole of G-U while we do not (see Table 2) although they ignore the fact that they also obtain an additional G-G-U in chickens.

In summary, the discrepancies derive from minor differences in analyses which we believe led others (6) to an incorrect structure.

The conservative nature of the structure of 5S RNA in higher organisms is somewhat surprising and must reflect the rather rigorous requirement for a specific structure (6). Nevertheless, sequences which differ between species are unlikely to have a key role in its function. However, the significance of the base changes must await a greater understanding of the structural-functional relationship of 5S RNA.

ACKNOWLEDGEMENTS

This work was undertaken as part of a UNESCO course for advanced students from South America, held under the direction of Prof. J.E. Allende in the Department of Biochemistry, University of Santiago, Chile. We particularly want to thank the following students for significant contributions to the results: B.O. Nascimento, F.D. Nobrega, A.M. Saburo, E. Rho, as well as Dr. A. Yudelevich.

We also thank Dr. C. Slater for essential help in preparing the primary culture of fibroblasts.

REFERENCES

1. Barrell, B.G. and Clark, B.F.C. (eds.) (1974) in Handbook of Nucleic Acid Sequences, Joynson-Bruvvers, Oxford, U.K.
2. Corry, M.J., Payne, P.I. and Dyer, T.A. (1974) FEBS Lett. 46, 63-70.
3. Miyazaki, M. (1974) J. Biochem. (Tokyo) 75, 1407-1410.
4. Williamson, R. and Brownlee, G.G. (1969) FEBS Lett. 3, 306-308.
5. Brownlee, G.G., Cartwright, E.M., McShane, T. and Williamson, R. (1972) FEBS Lett. 25, 8-12.
6. Pace, N.R., Walker, T.A., Pace, B. and Erikson, R.L. (1974) J. Mol. Evol. 3, 151-159.
7. Paul, J. (1965) in Cell and Tissue Culture (E. and S. Livingstone, Ltd., Edinburgh).
8. Brownlee, G.G. in Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 3, Part I, eds. T.S. & E. Work (North-Holland, Amsterdam, 1972).
9. Forget, B.G. and Weissman, S.M. (1967) Science 158, 1695-1699.
10. Sanger, F., Brownlee, G.G. and Barrell, B.G. (1965) J. Mol. Biol. 13, 373-398.
11. Wegnez, M., Monier, R. and Denis, H. (1972) FEBS Lett. 25, 13-20.
12. Hatlen, L.E., Amaldi, F. and Attardi, G. (1969) Biochemistry 8, 4989-5005.
13. Brownlee, G.G., Cartwright, E.M. and Brown, D.D. (1974) J. Mol. Biol. 89, 703-718.