THE SEQUENCE OF 5S RIBOSOMAL RNA FROM TWO MOUSE CELL LINES

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Received 16 May 1969

1. Introduction

No differences have been found in the T-1 and pancreatic ribonuclease "fingerprints" of ^{32}P-labelled 5S ribosomal RNA of Landschutz and LS mouse cell lines, and that of human KB tumour cell 5S RNA. It is therefore probable that the total sequence is the same in both mice and humans.

2. Experimental

The sequences of 5S ribosomal RNA from *E. coli* and from KB human epidermoid tumour cells have been published and show many differences [1,2]. In the course of other experiments we have isolated samples of ^{32}P-labelled 5S ribosomal RNA from two mouse lines and have prepared "fingerprints" using the technique of Sanger et al. [3]. Landschutz (mouse tumour line of ascitic origin) and LS (mouse line of subcutaneous connective tissue origin) both grow well in suspension culture. Landschutz cells are definitely mouse cells, as they can be transplanted to and from mice where they grow as an ascitic tumour; LS cells were karyotyped and the chromosome pattern closely resembled that of mouse, and was definitely not that of a human cell.

^{32}P-labelled 5S RNA was prepared as follows. Cells were labelled for 16 hr in a 100 ml suspension containing approximately 0.4×10^6 cells/ml in Waymouth's medium containing low phosphate Hank's balanced salt solution [4] and 0.05 mCi carrier-free ^{32}P-phosphate/ml. 5S RNA was isolated from the ribosomes according to the procedure of Reynier et al. [5], using G-100 Sephadex. The specific activity of the isolated RNA was about 10^5 dpm/μg. 5S RNA from Landschutz and LS cells was digested with T-1 RNase (Sankyo) and pancreatic RNase (Worthington) in separate experiments and fractionated using the standard two-dimensional ionophoretic method [3]. Fig. 1 and 2 show the autoradiographs of the "fingerprints" of the T-1, and pancreatic RNase digests, respectively. Oligonucleotides are identified by the same numbers as were used for human 5S RNA by Forget and Weissman [2]. The composition of each spot was determined by alkaline hydrolysis and where necessary further analysis was carried out using one or more of the following procedures: pancreatic RNase digestion of T-1 oligonucleotides; T-1 RNase digestion of pancreatic oligonucleotides; and partial spleen phosphodiesterase digestion on both T-1, and pancreatic oligonucleotides. The yields of the oligonucleotides were determined by counting in a liquid scintillation counter. (See ref. 3 for further details of methods used). The dry papers could also be counted without any scintillant. Counts in such a system arise from either fluorescence or Čerenkov emission of photons when a β-particle hits the glass wall of the vial. The efficiency of counting is approximately 30%, is very constant and is independent of the size of the paper spot, and its position in the vial, provided it is not folded over.

All oligonucleotides from the T-1 RNase digest (fig. 1) were positively identified as having identical sequences to those in KB cell 5S RNA with the exception of spots 56 (U-C-U-A-C-G) and 55 (C-C-A-U-A-C-C-A-C-C-C-U-G). In these two cases, however, further pancreatic ribonuclease digestion

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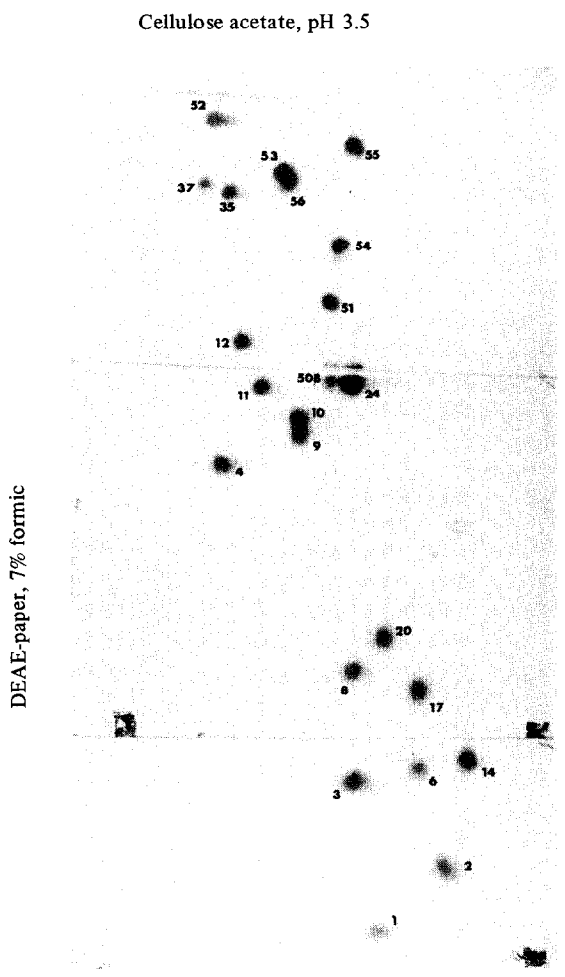

Fig. 1. Autoradiograph of a T-1 RNase digest of 5S RNA of Landschutz ascites cells fractionated by ionophoresis on cellulose acetate at pH 3.5 (direction 1) and ionophoresis on DEAE-paper using 7% formic acid (direction 2). The numbers are those of Forget and Weissman [2]. Their 50-A has run off in the second dimension. 53 and 56 separate only partially.

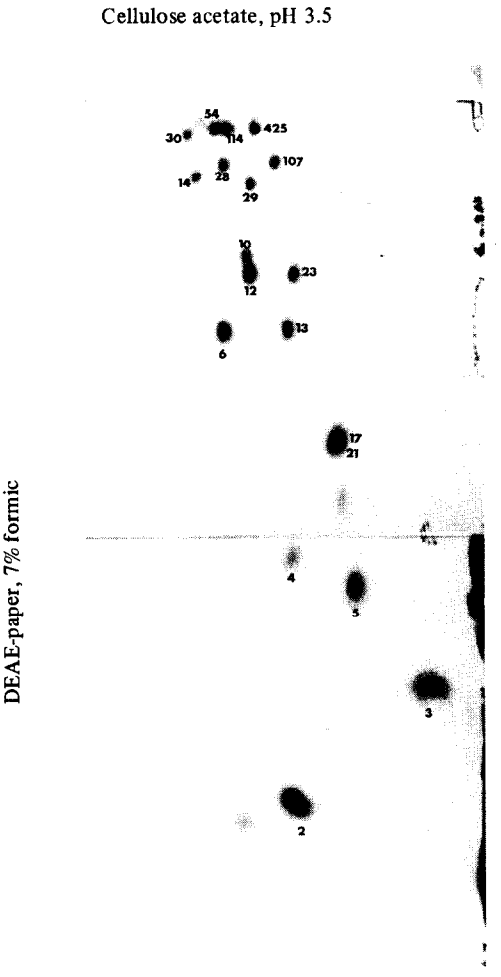

Fig. 2. Autoradiograph of a P-RNase digest of 5S RNA of Landschutz ascites cells fractionated by ionophoresis on cellulose acetate at pH 3.5 (direction 1) and ionophoresis on DEAE-paper using 7% formic acid (direction 2). The numbers refer to Forget and Weissman [2]. 17 and 21 do not separate. 6′ (pG-U) is not visible and 1(C) has run off.

gave the products predicted by those sequences and there was tentative evidence that 56 was U-(C,U)-A-C-G. There were no consistent differences in the molar yields of any of the fragments determined by counting in a scintillation counter from those for KB cell RNA except that the yield of pG was low. This probably indicates that the terminal 5′-phosphate is partially removed by hydrolysis during the preparation. As in KB cell RNA, we found that both C-U-U_{OH} and C-U-U-U_{OH} were present at the 3′-hydroxyl end, but we consistently found the latter present in greater amounts than the former, in a ratio of approximately 4:1. We suppose that C-U-U_{OH} derives from an incompletely modified 5S RNA, the last U being added by a "repair" enzyme after transcription. The pancreatic RNase digest (fig. 2) also

contains all of the sequences found in KB cell RNA. The molar yields were consistent with those reported for KB cell RNA, although owing to difficulties in controlling the extent of digestion, the yields of the smaller oligonucleotides were often high. The 5′-terminal oligonucleotide was confirmed as pG-U.

3. Discussion

Although our results do not rule out completely the possibility of minor sequence differences, specifically inversions, which could only be resolved by a complete sequence analysis, it is probable that there is no sequence difference between mouse and human 5S ribosomal RNA. Labrie [6] has also found a similar identity of the T-1 oligonucleotide sequences of normal rat pituitary and rabbit reticulocyte 5S RNA, which therefore suggests that the sequence of 5S RNA of these four mammalian species is probably the same.

It is therefore apparent that this represents a higher degree of conservation than is normally observed between functionally identical proteins. For example there are 23 amino-acid sequence differences between mouse and human haemoglobin-α [7,8,9], which is an 8% difference between the two molecules. Even between related primate haemoglobins there is a small difference, of the order of 1% [10]. These differences must reflect a similar although not an identical percentage difference in the gene. Thus in order to explain the sequence identities of the 5S RNAs, we can postulate that either there has not been sufficient time for natural selection to accumulate acceptable point mutations in the gene, or there must be a strong selective pressure in favour of the conserved structure. We can only speculate about rate of selection but, arguing by analogy with proteins, we would suggest that there has been ample time for the divergence of the structure of 5S RNA amongst mammals. We therefore conclude there has been a strong selective pressure to conserve the functional sequence of 5S RNA found in mammals. We would assume that this represented a conserved gene or genes for 5S RNA.

It is known that in *Xenopus laevis* (toad) there are at least 27,000 genes per haploid genome which will specifically hybridise with labelled 5S ribosomal RNA [11]. (This does not in any way show that this number of genes is actually active in synthesising 5S RNA, nor even, owing to possible non-exact matching during hybrid formation, that every one of these genes is exactly complementary to 5S RNA (cf. 12). If, however, the number of complementary genes is similar for mammals, and even a percentage of these genes are active in 5S RNA synthesis, such a reiterated genome might be thought to have an increased opportunity to mutate and to diverge in sequence in the absence of a mechanism to reassert the fidelity of the sequence at least once in each generation [13]. There remains the possibility, which we cannot exclude, that "mutant" sequences of 5S RNA are synthesised but in too low a proportion to be detected by the "fingerprint" technique we have used. A further possibility is that such "mutant" sequences may not be incorporated into the ribosome and are therefore not isolated by the method we used.

Acknowledgements

We would like to thank Dr. F.Sanger and Dr. J.Paul for discussions and advice, and Mrs. E.Cartwright and Miss T.Flanigan for valuable technical assistance. This work was supported by grants from the Medical Research Council and the British Empire Cancer Campaign for Research to the Beatson Institute for Cancer Research.

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