# NUCLEOTIDE SEQUENCES FROM HeLa CELL HnRNA

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## 1. Introduction

Studies on mammalian cell HnRNA, now regarded as a precursor of mRNA, have revealed several interesting chemical features such as the occurence of polyadenylic acid [1–6], oligouridylate regions [7, 8] and double stranded regions [9]. This paper presents a characterisation of the nucleotide sequences of HeLa cell HnRNA by the 'fingerprinting' technique of Sanger et al. [10]. The fingerprints obtained are extremely complex but reveal one highly distinctive feature, namely the low frequency of certain small oligonucleotides terminated by the dinucleotide, CG.

## 2. Methods

Exponentially growing monolayer cultured HeLa cells, in rotating 80 oz bottles, were washed with low phosphate Eagle's medium ($10^{-4}$ M) and then incubated in 50 ml of the same medium containing 0.04 $\mu$g $ml^{-1}$ actinomycin D, which selectively suppresses ribosomal RNA synthesis without apparent effect on Hn or messenger RNA synthesis without apparent effect on Hn or messenger RNA synthesis [11, 12]. After 0.5 hr, 10 mCi of [$^{32}$P] orthophosphate (carrier free) were added. After a further 2.5 hr the cells were harvested, fractionated to give nuclei [13], the nuclei digested with DNAase [13] and the nuclear RNA extracted using hot phenol–SDS [14].

Nuclear RNA was centrifuged, using a Spinco SW-27 rotor at 16000 rpm for 16 hr; through a gradient of 15–30% (w/v) sucrose in 0.1 M LiCl–0.01 M EDTA –0.2% SDS–0.01 M Tris–HCl, pH 7.4. Fractions sedimenting faster than 32 S were combined and precipitated with 2.5 vol of ethanol at –20°C.

$^{32}$P-labelled ribosomal RNA was prepared from the cytoplasm of cells labelled with [$^{32}$P] orthophosphate for 18 hr [15].

Ten-microgram samples of [$^{32}$P] RNA were subjected to ribonuclease T1 digestion followed by two dimensional electrophoresis on cellulose acetate and DEAE paperby the procedure of Sanger et al.[10]. After autoradiography (with Kodirex film for 9 days) oligonucleotides were excised, assayed for radioactivity in toluene–PPO scintillation fluid and, where indicated, were subjected to sequence analysis by standard procedures [10]. Samples of each $^{32}$P-labelled RNA were also subjected to alkaline hydrolysis and electrophoresis to determinetheir base compositions.

## 3. Results and discussion

Fig. 1 shows ribonuclease $T_1$ digests of $^{32}$P-labelled 28 S ribosomal RNA and HnRNA, separated by two dimensional electrophoresis. There are three main differences between the 28 S ribosomal RNA 'fingerprint', which is shown for reference, and that of HnRNA. First, 28 S ribosomal RNA yields a complex but finite array of discrete products, as might be expected for a fairly homogeneous high molecular weight RNA species. By contrast, the HnRNA pattern reveals diffuse regions (shaded in diagram) indicative of an extremely complex mixture of larger $T_1$ digestion products, and consistent with a heterogeneous mixture of high molecular weight RNA's. Secondly the HnRNA pattern clearly lacks the several weakly labelled, but distinctive, methylated 28 S ribosomal RNA digestion products, marked black in the key to 28 S rRNA. These products are also present in the nucleolar and nuclear precursors to rRNA [16] and their absence from HnRNA serves



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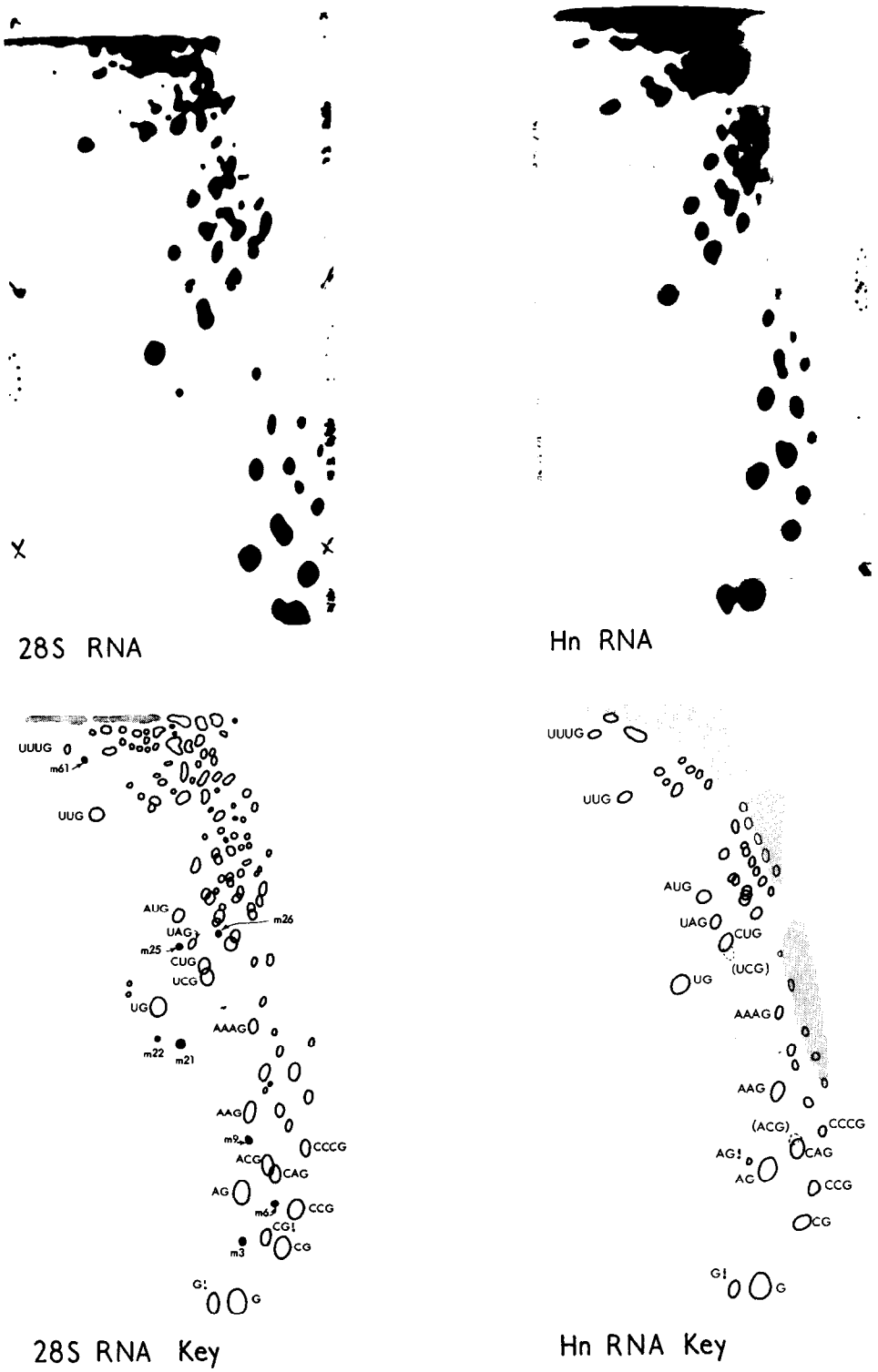


Fig. 1. Ribonuclease $T_1$ fingerprints of HeLa cell 28 S rRNA and HnRNA. RNA was prepared and fingerprinted as described in Methods. First dimension, right to left, cellulose acetate, pH 3.5 (5% acetic acid, 7 M urea). Second dimension, downwards, DEAE paper, 7% formic acid. Several 28 S rRNA methylated products are numbered and marked black in the 28 S rRNA key. The sequences of these products were determined previously [16]. G has run off the end in this 28 S fingerprint, but was obtained in fingerprints used for quantitation.

Table 1
Relative molar frequencies of oligonucleotides in $T_1$ RNAase digests of HnRNA and 28 S rRNA.

| RNA | Oligonucleotide | | | |
|---|---|---|---|---|
| | G | CG | CCG | CCCG |
| Hn | 100 (100) | 3.90(24.30) | 1.26( 5.90) | 0.47(1.43) |
| 28 S | 100 (100) | 39.0 (34.3 ) | 11.5 (11.76) | 3.00(4.03) |
| | | AG | AAG | AAAG |
| Hn | | 31.5 (27.80) | 7.46(7.73) | 2.65(2.15) |
| 28 S | | 13.4 (15.2 ) | 4.5 (2.31 ) | 0.99(0.35) |
| | | UG | UUG | UUUG |
| Hn | | 23.9 (28.40) | 8.06( 8.06) | 4.25(2.29) |
| 28 S | | 15.4 (17.1 ) | 2.52( 2.92) | 0.14(0.50) |
| | UCG | CUG | ACG | CAG |
| Hn | 0.33(6.90) | 8.07( 6.90) | 1.26( 6.75) | 6.53(6.75 |
| 28 S | 2.30(5.86) | 1.80( 5.86) | 3.26( 5.21) | 2.98(5.21) |

Oligonucleotides were assayed for radioactivity as described in Methods, and their respective molar yields calculated and expressed relative to an arbitrary value of 100 for G plus cyclic G. The values in parentheses are theoretical molar yields, also relative to 100 for G. These theoretical values were calculated from the base compositions of HnRNA and 28 S rRNA, assuming random nearest neighbour relationships between nucleotides within the limits imposed by these base compositions. The latter were determined by standard methods [10] and were, for HnRNA:- A, 27.8%; U, 28.5%; G, 19.3%; C, 24.3%. For 28 S rRNA:- A, 15.2%; U, 17.1%; G, 33.3%; C, 34.3%.

as useful evidence that the latter is uncontaminated by ribosomal precursor material.

The third difference between the two 'fingerprints' may be described as follows. To the right of the 28 S rRNA product, UG, is a pair of isomers, CUG (upper) and UCG (lower) (fig. 1). By contrast the HnRNA pattern contains only one strongly labelled product in this position, with a very weak 'satellite' spot just below. The strongly labelled spot was shown by standard procedures [10] to be CUG, and the lower, very weakly labelled spot was confirmed as UCG. Similarly in the ACG, CAG position HnRNA yields a single strongly labelled spot, CAG, with only very weak labelling of ACG.

These findings recall an earlier observation that the CG 'doublet' occurs very infrequently in the vertebrate genome [17]. This led to the speculation [18] based on subsequent analysis of dipeptide frequencies in proteins that CG 'doublets' might be under-represented in vertebrate mRNA. Two lines of evidence from the present study suggest that this is the case at least for HnRNA. First, the relative molar yields of various short oligonucleotides in table 1 show that the CG series of products indeed occurs in much lower yield than theoretically, and also than the AG and UG series. Secondly, when HnRNA fingerprints are prepared using longer separations, other deficiencies are seen within clusters of possible isomers, and in at least some of these cases the missing isomers seem to be the ones terminated in CG, as judged by their positions in the fingerprints (N.W. Fraser, unpublished observations). This distinctive feature of HnRNA may therefore prove to be useful in further structural studies on HnRNA itself and its relationship to mRNA.

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## References

[1] Edmonds, M. and Caramela, M.G. (1969) J. Biol. Chem. 244, 1314–1324.
[2] Edmonds, M., Vaughan, M.H. and Nakazato, H. (1971) Proc. Natl. Acad. Sci. U.S. 68, 1336–1340.
[3] Darnell, J.E., Wall, R. and Tushinski, R.J. (1971) Proc. Natl. Acad. Sci. U.S. 68, 1321–1325.
[4] Darnell, J.E., Phillipson, L. Wall, R. and Adesnick, M. (1971)Science 174, 507–510.
[5] Greenberg, J.R. and Perry R.P. (1972) J. Mol. Biol. 72, 91–98.
[6] Molloy, G.R. and Darnell,J.E. (1973) Biochemistry 12, 2324–2330.
[7] Molloy, G.R., Thomas, W.L. and Darnell, J.E. (1972) Proc. Natl. Acad. Sci. U.S. 69, 3684–3688.
[8] Burdon, R.H. and Shenkin A. (1972) FEBS Letters 24, 11–14.
[9] Ryskov, A.P. Saunders, G.F., Farashyan, V.R. and Georgiev, G.P. (1973) Biochim. Biophys. Acta 312, 152–164.
[10] Sanger, F., Brownlee, G.G. and Barrell, B.G. (1965) J. Mol. Biol. 13, 373–398.

[11] Penman, S., Vesco, C. and Penman M. (1968) J. Mol. Biol. 34, 49–69.
[12] Roberts, W.K. and Newman, J.F.E. (1966) J. Mol. Biol. 20, 63–73.
[13] Penman, S. (1969) in: Fundamental Techniques in Virology (Habel, K. and Sazman, N.P., eds), Ch. 5, Academic Press, New York.
[14] Scherrer, K. and Darnell, J.E. (1962) Biochem. Biophys. Res. Commun. 7, 486–490.
[15] Robertson, J. and Maden, B.E.H. (1973) Biochim. Biophys. Acta, in press.
[16] Maden, B.E.H., Salim, M. and Summers, D.F. (1972) Nature, New Biol. 237, 5–9.
[17] Subak-Sharpe, J.H. (1967) Brit. Med. Bull. 23, 161–168.
[18] Bullock, E. and Elton, R.A. (1972) J. Mol. Evol. 1, 315–325.