SOME METHYLATED SEQUENCES AND THE NUMBERS OF METHYL GROUPS IN HeLa CELL rRNA

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1. Introduction

We present here sequence data and molar yields of the methylated products 28 S 1–11 and 18 S 2–9′, observed in T_1 plus pancreatic RNAase fingerprints of ^{32}P-labelled Hela cell rRNA [1]. This information, combined with further quantitative data from [^{14}C]methyl fingerprints, permits estimation of the total numbers of methyl groups in Hela cell rRNA. [^{14}C]Methyl fingerprints of 32 S and 45 S RNA are also shown.

2. Methods

[^{14}C]Methyl-labelled RNA was prepared as described previously [2]. 2×10^{-5} M purine nucleosides and 10^{-2} M Na formate were added routinely to labelling medium to block purine ring labelling [2, 3]. Sequences were determined using both ^{32}P-labelled and ^{14}C-labelled material (in separate experiments), by a combination of some or all of the following procedures: alkaline hydrolysis, spleen phosphodiesterase digestion of alkali resistant products (sometimes but not always successful), and dephosphorylation followed by snake venom exonuclease digestion, either on the original material or on alkali resistant products.

3. Results and discussion

[^{14}C]Methyl fingerprints are shown in fig. 1. The methylated 28 S products 1–11 and 18 S products 2–9′ are clearly seen, as are an array of other products, only some of which are resolved in ^{32}P fingerprints. Table 1 gives sequence data and estimated molar abundances of products 1–11 and 2–9′. It will be seen that these products owe their distinctive electrophoretic mobilities to possession of more than one U and/or G residue, due to 2′-*O*-methylation and consequent enzyme resistance. Four of the smallest products (2–5) are common to both 28 S and 18 S RNA, though in different molar amounts. The others are unique to one or other rRNA species. Several of these "unique" products are doubly methylated. Absolute molar yields, determined from ^{32}P-labelling experiments, are in very good agreement with relative molar yields from [^{14}C]methyl labelling experiments. Most products are present in approximately integral molar amounts, but some A rich products were recovered in fractional molar yields of 2.5 and 0.5, were non-methylated A rich products (see [1] for discussion). 18 S Products 5 and 8′ were consistently recovered in fractional molar yields of 2.5 and 0.5, respectively. This could represent "point" sequence heterogeneity or incomplete methylation. 28 S Product 11 was at first thought to contain a doubly methylated trinucleotide, but relative recoveries of label in ^{32}P and ^{14}C experiments suggest the possibility of a triply methylated component. Similarly it is not completely clear whether 28 S product 4 (GmG) accounts for 8 or 9 methyl groups.

Within these limits of uncertainty, the numbers of methyl groups in 28 S products 1–11 and 18 S products 2–9′ may be summed as 29±1 and 14.5–15, respectively. The total numbers of methyl groups in rRNA can now be estimated from the distribution of radioactivity between these products and the remaining products in ^{14}C fingerprints, as shown in table 2.

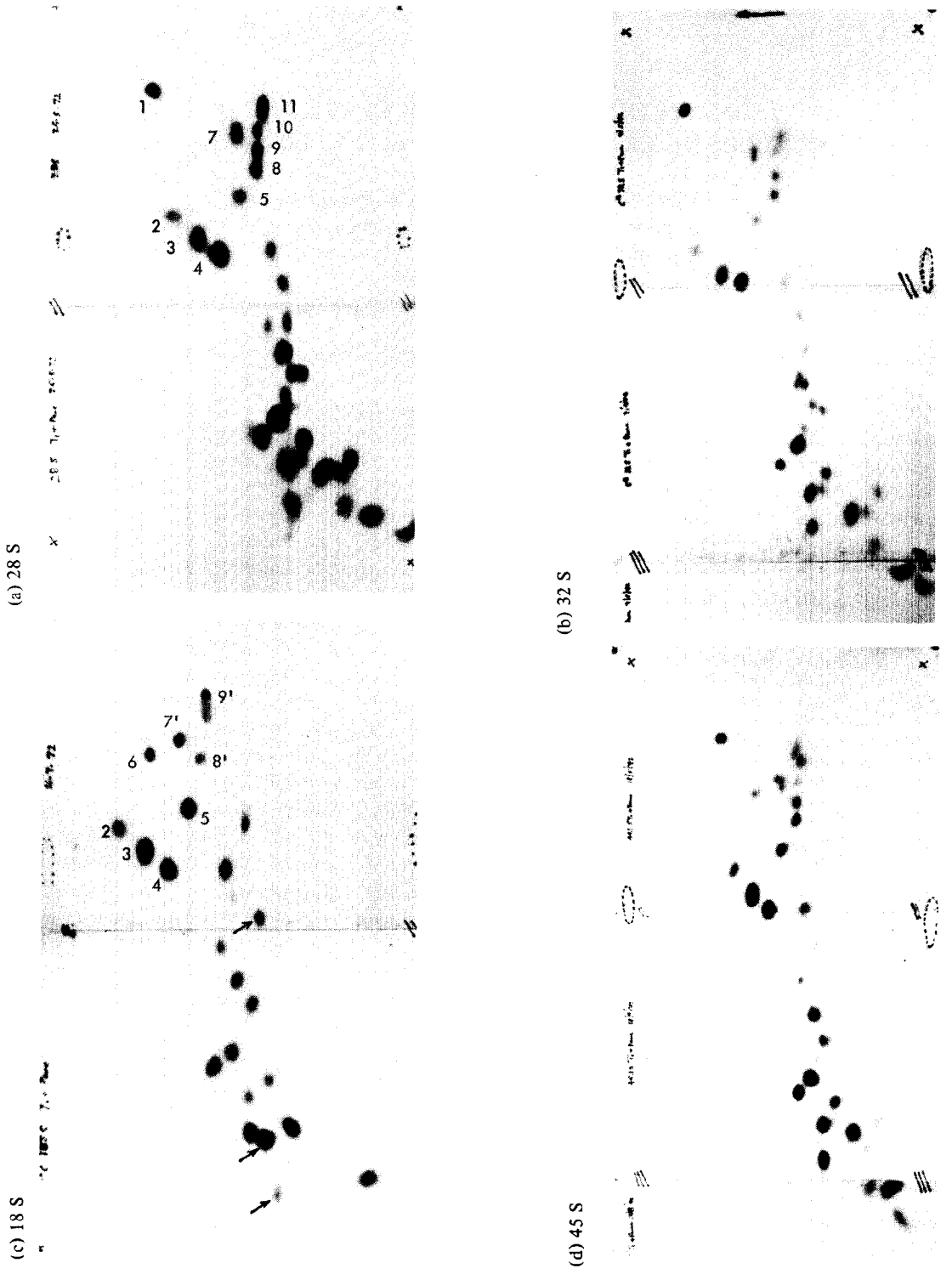

Fig. 1. T_1 plus pancreatic RNAase fingerprints of [^{14}C]methyl-labelled rRNA and precursors. The three 18 S spots marked with arrows are not present in 45 S RNA.

Table 1
Sequences and molar yields of 28 S products 1–11 and 18 S products 2–9.

28 S	Molar yield ^{32}P	[^{14}C]methyl	Suggested frequency, and number of CH_3 groups
1 UmGmU (+ UmUmG? (a))	2.1	2.10 (4.21)	2 (4)
2 UmU	1–1.5	1.15	1
3 UmG + GmU	4.86	5.27	5
4 GmG	8.24	9.17	8–9
5 AGmG	1.03	0.97	1
7 AAUmG (b)	1.90	1.68	2
8 CmAGmU	1.07	0.99 (1.98)	1 (2)
9 GmAAmAG	0.88	0.81 (1.61)	1 (2)
10 A(A,GmA)G	0.84	0.81	1
11 (A_2,X)U (c)	0.66	0.7–1.1 (2.28)	1 (2–3)
		Sum of CH_3 groups:	~ 29
18 S	Molar yield ^{32}P	[^{14}C]methyl	Suggested frequency, and number of CH_3 groups
2 UmU	1.2	1.03	1
3 UmG + GmU	3.8	4.15	4
4 GmG	2.65	2.76	2.5–3
5 AGmG	2.43	2.65	2.5
6 AUmU	1.03	0.94	1
7′ UmAAU	1.04	1.00	1
8′ AGmAG	0.45	0.52	0.5
9′ A(A,CmA,UmA)U	0.8	0.89 (1.78)	1 (2)
		Sum of CH_3 groups:	14.5–15

^{32}P values are based on 2–3 absolute determinations as described in table 1, [1]. ^{14}C values are based on relative distribution of methyl label (2–3 fingerprints), values in parentheses indicating relative numbers of methyl groups. Agreement between individual fingerprints was good. All products are 2′-O-methylated, e.g. UmG = 2′-O-methyl uridylyl guanylic acid, etc. (a) 28 S product 1 has proved difficult to degrade completely; present evidence indicates that only UmGmU is present. (b) 28 S product 7 seems to split into two components, and may be a mixture of AAUmG and another as yet unidentified methylated product. (c) Component X in product 11 is at least a trinucleotide with two or possibly three methyl groups.

Two 28 S determinations gave values which were in extremely good agreement (68 and 67). On this basis most individual 28 S products throughout the fingerprints appeared to occur in integral molar yields, one weakly labelled product occurring in half molar yield. Three 18 S determinations gave a slightly wider range of values (43–50 methyl groups), but again the mean value of 46 methyl groups was consistent with integral molar yields for the great majority of individual products.

These numbers of methyl groups are significantly higher than those suggested previously [4] on the assumption that there were two trinucleotides in 28 S RNA. Our data indicate that there are three, if the alkali stable component of product 11 is in fact a trinucleotide. In any case our values are related to the combined absolute molar abundances of all of the products 1–11, rather than to relative values for any one or two of them, and to this extent should be reliable.

Table 2
Calculation of approximate numbers of methyl groups in 28 S and 18 S RNA, based on data in table 1 and further data from [^{14}C]methyl fingerprints. (These contained 20–35,000 cpm, equivalent to 400–550 cpm per methyl group.)

	28 S	18 S
n = number of CH_3 groups in spots 1–11 (or 2–9′)	~ 29	15
$R = \frac{\text{cpm, all spots}}{\text{cpm, spots 1–11 (or 2–9′)}}$	2.335	~ 3.07
N = Total number of CH_3 groups = n × R	~ 68	~ 46

Alkali stable trinucleotides have been reported in 28 S RNA from Hepatoma cells [5] and yeast [6] as well as Hela cells [4, 7]. Doubly 2′-O-methylated sequences such as 28.8, 28.9 and 18.9′ have not previously been reported. In view of the relatively low overall methylation "density" of rRNA (~ 68 methyl groups per 5000 nucleotides in 28 S RNA) it is remarkable that several short products are doubly 2′-O-methylated.

The 32 S fingerprint in fig. 1 is qualitatively identical to that of 28 S RNA. Scintillation counting data showed good quantitative correspondence. 45 S RNA is similar to 28 S + 18 S RNA except for the absence of three 18 S spots (arrows in fig. 1c). These spots evidently represent late methylations, as also inferred from T_1 fingerprints [2, 8]. Some products (mainly dinucleotides) in T_1 plus pancreatic fingerprints are present in rather high molar yields, making exact quantitative comparison between rRNA and the precursors difficult. We are therefore pursuing a quantitative analysis of all the methylated products in T_1 (and T_1 plus alkaline phosphatase) fingerprints to facilitate definitive comparison between the methylation patterns of rRNA and the precursors ([8]; Salim and Maden, in preparation).

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