Use of ^{125}I in Fingerprinting RNA

IT is often difficult to obtain highly radioactive *in vivo* ^{32}P-labelled RNAs from higher organisms for fingerprinting[1]. In such situations, *in vitro* labelling methods can make a valuable contribution. One method using polynucleotide phosphokinase to introduce ^{32}P at the 5′ terminus of oligonucleotides has been reported[2,3]. Here I describe another method which is based on the fact that iodine has a high specificity for cytosine under certain conditions[4] and that iodine isotopes (^{125}I, ^{131}I) are easily detectable by autoradiography. I have used *Escherichia coli* formyl-methionine tRNA of known sequence[5,6] to test the suitability of this method for fingerprinting RNAs. The results show that a fingerprint of pmol amounts of RNA is obtainable within 2 d.

Figure 1*b* shows an RNase T_1 fingerprint of iodinated $tRNA_f^{Met}$ and Fig. 1*a* that of the *in vivo* ^{32}P-labelled $tRNA_f^{Met}$. The accompanying diagram gives the nucleotide sequences of the oligonucleotides. Several points in the figure are noteworthy. The fingerprint of iodinated tRNA is identifiable with that of the ^{32}P-labelled tRNA. Only cytosine containing oligonucleotides are prominent on the fingerprint and their intensity is in good agreement with their cytosine content indicating that, in the conditions used for labelling, the secondary structure of RNA is sufficiently opened up to make possible uniform iodination. The iodinated oligonucleotides move slightly faster in the first dimension and slower in the second relative to unmodified oligonucleotides (Fig. 1*a*), indicating a greater net negative charge on them in agreement with the observed mobility of iodinated cytidylic acid[7].

The results demonstrate the usefulness of ^{125}I for fingerprinting RNAs. The method is especially attractive because of its specificity, simplicity, speed and sensitivity. The fact that a fingerprint of a 'cold' RNA can be easily obtained by this method makes it a very useful procedure for studying homologies between closely related RNAs from different species, cell types and RNA tumour viruses.

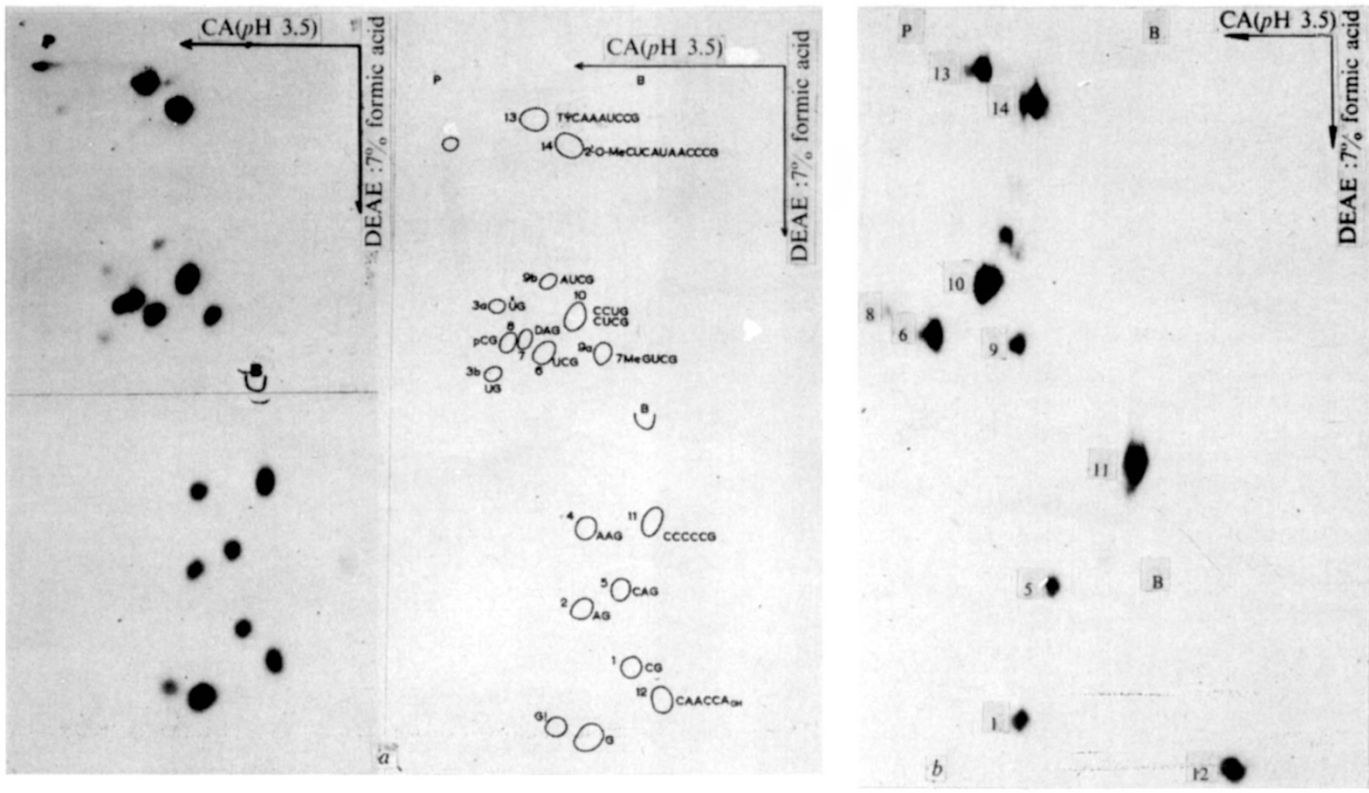

Fig. 1 A two-dimensional fractionation of an RNase T_1 digest of *in vivo* ^{32}P-labelled, (*a*), and *in vitro* ^{125}I-labelled, (*b*), *E. coli* $tRNA_f^{Met}$. $tRNA_f^{Met}$ was prepared as described[8]. The tRNA was labelled with ^{125}I in a total volume of 2 μl containing 0.2 μg tRNA, 10 mM sodium acetate acetic acid buffer, *p*H 5.0, 0.01 mM KI equilibrated with 10 to 100 μCi $Na^{125}I$ (Amersham, England) and 0.1 mM $TlCl_3$ (Merck, Germany). The reaction mixture assembled at 0° C, with reagents added in the above order, was taken up in a siliconised capillary tube which was afterwards sealed at both ends and incubated at 80° C for 10 min. The capillary tube was cooled on ice and broken open. Sodium thiosulphate (final concentration 1 mM) was added to reduce excess iodine and $TlCl_3$. Then 20 μg carrier RNA (*E. coli* mixed tRNA) and ammonium acetate ammonia buffer, *p*H 9 (final concentration 0.2 M), was added. The capillary tube was sealed again at both ends and heated at 45° C for 2 h to convert the unstable 5-iodo-6-hydroxy-5,6-dihydropyrimidines back to the unsaturated form. After this the RNA was passed over a Sephadex G-25 column made out of a siliconised capillary tube. The fraction eluting at the void volume was precipitated with ethanol. The tRNA was digested with RNase T_1 with an enzyme to substrate ratio of 1 : 20 for 30 min at 37° C[1]. The digest was fractionated on a two-dimensional high voltage electrophoretic system[1] using cellulose acetate, *p*H 3.5, for the first dimension and DEAE:7% formic acid for the second. The electrophoretogram was autoradiographed using Kodak X-Omatic H Film. The autoradiograph of the ^{125}I-labelled tRNA was obtained by exposure of the film for 8 to 12 h. The diagram shows the positions of the oligonucleotides (*a*) with their identification numbers and sequences[8]. B is the blue marker; P is the dye marker peak.

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