COMMUNICATIONS

³²P-Labeling of Haemoglobin Messenger and Other Reticulocyte Ribonucleic Acids with Polynucleotide Phosphokinase *in vitro*

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The fractionation procedures developed by Sanger, Brownlee & Barrell (1965) for the separation of nucleotides derived from uniformly ³²P-labelled nucleic acids have already permitted the determination of the sequence of some low-molecular-weight RNA species. These methods may be extended to the study of nucleic acids that cannot be sufficiently labelled *in vivo* or in tissue culture by the use of polynucleotide phosphokinase (Richardson, 1965; Murray & Wu, 1968; Szekely & Sanger, 1969), which catalyses the transfer of orthophosphate from [γ -³²P]ATP to the 5'-hydroxyl termini of oligo- and poly-nucleotides (Richardson, 1965; Novogrodsky & Hurwitz, 1966). We have studied the optimum conditions of this reaction for ³²P-labelling of ribonuclease digestion products and applied the technique to the study of reticulocyte RNA isolated from polyacrylamide gels (Labrie, 1969).

RNA stained with Methylene Blue was extracted by homogenization of the individual or pooled gel fraction. RNA (0.1–0.5 μ g.) was incubated successively with T-1 or pancreatic ribonuclease and bacterial alkaline phosphatase. A large proportion of the enzymic activity was removed by extracting with aqueous phenol. The nucleotides were then ³²P-labelled at their 5'-hydroxyl termini by incubating with polynucleotide kinase and a two- to three-fold excess of [γ -³²P]ATP. Unchanged ATP was removed with myosin. The nucleotides were then fractionated by the standard two-dimensional procedures (Sanger *et al.* 1965).

We have shown previously that 10s reticulocyte RNA labelled *in vivo* had a specific 'fingerprint' different from those of 18s and 29s RNA. By labelling with polynucleotide kinase, we have obtained 'fingerprints' of the minor bands previously observed. The pattern of 15s and 11s RNA indicates that they are probably degradation products of 18s RNA, and 9s RNA probably originates from 29s RNA. A specific pattern was again observed for 10s RNA similar to that found by labelling *in vivo*.

The double bands observed for 5s and 7s RNA on 7.5% polyacrylamide gels gave identical 'fingerprints', indicating that they represent different conformations of the same molecule. The complete T-1 ribonuclease digests of 5s RNA from

rabbit reticulocyte and from rat pituitary were analysed by partial hydrolysis with venom phosphodiesterase and found to be identical with those published for human KB-cell carcinoma (Forget & Weissman, 1967).

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5s Ribosomal Ribonucleic Acid from Two Mouse Cell Lines Gives 'Fingerprints' Identical with Human 5s Ribosomal Ribonucleic Acid

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5s Ribosomal RNA labelled with ³²P was isolated from Landschütz cells (a mouse ascites-tumour line) and LS cells (a line derived from mouse subcutaneous tissue) in culture. The RNA was 'fingerprinted' by using the techniques developed by Sanger, Brownlee & Barrell (1965). The 'fingerprints' of T-1 ribonuclease, T-1 ribonuclease plus phosphatase, and pancreatic-ribonuclease digests show no detectable differences in sequence or amount of oligonucleotides from those determined for human KB-cell 5s ribosomal RNA by Forget & Weissman (1967), with the exception of a possible difference in the proportions of the two 3'-chain termini -C-U-U-OH and -C-U-U-U-OH.

For a molecule of this size identical 'fingerprints' are very strong evidence for identical sequence. It is possible that this invariance points to a very exact function for 5s RNA, demanding for molecular reasons this particular nucleotide sequence and none other. It would appear that the 5s ribosomal RNA system, with an implied gene multiplicity for an invariant and non-diverging small RNA of known base sequence, would prove an interesting system for hybridization experiments with mammalian material.

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