in vivo to high specific radioactivity with [*Me*-¹⁴C]-methionine (Salim, Williamson & Maden, 1970). Results of this analysis were as follows. (i) Ribonuclease T₁ digests of 18S and 28S rRNA yielded characteristic 'methyl' 'fingerprints'. The 18S RNA yielded 15 well-resolved spots, and 28S RNA about 28 spots. Several spots are unique to 28S RNA, and four spots unique to 18S RNA. In addition, both 'fingerprints' showed some incompletely resolved material near the origin of the second dimension. (ii) The 'fingerprint' of 32S nucleolar RNA was similar to that of 28S rRNA. (iii) The 'fingerprint' of 45S nucleolar RNA resembled the 'fingerprints' of 28S and 18S RNA superimposed.

Further work has been aimed at structural characterization of some of the methylated oligonucleotides liberated from ribonuclease T₁ digestion of rRNA and nucleolar RNA. Alkaline hydrolysis of [¹⁴C]methyl-labelled material yields methyl-labelled mononucleotides or alkali-resistant (2'-*O*-methyl) dinucleotides. In most, but not all, cases examined a single spot yields a single methyl-labelled derivative on alkaline hydrolysis. When alkaline hydrolysis products from corresponding spots in 18S, 28S and 45S RNA are subjected to high-voltage electrophoresis it has been found, in all cases so far examined, that the material liberated from 18S or 28S RNA co-migrates with material from the corresponding spots in 45S RNA. These observations provide further chemical evidence on the nature of the relationship between rRNA and 45S nucleolar RNA in HeLa cells, previously deduced from several other lines of evidence (for reviews see Darnell, 1968; Maden, 1971).

These results from [¹⁴C]methyl-labelled material, together with analyses of rRNA double-labelled with [³²P]phosphate and [*Me*-³H]methionine, have permitted tentative sequence assignments for some of the smaller methylated oligonucleotides in HeLa-cell rRNA.

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'Fingerprinting' Studies on ³⁵S-Labelled Mammalian-Cell Ribosomal Proteins

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Studies on the structure and assembly of animal-cell ribosomes have frequently relied on the use of one-dimensional protein fractionation techniques such as polyacrylamide-gel electrophoresis. In the present work a ³⁵S-labelled peptide 'mapping' technique previously applied to bacterial ribosomes (Traut, Moore, Delius, Noller & Tissières, 1967) has been adapted for use with mammalian-cell ribosomes. HeLa or other cultured cells were labelled to high specific radioactivity with [³⁵S]methionine or [³⁵S]cystine, ribosomal proteins were extracted and digested with trypsin, and the products were separated by two-dimensional electrophoresis and chromatography. Under standard experimental conditions reproducible 'fingerprints' were obtained. Results that relate primarily to ribosome structure are as follows. (i) HeLa-cell large (50S) and small (30S) subunits, labelled with [³⁵S]methionine and separated in EDTA-containing buffer, yield dissimilar and characteristic 'fingerprints'. [³⁵S]-Cystine-labelled ribosomal subunits also yield 'fingerprints' that are dissimilar both from each other and from the 'fingerprints' of [³⁵S]methionine-labelled subunits. (ii) [³⁵S]Methionine-labelled ribosomal subunits separated by dissociation at high salt concentration yield substantially similar 'fingerprints' to those yielded by subunits separated in EDTA. Together these findings imply the existence of major differences in the primary structures of HeLa-cell 50S and 30S ribosomal proteins, a result not fully evident from previous analyses using polyacrylamide-gel electrophoresis. (iii) Ribosomal subunits from methionine-labelled BHK (baby-hamster kidney) and HeLa (human) cells give very similar 'fingerprints'.

[³⁵S]Methionine-labelled peptide 'mapping' has also yielded information on ribosome assembly in HeLa cells. (i) The methionine-containing peptide 'map' of 55S nucleolar ribosomal precursor particles is similar to that of 50S ribosomes. The 55S nucleolar particles have previously been identified as direct precursors to cytoplasmic 50S ribosomes by various criteria (Warner & Soeiro, 1967). The methionine-containing peptide content of the kinetically more immature 80S nucleolar particle is presently being investigated. (ii) When formation of new ribosomes is inhibited by actinomycin D, a small number of distinctive methionine-containing peptides are found in 'fingerprints' of EDTA-separated 50S ribosomal subunits. This implies the

existence of one or more exchangeable cytoplasmic proteins that can firmly associate with pre-existing 50S ribosomes, as concluded by Warner (1966). In similar experiments there is no evidence for the existence of firmly bound but exchangeable 30S ribosomal proteins.

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Effects of Amino Acid Starvation on Ribosome Formation in HeLa Cells

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Balanced ribosome production requires co-ordinated synthesis and assembly of a large number of macromolecular components. Amino acid-starvation experiments should assist in identifying some of the interactions involved in this process in animal as well as in bacterial cells.

Recognized effects of amino acid starvation on ribosome formation in HeLa cells are as follows. (i) Methionine starvation results in complete arrest of formation of mature ribosomes, with deficient methylation of ribosomal precursor RNA (Vaughan, Soeiro, Warner & Darnell, 1967). Synthesis of ribosomal proteins continues at an appreciable rate during methionine starvation through protein turnover (Maden & Vaughan, 1968). (ii) Starvation for other essential amino acids, such as valine, results in slowdown of synthesis of 45S ribosomal precursor RNA and of its conversion into 32S RNA, but newly formed ribosomes continue to appear in the cytoplasm at an appreciable rate and to associate with polyribosomes (Maden, Vaughan, Warner & Darnell, 1969). (iii) Although both types of ribosomal subunit continue to be produced during valine starvation, production becomes imbalanced, fewer large than small subunits appearing in the cytoplasm. Lysine starvation exerts an opposite effect, fewer small than large ribosomal subunits appearing in the cytoplasm (Maden, 1969). The missing particles in each case cannot be found in the nucleus and are presumably selectively degraded. Each of these effects is reversible after several hours of amino acid starvation on restoration of the missing amino acid to the medium.

In the present work the analysis of imbalanced subunit production during amino acid starvation was extended. Starvation for any one of ten essential amino acids (arginine, histidine, isoleucine,

leucine, lysine, phenylalanine, threonine, tryptophan, tyrosine or valine) resulted in considerable slowdown of ribosome production. In most cases there was no major disturbance in the relative rates of formation of the two types of ribosomal subunit. Effects of leucine starvation resembled those of lysine starvation, but imbalance was less marked. The characteristic lysine- and valine-starvation effects were confirmed both in suspension and in monolayer HeLa-cell lines.

A possible explanation for the observed imbalance in ribosomal-subunit maturation during starvation for particular amino acids might be a disturbance in relative rates of synthesis of individual ribosomal proteins under these conditions. Results of experiments designed to test this possibility, by using polyacrylamide-gel electrophoresis of radioactively labelled ribosome proteins, will be presented.

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Methylation of Newly Synthesized Ribonucleic Acid by Isolated Rat Liver Nuclei: a Means of Characterizing Ribonucleic Acid Synthesized *in vitro*

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Ribosomal precursor RNA is the predominant species of RNA synthesized by nuclei *in vitro* in the presence of Mg^{2+} and *in vivo*. Ribosomal precursor RNA is methylated during synthesis in the cell and contains approx. 30 uridylylate residues/methyl group (Wagner, Penman & Ingram, 1967; Willems, Wagner, Laing & Penman, 1968). In this report an attempt is made to calculate the proportion of ribosomal precursor RNA synthesized by nuclei *in vitro* by comparing the incorporation of radioactivity into RNA from $[4-^{14}C]$ UTP and *S*-adenosyl-L- $[Me-^{14}C]$ methionine.

Radioactive RNA could be extracted with phenol from nuclei that had been incubated with labelled *S*-adenosyl-L-methionine and all four ribonucleoside triphosphates. The incorporation of radioactivity into RNA from UTP and *S*-adenosyl-L-methionine was measured in hot-trichloroacetic acid extracts of nuclei that after incubation had been washed with 0.4M-perchloric acid at 0°C and with lipid solvents