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Chemical Studies on Ribosomal Ribonucleic Acid and Ribosome Formation in HeLa Cells

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The chemical relationship between HeLa-cell rRNA and its nucleolar precursors was examined by methods for the determination of RNA sequences. The various RNA species were purified after methyl labelling *in vivo* and digested to completion with T₁ ribonuclease or with a combination of T₁ ribonuclease plus alkaline phosphatase. The products were separated by electrophoresis on cellulose acetate at pH 3.5 followed by DEAE-cellulose paper in 7% formic acid. The 28S rRNA yielded over 50 different methylated oligonucleotides, and 18S rRNA over 30 methylated oligonucleotides. Nearly all of the larger of these oligonucleotides were unique to one or other RNA species, although a number of the smaller ones were common to both species. A double-label technique, with ³²P-labelled and Me-¹⁴C-labelled RNA, was used for identifying the methylated oligonucleotides for subsequent sequence analysis with ³²P-labelled RNA. The sequences of many of the methylated oligonucleotides were then determined, and each of these sequences was found to contain a 2'-O-methylribose group.

The distribution of methylated oligonucleotides between the various nucleolar RNA species was consistent with the following pathway for rRNA maturation in the HeLa-cell nucleolus:

There were no methylated sequences of non-ribosomal type in the precursor molecules (with one possible exception in 45S RNA that is still being investigated), but there was evidence for a few (probably three) late methylation events on 18S rRNA after its appearance in the cytoplasm as an intact ribosomal subunit. One of these late methyl-

ation steps results in the formation of dimethylaminopurine within a specific oligonucleotide of the 18S molecule.

³²P-labelled rRNA was also digested with a combination of T₁ and pancreatic ribonucleases. The resultant 'fingerprints' yielded a number of pure methylated oligonucleotides containing more than one uridine and/or guanosine residue (due to 2'-O-methylation) and separated from all other ³²P-labelled digestion products by their greater mobility at pH 3.5. This technique has revealed two short doubly methylated oligonucleotides in 28S RNA, and has other useful applications, which will be described.

Finally, a methionine peptide 'mapping' method was used to examine the assembly of ribosomal proteins with ribosomal precursor RNA in the nucleolus. Cells were labelled with [³⁵S]methionine, ribosomal subunits or nucleolar ribonucleoprotein particles were prepared, and the proteins were digested with trypsin without further prior fractionation. Proteins from the large and the small ribosomal subunits yielded different methionine peptide 'maps'. The 55S nucleolar particle (containing 32S RNA) contained most of the methionine peptides characteristic of the large ribosomal subunit. The 80S nucleolar particle (containing 45S RNA) also contained most of the methionine peptides characteristic of the large ribosomal subunit, together with some weakly labelled peptides characteristic of the small subunit. ³⁵S-labelled peptides could be 'chased' from nucleolar particles to cytoplasmic ribosomes by addition of excess of unlabelled methionine. In addition, a small number of peptides were added to large ribosomal subunits after their appearance in the cytoplasm. Possible implications of these findings on the mechanism of ribosome assembly in the HeLa-cell nucleolus will be discussed.

Structure and Function of 70S Ribosomal Proteins

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The 70S ribosomes are present in prokaryotes (bacteria and blue-green algae) and in cell organelles (chloroplasts and mitochondria) of eukaryotes. The best known ribosomes are those of *Escherichia coli*. Therefore our present knowledge on the structure and function of ribosomal proteins from *E. coli* is presented below and discussed. Also, *E. coli* ribosomes are compared with those of other bacteria and of cell organelles.

(1) *Number of E. coli ribosomal proteins.* As shown by two-dimensional polyacrylamide-gel electrophoresis and by isolation techniques, 30S subunits contain 21 proteins and 50S subunits contain 34 proteins.