Biosynthèse des ribosomes
électrophorèse: analyse des acides ribonucléiques et des
ribonucléoprotéines en gels exponentiels de polyacrylamide

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BIOSYNTHESE DES RIBOSOMES:
MATURATION DES PRERIBOSOMES
DE CELLULES HELA
IN VIVO ET IN VITRO

ELECTROPHORESE:
ANALYSE DES ACIDES RIBONUCLEIQUES
ET DES RIBONUCLEOPROTEINES
EN GELS EXPONENTIELS DE POLYACRYLAMIDE

Thèse
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SUMMARY

a) Electrophoresis on Exponential Gradient Polyacrylamide Gels.

Exponential gels facilitate the high resolution of RNA molecules over a wider range of molecular weights than do conventional gels of uniform concentration. Since the relative migration rates in exponential gels remain identical to those in uniform gels during electrophoresis, the molecular weights of RNA molecules can be as readily estimated as on uniform gels. Gradient gels have a focusing effect on each set of identical molecules migrating through the gel, due to the increasing concentration of acrylamide. They provide optimal resolution, even when a large sample volume is loaded, since the resolution becomes independent of it at the limit where the molecules approach a zero migration rate, near the specific "exclusion" gel concentration. Using quartz tubes, direct scanning of UV-absorbance is possible without handling the gels; thus, very low concentration (semi-liquid) gels can be used. Exponential gels of 1.8 to 20 % acrylamide allow the fine analysis of the whole spectrum of cellular RNA simultaneously, from the giant nuclear extranucleolar RNA, down to the small 4S RNA with equal resolution in the 10^4, 10^5 and 10^6 MW range. Ribonucleoprotein particles such as ribosomal subunits and their precursors can also be analysed on such gels. In principle, exponential gradients gels can be applied advantageously to the electrophoretic fractionation of any charged macromolecules or stable macromolecular complexes.

b) In vivo and in vitro Processing of HeLa Cell Preribosomes.

The formation of eukaryotic ribosomal subunits by post-transcriptional processing of a common precursor (preribosome) has been investigated in HeLa cells. Our investigation was focused on the enzymatic mechanisms involved in the stepwise cleaving of the preribosomes. In an attempt to understand the molecular basis of this process, an in vitro system was developed to characterize the enzyme(s) and possibly other factors involved in these specific reactions.

A new cell fractionation method is presented for the isolation of nucleoli from which intact and stable preribosomes could be extracted under mild conditions, in presence of Mg^{++} (no chelating agents) and at low salt concentration. These nucleolar preribosomes were characterized by sedimentation in sucrose gradients, by CsCl-density-
gradient centrifugation and by electrophoresis in polyacrylamide gels. Using this method of isolation, nucleolar particles are stable enough to serve as substrate in an in vitro assay for the processing enzyme(s).

The kinetics of in vivo maturation of the preribosomes was studied by following uridine incorporation into the nucleolar ribosomal precursors and by pulse-chase experiments with actinomycin D. Both the nucleolar particles and their RNA were analysed by gel-electrophoresis, which made it possible to identify the whole series of intermediate ribosomal precursor particles. From these in vivo kinetics, as well as from the molecular weights of the distinct pre-rRNAs as estimated by gel-electrophoresis, a 45 S RNA-processing-scheme can be deduced which is similar to that recently proposed by Weinberg and Penman (43). We present the corresponding scheme for the ribonucleoproteins also.

In addition, the inhibition of ribosome formation by different types of drugs was studied. An analogue of adenosine, toyocamycin, which is incorporated into 45 S RNA and prevents its conversion to ribosomal RNA (62), and chloroquin, a quinolin-derivative which binds to DNA and RNA, both drastically reduce the rate of 45 S RNA processing. Toyocamycin apparently causes an abortive maturation of both 32 S and 20 S pre-rRNAs, which are no longer converted to 28S and 18S ribosomal RNAs. Chloroquin inhibits both nucleolar transcription and maturation of the ribosomal precursors; these effects are very concentration dependant.

Pulse labelled preribosomes were used in vitro as substrate for the processing activity. With this assay, a ribonucleolytic activity could be isolated from the nucleoli; this crude enzyme was able to convert the preribosomes containing 45S RNA into particles containing RNAs which correspond in size to the nucleolar RNAs normally found in vivo. The in vitro processing rate is shown to be both time and enzyme dependent; the reaction proceeds through essentially the same sequence of cleaving steps as that observed in vivo. Since virtually no alcool-soluble reaction products are found, we conclude that the enzyme is an endonuclease.