recently in the scanning-[11] electron microscope. Secondly, even without negative staining, the gold colloid and the ferritin molecules (iron nucleus) can easily be detected and distinguished in the transmission electron microscope. Double labelling experiments, using colloidal gold particles coated with a protein (phytohemagglutinin, antibodies, etc.) and ferritin-protein conjugates can therefore be performed simultaneously.

Summary. The morphology of model complexes between colloidal gold, ferritin and anti-ferritin antibodies has been studied in order to evaluate the potential of colloidal gold as a cytochemical marker.

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Hybridization of Mitochondrial and Cytoplasmic Ribosomal RNA with Mitochondrial and Nuclear DNA

The genetic origins of mitochondrial and cytoplasmic rRNAs from rat liver were investigated by RNA-DNA hybridization experiments. It was shown in earlier studies[1] that mitochondrial rRNAs from rat or mouse liver were different from their cytoplasmic counterparts in several of their physico-chemical properties. If in addition mitochondrial rRNAs hybridize specifically with mitochondrial DNA and not with nuclear DNA, this would be an indication of a different genetic origin from both types of rRNAs.

Material and methods. Mitochondria and RNA were prepared from adult rat liver as described in earlier studies[1]. During subcellular fractionation and RNA extraction, 2 ribonuclease inhibitors were used to avoid RNA degradation: a natural one prepared from rat liver the day before, and diethylpyrocarbonate. After ethanol precipitation, the RNAs were purified by 2 centrifugations through 5–20% sucrose gradients in order to completely eliminate tRNAs.

The cytoplasmic ribosomal RNAs were prepared according to Stevenin et al.[2], except that for the ribonuclease treatment, 0.05 µg ribonuclease per ml for 20 min at 0 °C was used to remove mRNA.

Mitochondrial DNA was prepared according to Ittel et al.[3] and the nuclear DNA according to Marmur[4], except that DNA was precipitated by cold ethanol and not by isopropanol. Both DNAs were fragmented by sonication as described by Mori et al.[5], in order to obtain fragments with a length of about 500 base pairs, which corresponds approximatively to a gene size[6,7]. The two species of DNAs were tritiated in vitro with sodium ^{3}H-borohydride as described by Lee and Gordon[8]. The specific activities of DNAs obtained varied from 5–1500 cpm/µg DNA.

The melting temperatures (Tms) of the DNA fragments and hybrids were determined by thermic elutions at increasing temperatures on hydroxyapatite columns. This method of temperature measurements[5] is very rapid and several determinations can be done simultaneously. Hydroxyapatite was prepared according to Tiselius[9] and modified by Levin[10]. The elution was performed with 0.12 *M* sodium phosphate buffer.

Nuclease S_1 was prepared according to Sutton[11] in our laboratory from takadiastase. Enzymatic activity was determined according to Sutton[11] except that the incubation was performed at 37 °C and not at 56 °C. This enzyme preferentially attacks single-stranded DNA.

Hybridization conditions were those of our laboratory. Tritiated DNA (12,000–15,000 cpm) was incubated with a large excess of RNA (100 to 600 times) in order to decrease strongly DNA-DNA reassociations, in 0.75 *M* sodium phosphate buffer for 48 h at 65 °C after heat denaturation. Hydroxyapatite (2 ml per column) was

Table I. Hybridization percentages of mitochondrial and cytoplasmic rRNAs with mitochondrial and nuclear DNA

	Mitochondrial DNA (% DNA hybridized)	Nuclear DNA (% DNA hybridized)
Mitochondrial rRNA	1.74 (10)	0.01 (3)
Cytoplasmic rRNA	0.02 (3)	0.42 (8)

The values in brackets are the number of determinations.

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Table II. Temperature values from nuclear and mitochondrial DNA, and RNA-DNA hybrids

	Temperature (°C)		Temperature (°C)
Mitochondrial DNA	82	Nuclear DNA	86
Mitochondrial DNA-mitochondrial rRNA hybrids	74.8	Nuclear DNA-cytoplasmic rRNA hybrids	78.5

equilibrated in 0.18 *M* sodium phosphate buffer (equimolar mixture of Na_2HPO_4 and NaH_2PO_4) at pH 6.8. Before adsorption on the hydroxyapatite columns, the ionic strength of the hybridization mixture was decreased to a final concentration of 0.18 *M* sodium phosphate buffer. Formamide was also added to a final concentration of 50% v/v to prevent non-specific hydrogen bond interactions between nucleic acids, thus allowing the adsorption and elution to be performed at a lower temperature than the incubation temperature. The double-stranded material was eluted from hydroxyapatite columns with 0.25 *M* sodium phosphate buffer at 100°C after washing extensively with 0.18 *M* sodium phosphate buffer at room temperature and at 40°C to eliminate single-stranded material.

DNA alone was incubated in the same conditions as hybridization assays and gave the blank value which was substracted from the RNA-DNA hybridization. Radioactivity of the hybrids was measured by liquid scintillation counting in 0.25 *M* sodium phosphate buffer eluates using Instagel (Packard) in a SL 40 Spectrometer Intertechnique.

Results and discussion. The results of hybridization experiments are presented in Table I. The values for mitochondrial DNA-rRNA hybridizations (Figure 1) and for nuclear DNA-cytoplasmic rRNA hybridizations (Figure 2) were graphically determined. We plotted 1/H (where H = % of hybridization) against the DNA/RNA ratio. This representation suggested by BISHOP[12] for hybridization done in the presence of an excess of RNA, leads to a straight line, and its extrapolation to zero value corresponds to the inverse of the percentage of DNA hybridized at saturation level (1/H). Our results show that mitochondrial rRNAs hybridized specifically with mitochondrial DNA and cytoplasmic rRNAs with nuclear DNA. Competition hybridization assays were also performed. Cytoplasmic rRNA did not compete with mitochondrial rRNA when mitochondrial DNA was incubated with both types of rRNAs. Mitochondrial rRNA did not compete with cytoplasmic rRNA when incubated together with nuclear DNA[13].

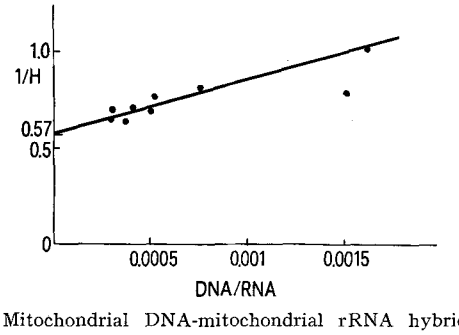

Fig. 1. Mitochondrial DNA-mitochondrial rRNA hybridization. H represents the percent of rRNA-DNA hybrids for a given RNA/DNA ratio.

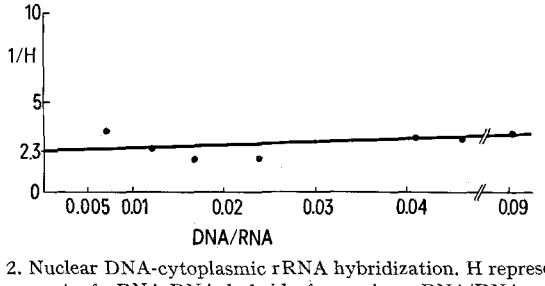

Fig. 2. Nuclear DNA-cytoplasmic rRNA hybridization. H represents the percent of rRNA-DNA hybrids for a given RNA/DNA ratio.

The lower hybridization values found for mitochondrial rRNA-mitochondrial DNA than those reported in the literature[14,15], were certainly due to the experimental conditions used and to the intrinsic properties of mitochondrial DNA, e.g. rapid self reassociation. Strand separation would have given a higher hybridization value for mitochondrial DNA but the techniques of strand separation cannot be applied with success to nuclear DNA of eukaryotes. Our experiments were designed to analyze the specificity of locus transcription of the rRNAs studied by hybridization with DNA fragments from homologous or heterologous subcellular fractions, rather to determine the number of ribosomal cistrons.

The stringency of the hybridization conditions allowed a better comparison of hybridization assays from nuclear and mitochondrial DNAs with their ribosomal rRNAs to be made.

The Tms determined for mitochondrial and nuclear DNA and for the hybrids RNA-DNA are summarized in Table II. The Tms of hybrids were lower than those of the corresponding DNAs.

According to LAIRD[16], hybrids show 1.5% mismatching for a temperature decrease of 1°C. In our case, the hybrids showed an average of 13% mismatching. Likewise the DNA-RNA hybrids were 80 to 90% resistant to S_1 nuclease activity. Temperature measurements and resistance toward nuclease S_1 showed that the hybrids possessed a double-stranded structure with a low percentage of mismatching.

These hybridization experiments lead to the conclusion that mitochondrial rRNAs constitute a class of RNA different from their cytoplasmic counterparts and that they are genetically distinctly determined.

Summary. Hybridization assays of rat liver mitochondrial and cytoplasmic rRNAs with in vitro labelled mitochondrial and nuclear DNA were performed in liquid medium. Sensitivity towards S_1 enzyme and Tms of the RNA-DNA hybrids were studied. Our results are in favour of a distinct genetic origin of the two types of cellular rRNAs.

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