TRANSPORT OF NUCLEAR DNA INTO THE CYTOPLASM IN CULTURED HUMAN LIVER CELLS

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1. Introduction

In eucaryotic cells DNA is associated with cell organelles, i.e. mitochondria [1] and chloroplasts [2]. There have also been several recent reports on DNA being associated with the microsomal fraction of rodent liver [3, 4] and cultured embryonic muscle [5] from an unidentified source. The latter has been suggested to have an informational role [5]. In this communication we report on the detection of a DNA associated with the membrane fraction of cultured human cells. Furthermore the labeling kinetics and the preliminary characterization of the DNA are described. The results indicate that the DNA is replicated within the nucleus and transported into the cytoplasm.

2. Materials and methods

A serially propagated cell line derived from human liver (Chang) was used for these experiments. The growth conditions were described previously [6, 7]. The cell line was tested regularly for mycoplasma contamination with negative results [6]. Thymidine-methyl-^{3}H (26 Ci mmole^{-1}) and thymidine-2-^{14}C (60 mCi mmole^{-1}) were obtained from The Radiochemical Centre Amersham, GB. Mitochondrial and membrane fractions were prepared as described previously [6]. Total cytoplasmic extracts were prepared by lysing the cells ($\sim 10^{7}$) in 0.9 ml of 0.001 M EDTA, 0.001 M spermidine, 0.01 M Tris-HCl pH 7.6, 0.5% Triton X-100 (or 2% Brij 35). After standing for 30 sec (0°) the suspension was briefly agitated with a Vortex mixer and the nuclei were removed by centrifugation at 2000 *g* for 10 min. Mitochondrial and membrane fractions and lysates were made 1% SDS (0.1 M NaCl, 0.001 M EDTA, 0.01 M Tris-HCl pH 7.6) and further processed by a modified Hirt procedure [6] or layered on top of a gradient of 15–30% sucrose in 0.1 M NaCl, 0.001 M EDTA, 0.01 M Tris-HCl pH 7.6, 0.5% SDS (Spinco SW 27, 25000 rpm, 25°, 12 hr). All further analytical methods have been described in detail [6].

3. Results

Following the incorporation of thymidine-methyl-^{3}H into the nuclear and total cytoplasmic DNA in cultured human liver cells (Chang) we observed that the incorporation of radioactivity into the nuclear DNA remained linear during one doubling time (24 hr) of the cells. In contrast the incorporation of radioactivity into the total cytoplasmic DNA showed a biphasic pattern (fig. 1). At the end of one doubling time the amount of isotope incorporated into the total cytoplasmic DNA was about 0.5% of the radioactivity incorporated into the nuclear DNA.

Pulse (thymidine-2-^{14}C)–chase–pulse (thymidine-methyl-^{3}H) experiments and band sedimentation through sucrose gradients were used for analysis of the cytoplasmic DNAs. The radioactivity of a short pulse (6 hr) before harvest was located mainly in the mitochondrial supercoiled (39 S) [8] and open circular (27 S) [8] DNA. The radioactivity of the early

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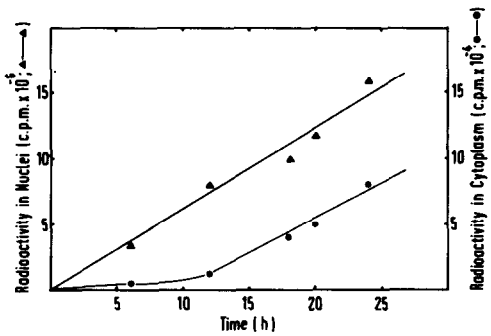

Fig. 1. Incorporation of thymidine-methyl-^{3}H into nuclear and total cytoplasmic DNA. Cells in logarithmic growth phase were labeled with thymidine-methyl-^{3}H (6 μCi ml^{-1}) for the time indicated. Total cytoplasmic extracts were prepared by the detergent method (Materials and methods). Nuclear and cytoplasmic fractions were made 1% SDS (0.15 M NaCl, 0.001 M EDTA, 0.01 M Tris-HCl pH 8). The nuclei were broken by brief sonication (Branson J-17V). Acid precipitable radioactivity was determined.

Table 1

Durations of pulse–chase–pulse (hr)	early pulse / late pulse	
	Nuclear DNA	18 S DNA
24–24– 6	1	5.1
24–24–12	1	1.8

^{14}C/^{3}H ratios of 18 S DNA in comparison with those of nuclear DNA. 18 S DNA was isolated from total cytoplasmic extracts by sedimentation through sucrose gradients. 18 S DNA and nuclear DNA were banded in CsCl density gradients. ^{14}C/^{3}H ratios were determined over the peak fractions and averaged.

pulse, however, was found mainly in a DNA sedimenting heterogeneously with a peak at 18 S (fig. 2, frame A). Treatment of the mitochondrial and membrane fraction with pancreatic DNase before analysis of the DNAs by means of band sedimentation resulted in a complete loss of the 18 S species (fig. 2, frame B). When the late pulse was extended to 12 hr duration, the 18 S DNA also showed considerable labeling (fig. 2, frame C). Table 1 compares the ratios early pulse (^{14}C)/late pulse (^{3}H) of the 18 S DNA with those of the nuclear DNA taken as unity. These results indicate that (1) the 18 S DNA is located in the membrane fraction not protected against

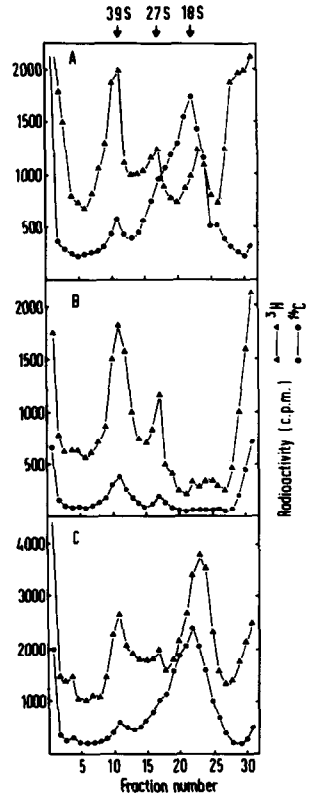

Fig. 2. Sedimentation of pulse–chase–pulse labeled DNA isolated from the mitochondrial and membrane fraction through sucrose gradients. (A) Cells were labeled for 24 hr with thymidine-2-^{14}C (0.5 μCi ml^{-1}). The medium was replaced by a medium which did not contain the radioisotope for another 24 hr. The cells were subsequently exposed for 6 hr to a medium containing thymidine-methyl-^{3}H (2.5 μCi ml^{-1}). (B) Labeling conditions as in (A). Mitochondria and membrane fraction was treated for 1 hr at 25° with 200 μg ml^{-1} pancreatic DNase (0.25 M sucrose, 0.005 M $MgCl_2$, 0.01 M Tris-HCl pH 7.0). (C) Conditions as in (A), but the duration of the thymidine-methyl-^{3}H pulse was extended to 12 hr. Gradients were fractionated into 1 ml fractions. Aliquots were used to determine the acid precipitable radioactivity.

the action of DNase, (2) the 18 S DNA appears late in the cytoplasm, i.e. the cells have to be exposed at least for 10 hr to the radioactive precursor before this DNA shows considerable labeling, (3) the radioactivity in the 18 S DNA represents the major part of the radioactivity in the cytoplasmic DNAs after long labeling periods, (4) the radioactivity in the 18 S

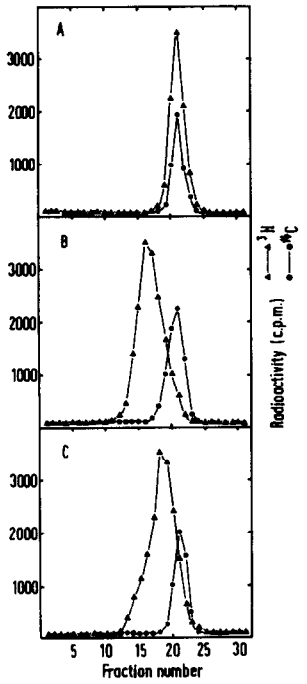

Fig. 3. Buoyant density of thymidine-methyl-^3H labeled 18 S DNA in CsCl-gradients. (A) Native 18 S DNA. (B) 18 S DNA heated in 0.01 M NaCl, 0.001 M EDTA, 0.001 M Tris-HCl pH 7 (10 min, 100°) and rapidly cooled. (C) Denatured 18 S DNA reannealed for 18 hr in 0.5 M CsCl at 60°. Nuclear DNA labeled with thymidine-2-^{14}C was used as density marker. Starting density 1.700 g ml^{-1}, 60 hr, 20°, 30,000 rpm, Spinco SW50L. The field is directed to the left.

DNA persists in the cytoplasm over more than one cell generation.

In CsCl-gradients the 18 S DNA had a buoyant density not to be distinguished from that of nuclear DNA (1.699 g ml^{-1}) [6]. Thermal denaturation resulted in an increase in buoyant density of approximately 0.020 g ml^{-1}. The reannealing characteristics of the 18 S DNA are similar to those of the nuclear DNA (fig. 3). No closed circular DNA was detected in the 18 S DNA-fraction by centrifugation in ethidium bromide-CsCl-gradients [9].

4. Discussion

Evidence for the association of DNA with the membrane fraction of human cells has been presented. In view of the labeling pattern of this DNA, it seems unlikely that this membrane bound DNA stems from any random leak of nuclear DNA during cell fractionation. Moreover, cell fractionation by mechanical disruption or lysis with detergents (Triton X-100, Brij 35) gave identical results, and extraction of nuclei yielded faster sedimenting DNA with the same $^{14}C/^3H$ ratio as nuclear DNA in the pulse–chase–pulse experiments. Buoyant density and reannealing properties distinguish the 18 S DNA clearly from the mitochondrial DNA (1.706 g ml^{-1}) and the light cytoplasmic DNA (1.688 g ml^{-1}) [6]. The latter is rapidly labeled and sediments with S-values larger than 60 S [10].

The notion that the 18 S DNA is replicated within the nucleus and is actively transported into the cytoplasm is in good agreement with the reported results. The question whether this DNA is transcribed in the cytoplasm cannot be answered at present. The possibility that by means of gene amplification [7] in the nucleus information could be transferred into the cytoplasm in form of DNA would be of considerable interest.

Acknowledgements

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