TRANSPORT OF NUCLEAR DNA INTO THE CYTOPLASM IN CULTURED ANIMAL CELLS. A SURVEY

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1. Introduction

Recently several observations of a special class of cytoplasmic DNA in animal cells have been reported [1–5]. Apparently this DNA is replicated in the nucleus and is later transported to the cytoplasm. Results obtained with cultured human liver cells seem to rule out the possibility that this DNA stems from any random contamination of the cytoplasmic fractions with nuclear DNA during cell fractionation [1]. So far a defined biological role could not be assigned to this DNA. However, the assumption seems reasonable that this DNA has a function in the transfer of information from the nucleus to the cytoplasm in the eukaryotic cell [3]. The importance of this notion prompted us to survey cells of several different species in order to see whether the phenomenon of the transport of DNA from the nucleus to the cytoplasm is a general one in animal cells.

2. Materials and methods

Mammalian cells were grown in Basal Medium Eagle supplemented with 10% fetal calf serum and 30 μg/ml tetracycline and 200 μg/ml gentamycin (screw cap bottles, monolayers, 37°). Chick embryo cells were prepared from 10 days old, decapitated chick embryos. The chick embryo cells were grown in Basal Medium Eagle supplemented with 10% fetal calf serum, 1% chick serum, 10% tryptose phosphate broth, 30 μg/ml tetracycline and 200 μg/ml gentamycin (screw cap bottles, monolayers, 38°). Cells were labelled with thymidine-methyl-^{3}H (20.4 Ci/mmole) and thymidine-2^{14}C (57 mCi/mmole) obtained from the Radiochemical Centre, Amersham, England. The methods of cell fractionation and the analytical methods have been described in detail [1, 2].

3. Results

In an established cell line derived from human tissues (Chang liver) a special cytoplasmic DNA can be detected by means of the technique of pulse (^{14}C-thymidine)-chase-pulse(^{3}H-thymidine) labeling and sedimentation through sucrose gradients [1, 2]. In these experiments this species of DNA has a characteristic early pulse/late pulse ratio which is higher than that of the mitochondrial DNA as well as that found in the nuclear DNA. Considering these results and kinetic data it has been concluded that this DNA is synthesized in the nucleus and appears in the cytoplasm after a considerable lag period (approx. 10 hr) by means of an active transport mechanism [1]. The sedimentation constant of this DNA is 18 S if related to that of the supercoiled mitochondrial DNA taken as 39 S [6]. With supercoiled Col E_1 DNA as a standard (23 S) [7] the sedimentation constant becomes 16 S (supercoiled mitochondrial DNA 35 S). These sedimentation values correspond to a molecular weight of approximately 3×10^6 daltons for a double stranded linear DNA molecule [8].

The same technique also revealed a DNA with similar features in respect to sedimentation behaviour and mode of labeling in the cytoplasm of HeLa cells (fig. 1A). Quite different results were obtained with a permanent cell line derived from human amnion

(WISH). In the cytoplasm of these cells no DNA could be detected with the labeling characteristics described for the cytoplasmic 16 S DNA of Chang liver cells [1] and HeLa cells (fig. 1B). Several experiments with explants of normal human tissues also did not give any indication of the existence of such a cytoplasmic DNA in cultured human embryonic lung cells and human embryonic kidney cells (fig. 1C).

Moreover, no discrete species of a cytoplasmic DNA with a considerably higher early pulse/late pulse-ratio than that of the nuclear DNA was detectable by means of these pulse-chase-pulse experiments neither in cells of a permanent cell line derived from a kidney of an African green monkey (fig. 2A), nor in cells of cell lines from rodent tissues such as mouse connective tissue (NCTC 929) or hamster kidney (BHK 21). Exactly the same experiments performed with cultured cells of chick embryos indicated that a DNA is present in the cytoplasm of these cells with labeling characteristics similar to those described for the 16 S DNA's of Chang liver cells and HeLa cells. However, the chick cell cytoplasmic DNA had a sedimentation constant of 7 S (fig. 2B). Table 1 summarizes the described results and gives the early pulse/late pulse ratios of the "transported DNA" and the supercoiled mitochondrial DNA in relation to that of the nuclear DNA.

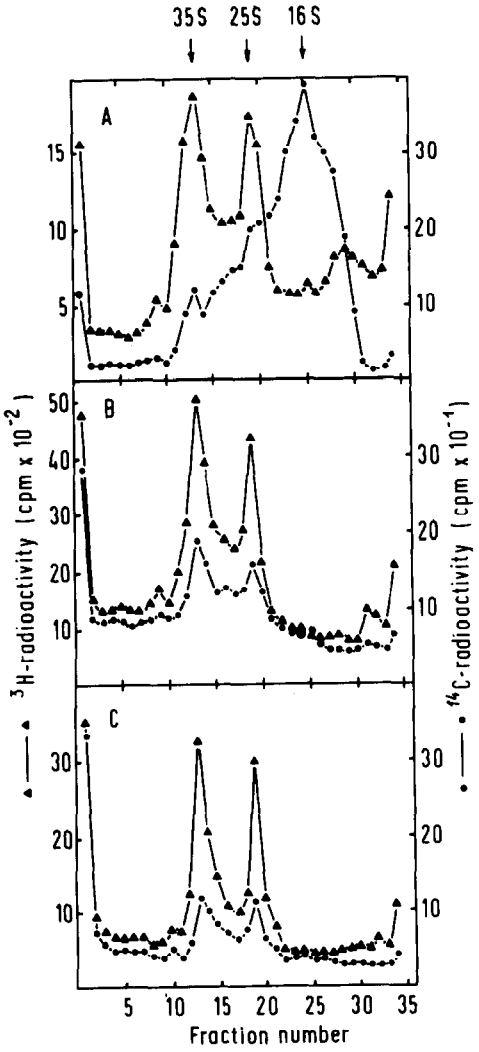

Fig. 1. Sedimentation of pulse-chase-pulse labeled total cytoplasmic DNA through sucrose gradients. Cells were labeled for 24 hr with thymidine-2-^{14}C (0.5 μCi/ml). The medium was replaced by a medium which did not contain the radioisotope for another 24 hr. The cells were subsequently exposed for 6 hr to a medium containing thymidine-methyl-^{3}H (5 μCi/ml). Total cytoplasmic extracts were prepared by lysing the cells 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. After standing for 30 sec (0°) the suspensions were briefly agitated with a Vortex mixer and the nuclei were removed by centrifugation at 2000 *g* for 10 min. The lysates were made 1% SDS (0.1 M NaCl, 0.001 M EDTA, 0.01 M Tris-HCl pH 7.6) and layered on top of gradients 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, 25,000 rpm, 25°, 12 hr). Gradients were fractionated into 1 ml fractions and acid precipitable radioactivity was determined: (●—●—●): ^{14}C; (▲—▲—▲): ^{3}H. A) HeLa cell cytoplasmic DNA. B) WISH cell cytoplasmic DNA. C) Cytoplasmic DNA of first passage cells of a primary culture of human embryonic kidney.

4. Discussion

In some of the cells under study a special class of cytoplasmic DNA has been detected. The complexity of this DNA is approximately the same as that of the nuclear DNA [9] and the labeling kinetics suggest that this DNA is of nuclear origin [1]. Working with chick cells, Bell [3, 4] has claimed that this DNA might be the intermediate in the transfer of genetic information from the nucleus to the cytoplasm (informational DNA). The work presented here confirms the observation of Bell as far as chick cells are concerned. Although several lines of evidence support the view that this DNA is not an artefact, it cannot be ignored that this cytoplasmic DNA is not a common feature of all the

Table 1

$^{14}C/^{3}H$ ratios of the cytoplasmic DNA's in comparison with those of the nuclear DNA's taken as unity of different cells.

Species, cell line			S-value of the "transported DNA"	Early pulse / Late pulse		
				Nuclear DNA	"Transported DNA"	Mitochondrial DNA
Human	HeLa	(ATCC;CCL 2)	16	1	5.3	0.47
	Chang liver	(ATCC;CCL 13)	16	1	5.1	0.58
	WISH	(ATCC;CCL 25)	–	1	–	0.50
	Embryonic lung		–	1	–	0.42
	Embryonic kidney		–	1	–	0.39
Monkey	Vero	(ATCC;CCL 81)	–	1	–	0.40
Mouse	NTCT 929	(ATCC;CCL 1)	–	1	–	0.25
Hamster	BHK 21	(ATCC;CCL 10)	–	1	–	0.21
Chick	Decapitated embryo		7	1	4.1	0.25

Experimental conditions for the labeling and the isolation of the cytoplasmic DNAs as described in fig. 1. Nuclear DNA was prepared as described previously [10].

cells under study. This should be expected if such a fundamental task is to be accomplished by this DNA. Therefore, the significance and function of this DNA is still questionable. At the moment not even a clear-cut pattern is recognizable in what kind of cells this DNA can be found. The reported results indicate that within one species this DNA is present in some established cell lines, while it is absent in others and is also absent in explants of several tissues. On the other hand, explanted cells of tissues of another species do contain an "informational DNA".

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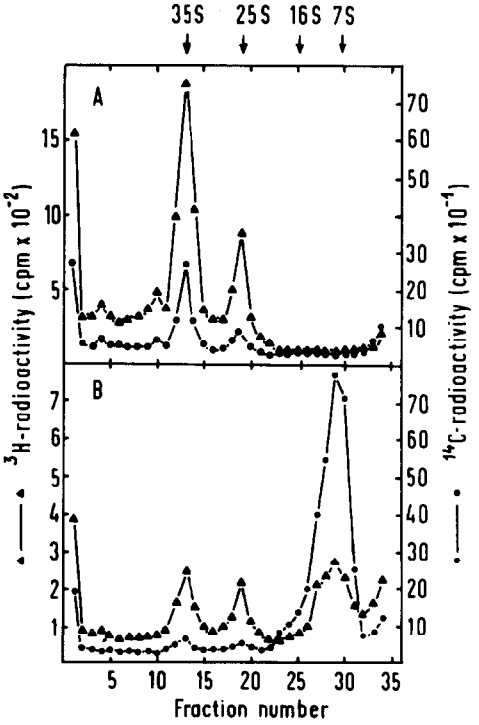

Fig. 2. Sedimentation of pulse-chase-pulse labeled total cytoplasmic DNA through sucrose gradients. Experimental conditions as described in fig. 1. Acid precipitable radioactivity: (●—●—●) ^{14}C; (▲—▲—▲) ^{3}H. A) Cytoplasmic DNA of cells of an established cell line (Vero) derived from a kidney of an African green monkey. B) Cytoplasmic DNA of first passage cells of a primary culture of a decapitated 10 days old chick embryo.

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