CYTOPLASMIC DNA's CONSISTING OF UNIQUE NUCLEAR SEQUENCES IN HAMSTER CELLS

A possible rôle in cell differentiation and oncogenesis

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Received 19 March 1973

1. Introduction

A special class of cytoplasmic DNA (cDNA = communication DNA [1]) is found in some animal cell lines [2]. The size of these DNA molecules ranges between a molecular weight of approx. 3×10^5 (7 S) [2, 3] for chick cells and approx. 3×10^6 (16 S) for human cells [2, 4, 5]. There is considerable evidence that this cDNA is of nuclear origin and that it is transported to the cytoplasm [4]. The base composition of the cDNA is similar to that of the bulk of the homologous nuclear DNA as judged by comparison of the buoyant densities in CsCl-gradients [4]. However, reassociation studies indicate that cDNA is not a random selection of nuclear DNA, rather the cDNA consists almost exclusively of unique nuclear sequences [1].

I report here studies employing different cell lines derived from tissues of the syrian hamster which indicate that the occurrence of cDNA in hamster cells depends on the history of the cell line and on the history of the tissue from which the cell line is derived. The size distribution of hamster cDNA centers around two maxima, one at 16 S and the other at 10 S.

2. Materials and methods

All hamster cells were grown in Basal Medium Eagle supplemented with 10% fetal calf serum, 30 $\mu g \times ml^{-1}$ tetracycline and 200 $\mu g \times ml^{-1}$ gentamycin (screw cap bottles, monolayers, 37°). Hamster embryo cells were prepared from 10 day old whole embryos. For cultures of lung and kidney cells 10 day old hamsters were used. Polyoma-transformed hamster cell lines BHK wt Cl 1a and BHK wt Cl 2a were kindly provided by Dr. K.L. Skoog, Biokemiska Avdelningen, Karolinska Institutet, Stockholm. Cells were labeled with [methyl-^{3}H] thymidine (18.4 $Ci \times mmole^{-1}$) and [2-^{14}C] thymidine (62 $mCi \times mmole^{-1}$) obtained from the Radiochemical Centre Amersham, GB.

The methods of cell fractionation (detergent method) and isolation of DNA have previously been described [6, 7]. DNA was dissolved in 0.12 M phosphate buffer (0.06 M monobasic sodium phosphate, 0.06 M dibasic sodium phosphate), cooled in ice and sheared by 6 cycles of sonication (10 sec, Bransom Sonifier J17V). Denaturation was carried out by heating the DNA solution for 15 min in a boiling water bath. The DNA was reassociated at 60°. Chromatography on hydroxyapatite columns was used to separate denatured and reassociated DNA as described by Britten and Kohne [8, 9]. All further analytical methods have been described elsewhere [6].

3. Results and discussion

The sedimentation pattern of the labeled DNA's being found in the cytoplasm of a polyoma-transformed hamster cell line (BHK wt Cl 1a) depends strongly on the length of time to which the cells are exposed to [methyl-^{3}H] thymidine. Thus after short

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to achieve in the sense of chemical exchange, but we can arrive at a fast exchange situation by making use of electron exchange between the protein in its copper(II) and copper(I) forms. The fast electron exchange situation is approached on titration of the holoenzyme with potassium ferrocyanide, which approximates to the "poised-potential" situation [9]. In the NMR spectrum a gradual change is observed from the broadened spectrum of the holoenzyme to the characteristically sharp spectrum of the diamagnetic protein. It may therefore be possible to relate the position of these residues which give rise to assignable resonances to that of the copper(II) ions.

Such techniques, combining the use of difference spectroscopy [10] and the intrinsic probe properties of Cu^{2+} should be applicable to any copper protein which exists in the paramagnetic and diamagnetic states.

Acknowledgements

We thank the SRC (A.M.S. and H.A.O.H.), the Wellcome Trust and the Nuffield Foundation (W.H.B.) for financial support. H.A.O.H. is a member of the Oxford Enzyme Group. J.V.B. thanks Dr. P.J.R. Phizackerley and Mr. J.R.P. O'Brien for much encouragement.

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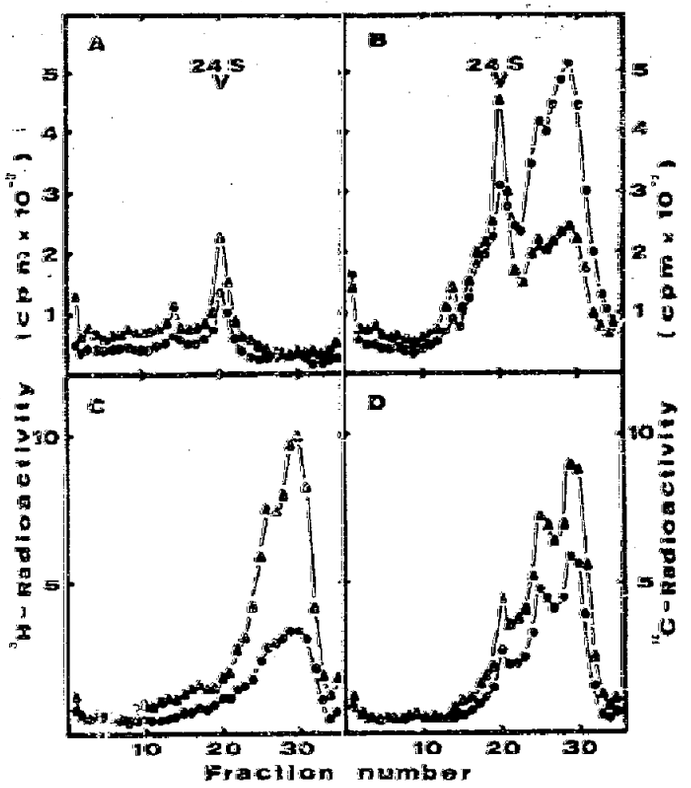

Fig. 3. Sedimentation of pulse-chase-pulse labeled cytoplasmic DNA's from different hamster cell lines through sucrose gradients. Cells were labeled for 24 hr with [2-^{14}C] thymidine (0.5 μCi × ml^{-1}). The medium was replaced by a medium which did not contain the radioisotope for another 24 hr. The cells were subsequently exposed for 6 hr to a medium containing [methyl-^{3}H] thymidine (5 μCi × ml^{-1}). Further experimental conditions as described in fig. 1. Acid precipitable radioactivity: (●—●—●) ^{14}C; (▲—▲—▲) ^{3}H. A) Cytoplasmic DNA's of first passage cells of a primary culture of kidneys from four 10 days old hamsters. B) Cytoplasmic DNA's of a polyoma-transformed hamster cell line (BHK wt Cl 1a). C) Cytoplasmic DNA's of first passage cells of a primary culture of five 10 day old whole hamster embryos. D) Cytoplasmic DNA's of a cell line derived from a polyoma induced hamster tumour.

type of experiments the distribution of the early pulse radioactivity and that of the late pulse radioactivity is different in the individual cytoplasmic DNA's. Fig. 3 shows a few representative sedimentation profiles of cytoplasmic DNA's of different hamster cells labeled in such a way. The sedimentation pattern and the occurrence of cDNA which is relatively rich in early pulse radioactivity is a characteristic feature of each cell line. Table 1 summarizes the results obtained with a number of hamster cells of different history. Considering these data two points can be made. First, the highest amount of cDNA in the cytoplasm is found in the cell lines which are derived from tumour tissues or from whole embryos. The other extreme is represented by cell lines which have not acquired the status of a permanent cell line and are derived from fully differentiated tissues. In these cell lines no cDNA is detectable in the cytoplasm. Virus-transformed cell lines occupy an intermediate position. Secondly, in cell lines derived from whole embryos or from virus-induced tumours the cDNA is by far the most prominently labeled DNA species in the cytoplasm. Because of the abundance of cDNA labeled mitochondrial DNA is hardly detectable in these cell lines even after short labeling periods.

The informational content of DNA can be estimated from reassociation rate measurements [8]. An uneven distribution of repetitive and unique sequences in cDNA and in nuclear DNA is revealed by measuring the extent of reassociation of trace amounts of labeled cDNA in the presence of large amounts of nuclear DNA (fig. 4). The cDNA of polyoma-transformed BHK cells reassociates almost in its entirety with the non-repetitive fraction ($c_0t_{1/2} \approx 10^3$ M × sec) of the nuclear DNA of untransformed BHK cells. This $c_0t_{1/2}$-value is within the range of values expected for the single-copy DNA of a mammalian genome.

The observation that cDNA is not a random selection of nuclear DNA minimizes arguments about cDNA being a mere artifact created by damaging nuclei during cell fractionation. Furthermore, this observation suggests a significant role of this DNA in the transfer of information across the nuclear membrane. However, this suggestion raises again the question why a DNA species with such a fundamental task is not found in all animal cells under study [2]. From the observations described in this report and our earlier observations [2] a general pattern seems to emerge about the species specificity and the cell specificity of cDNA. The size distribution is species specific and cDNA is found in at least some fetal tissues and in cancer tissues which both are, to varying degrees, composed of undifferentiated cells. On the other hand, fully differentiated embryonic tissues such as human embryonic lung and human embryonic kidney as well as adult tissues do not contain cDNA. This analogy between fetal and cancer tissues has a remarkable

Table 1
Occurrence of cDNA and amount of radioactivity recovered in the cytoplasm of pulse–chase–pulse labeled hamster cells.

Cell line	% of total radioactivity (early pulse)		% of total radioactivity (late pulse)		Occurrence of cDNA 16 S and 10 S DNA
	Cytoplasm	Nuclei	Cytoplasm	Nuclei	
Hamster kidney (10 day old hamster) Passage No. 1	0.15	99.85	0.36	99.64	–
Hamster Embryo (10 day old embryo) Passage No. 1	0.22	99.78	0.68	99.32	+++
BHK 21 Cl 13 (ATCC; CCL 10) Passage No. unknown	0.10	99.90	0.16	99.84	+
BHK wt Cl 1a (Polyoma transformed) Passage No. unknown	0.33	99.67	0.57	99.43	++
BHK wt Cl 2a (Polyoma transformed) Passage No. unknown	0.12	99.88	0.27	99.73	++
Polyoma induced Hamster Tumour Passage No. 17	0.30	99.70	0.44	99.56	+++
SV 40 induced Hamster Tumour Passage No. 83	0.57	99.43	0.76	99.24	+++

Cells were labeled and cytoplasmic DNA's were analyzed as described in fig. 3. Occurrence of cDNA: –, not detectable; +, weak; ++, medium; +++, strong

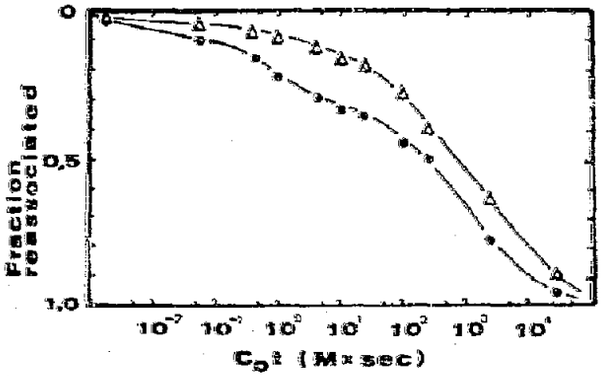

Fig. 4. Reassociation kinetics of trace amounts of cDNA in presence of nuclear DNA. cDNA was isolated from BHK wt Cl 1a cells labeled with [methyl-^{3}H] thymidine (10 μCi × ml^{-1}) for 24 hr as described in fig. 1 and further purified by RNAase treatment and phenol extraction. Nuclear DNA from BHK 21 cells labeled with [2-^{14}C] thymidine was used. The concentration of cDNA was indeterminate but negligible in comparison to that of the nuclear DNA. Consequently, the extent of the reassociation is plotted against the c_0t of nuclear DNA. (Δ—Δ—Δ), Reassociation of cDNA. (●—●—●), Reassociation of nuclear DNA.

parallel in some tumour specific fetal antigens i.e. α-fetoglobulin [10] and carcinoembryonic antigen [11] which are present in some fetal tissues, disappear shortly before or after birth and recur in association with malignancy. Whether this parallel is merely coincidental or has a causal relationship cannot be decided at present.

The transport of nuclear DNA into the cytoplasm calls for a mechanism of gene amplification in the nucleus. Otherwise, the nucleus of a differentiating embryonic cell or of a cancer cell would necessarily suffer a loss of information. There is an increasing amount of evidence that gene amplification is neither restricted to the rDNA (ribosomal DNA) in the oocyte [7, 12] nor to the rDNA itself [13]. Gene amplification might be an important regulation mechanism in the undifferentiated cell or during the process of cellular differentiation and rapid growth. Consequently,

our data suggest that this mechanism may be normally repressed in a fully differentiated cell, but can be reestablished by a cell transforming agent, i.e. an oncogenic virus.

cDNA molecules have approximate molecular weights between 4×10^5 (chick 7 S cDNA) and 3×10^6 (human and hamster 16 S cDNA) containing sufficient genetic information to code for one to five polypeptide chains of average length. The data accumulated so far do not indicate the type of information that cDNA may contain. The fact that unique nuclear sequences are observed does not rigorously exclude the possibility that virus-specific sequences are also present in cDNA isolated from virus induced tumours.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft, Bad Godesberg. I wish to thank Dr. K.L. Skoog, Stockholm for the kind gift of polyoma-transformed hamster cells and the E. Merck AG, Darmstadt for the generous supply of gentamycin.

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