TRANSPORT OF NUCLEAR DNA INTO THE CYTOPLASM IN ANIMAL CELLS
The complexity of the transported DNA

Jürgen KOCH and Dieter GÖTZ
Institut für Biologische Chemie und Ernährungswissenschaft, Universität Hohenheim
Stuttgart-Hohenheim, W. Germany

Received 22 August 1972

1. Introduction

The concept that the genetic information flows from DNA to RNA by means of a transcription process before it is expressed as protein (translation) stems from experiments with prokaryotic organisms [1, 2]. In these organisms transcription and translation proceed in one spacial unity, a ternary association of DNA, RNA and ribosomes [3]. This concept has also been adopted for eukaryotic cells with the modification that a messenger RNA (mRNA) is synthesized in the nucleus and subsequently migrates to the cytoplasm where translation occurs. In other words transcription and translation are spacially separated by the nuclear membrane. However, the fraction of rapidly labeled heterogeneous RNA in the nucleus of animal cells that behaves as a precursor of cytoplasmic polyribosomal messenger RNA is quite small and the results of the experiments intended to demonstrate this migration of mRNA across the nuclear membrane were not altogether equivocal [4–7].

More recently a special class of cytoplasmic DNA has been observed in chick cells [8, 9] and in human cells [9–12], the origin of which apparently is the cell nucleus [10, 13]. Bell [14] already has hypothesized that this cytoplasmic DNA which he has called informational DNA (I-DNA) represents a fraction of the total genome and acts as a template for the synthesis of mRNA in the cytoplasm. In view of the experiments which indicate that this DNA is replicated within the nucleus and is later transported to the cytoplasm [10, 13] an alternative model for the information transfer across the nuclear membrane can be envisaged. Because of the role which will be attributed by this model to this special class of DNA, we shall call this DNA in the following: "communication DNA".

By studying the reassociation characteristics of the "communication DNA" isolated from HeLa cells and chick fibroblasts, we intended to answer the question whether the "communication DNA" represents a random selection of the total nuclear DNA.

2. Materials and methods

HeLa cells (ATCC; CCL 2) 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°). Chick embryo cells were prepared from 10 days old, decapitated chick embryos. Chick cells were grown in Basal Medium Eagle supplemented with 10% fetal calf serum, 1% chick serum, 30 $\mu g \times ml^{-1}$ tetracycline and 200 $\mu g \times ml^{-1}$ gentamycin. Cells were labeled with [^{3}H]methyl-thymidine (20.4 Ci × mmole^{-1}) or [2-^{14}C]thymidine (57 mCi × mmole^{-1}) obtained from the Radiochemical Centre Amersham, GB.

The methods of cell fractionation (detergent method) and isolation of DNA have previously been described [11, 15]. DNA was dissolved in 0.12 M phosphate buffer (0.06 M monobasic sodium phosphate), cooled in ice and sheared by 6 cycles of sonication (10 sec, Branson Sonifier J17V). Denaturation was at 100° for 10 min. Reassociation was carried out at 60°. Chromatography on hydroxyapatite columns was used to separate denatured and reassociated DNA as described

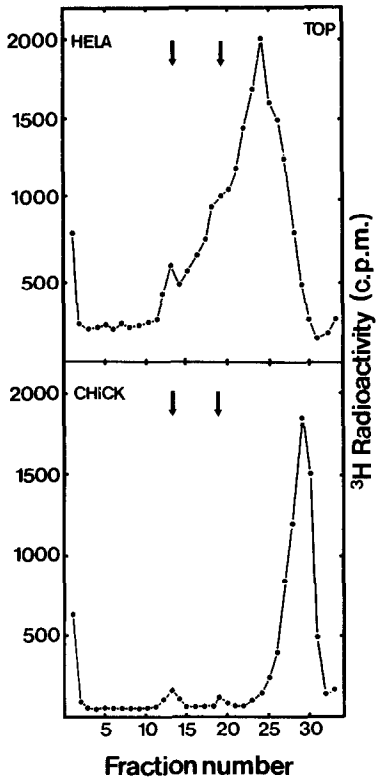

Fig. 1. Sedimentation of pulse-chase labeled total cytoplasmic DNA through sucrose gradients. HeLa cells and chick embryo cells (secondary culture) were labelled in exponential growth phase for 24 hr with [^{3}H]methyl-thymidine (10 μCi × ml^{-1}). The medium was replaced by a medium which did not contain the radio isotope for another 24 hr. 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, 25000 rpm, 25°, 12 hr). Gradients were fractionated into 1 ml fractions and acid precipitable radioactivity was determined. Direction of sedimentation is from right to the left. Arrows indicate the position of the closed circular (34 S) and open circular (24 S) mitochondrial DNA.

by Britten and Kohne [16, 17]. All further analytical methods have been described in detail elsewhere [11].

Results and discussion

After pulse-chase labeling with [^{3}H]methyl-thymidine the most prominently labeled cytoplasmic DNA sediments with 16 S in case of HeLa cells and 7 S in case of chick cells (fig. 1). Previous experiments have indicated that these DNA's are synthesized in the nucleus and after a considerable lag period appear in the cytoplasm [9, 10]. Therefore, these DNA's ("communication DNA's") comply with one indispensable requirement which has to be met by a potential communication medium between the nucleus and the cytoplasm, i.e. movement across the nuclear membrane.

Reassociation rate measurements can give an indication of the informational content of a DNA, in as much as the rate of renaturation is inversely proportional to the number of different nucleotide sequences present at a given concentration of DNA [16]. But the measurement of the reassociation of complex DNA's requires large amounts (high concentrations) of DNA which were not available in case of the "communication DNA's". Therefore, we decided to use trace amounts of highly labeled "communication DNA" ([^{3}H]methyl-thymidine) isolated as described in fig. 1 and to study their reassociation in the presence of a large excess of nuclear DNA. By this method really the sequence homology between the "communication DNA" and nuclear DNA was determined. Accordingly, this approach should enable us to decide whether populations of individual sequences are present in both types of DNA with approximately the same distribution. As shown in fig. 2 the "communication DNA" does not seem to be a random selection of nuclear DNA. The "communication DNA" of HeLa cells as well as that of chick cells contains less repetitive sequences than the homologous nuclear DNA.

The haploid genome of a human cell contains roughly 3×10^9 nucleotide pairs (6 pg DNA per diploid cell). Thus the human genome is about 670 times as large as that of *Escherichia coli* with 4.5×10^3 nucleotide pairs [18]. Since the $(C_0t)_{\frac{1}{2}}$ of *E. coli* DNA has a value of approx. 6 M × sec [17] the theoretical $(C_0t)_{\frac{1}{2}}$ for the human single-copy DNA can be calculated to be 4.0×10^3 M × sec. Experimentally, 80% of the "communication DNA" of HeLa cells has been found to reassociate together with nuclear DNA with a $(C_0t)_{\frac{1}{2}}$ value of 5.5×10^3 M ×

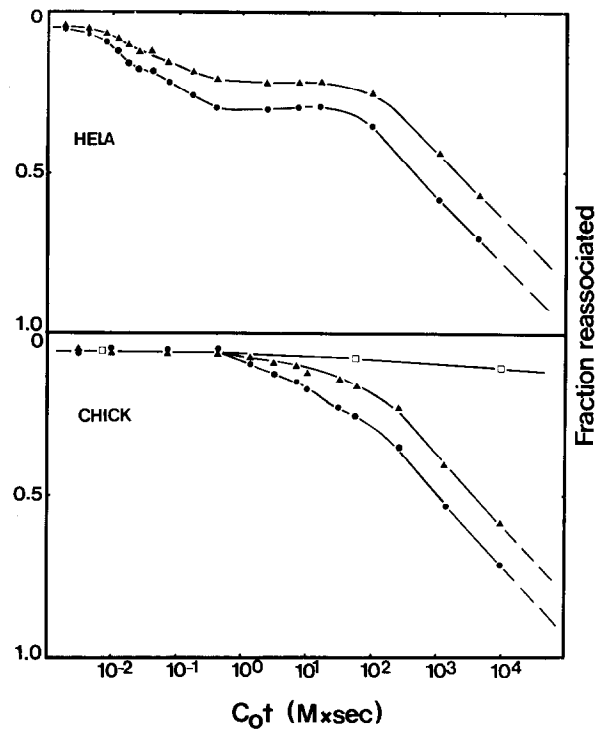

Fig. 2. Reassociation kinetics of trace amounts of HeLa "communication DNA" (cytoplasmic 16 S DNA) and chick "communication DNA" (cytoplasmic 7 S DNA) in presence of nuclear DNA. In these experiments ^{14}C-labeled nuclear DNA's and ^{3}H-labeled cytoplasmic DNA's were used. Cytoplasmic DNA's were isolated as described in fig. 1 and further purified by RNAase treatment and phenol extraction. The true concentration of the "communication DNA's" was indeterminate but negligible in comparison to that of the nuclear DNA. Consequently, the extent of the reassociation is plotted against the C_0t (product of initial concentration and time of incubation) of nuclear DNA. (▲—▲—▲) Reassociation of "communication DNA" in presence of homologous nuclear DNA (●—●—●). (□—□—□) Reassociation of HeLa "communication DNA" in presence of chick nuclear DNA (●—●—●).

sec (fig. 2). We can therefore assume that 80% of the "communication DNA" consists of copies of unique sequences of the human genome.

In human cells the "communication DNA" in the cytoplasm amounts to approx. 0.5 to 1% of the total cell DNA [10]. On the other hand, it has been concluded that roughly 2% of the total DNA represent functional gene loci in a mammalian cell [19]. That means that a considerable amount if not all of the active information of a human genome necessary to maintain the viable functions of a cell might be available in the cytoplasm in form of the "communication DNA".

This still hypothetical model of information transfer across the nuclear membrane bears three important conclusions: i) gene amplification may be a key event if not a pre-requisit in gene expression, ii) regulation of gene expression may occur at the level of DNA replication rather than transcription and iii) there must exist a transcription mechanism in the cytoplasm. There is an increasing amount of evidence that gene amplification is neither restricted to the rDNA (ribosomal DNA) in the oocyte [15, 20] nor to the rDNA itself [21]. Recently a cytoplasmic DNA dependent RNA polymerase (RNA polymerase C) has been described [22] which could be involved in the transcription of the "communication DNA" in the cytoplasm. Furthermore, there is evidence that no precise transcriptional control is executed within the nucleus [5, 6].

Thus, the "communication DNA" does not seem to be a completely unattractive alternative to the elusive messenger RNA. Nevertheless, it should be mentioned that a discrete species of DNA with the properties of the "communication DNA" was not detectable in the cytoplasm of all eukaryotic cells under study [9]. There is preliminary experimental evidence that in the cells which gave negative results an extrachromosomal DNA is present with properties similar to those of the cytoplasmic "communication DNA" of HeLa cells and chick cells [20]. But the part of the DNA which becomes labeled stays in the nucleus under the experimental conditions so far used. The conditions which determine the transport of this DNA across the nuclear membrane are the subject of further investigation.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft, Bad Godesberg. The authors wish to thank the E. Merck AG, Darmstadt for the generous supply with gentamycin.

References

[1] S. Brenner, F. Jacob and M. Meselson, Nature 190 (1961) 576–581.
[2] F. Gros, H. Hiatt, W. Gilbert, C.G. Kurland, R.W. Risebrough and J.D. Watson, Nature 190 (1961) 581–585.
[3] O.L. Miller, Jr., B.A. Hamkalo and C.A. Thomas, Jr., Science 169 (1970) 392–395.
[4] S. Penman, C. Vesco and M. Penman, J. Mol. Biol. 34 (1968) 49–69.
[5] K. Scherrer, G. Spohr, N. Granboulan, C. Morel, J. Grosclaude and C. Chezzi, Cold Spring Harbour Symp. Quant. Biol. 35 (1970) 539–554.
[6] J.E. Darnell, G.N. Pagoulatos, U. Lindberg and R. Balint, Cold Spring Harbour Symp. Quant. Biol. 35(1970) 555–560.
[7] C. Morel, B. Kayibanda and K. Scherrer, FEBS Letters 18 (1971) 84–88.
[8] E. Bell, Science 174 (1971) 603–606.
[9] J. Koch and H. v. Pfeil, FEBS Letters 24 (1972) 53–56.
[10] J. Koch and H. v. Pfeil, FEBS Letters 17 (1971) 312–314.
[11] J. Koch, European J. Biochem. 26 (1972) 259–266.
[12] R.A. Lerner, W. Meinke and D.A. Goldstein, Proc. Natl. Acad. Sci. U.S. 68 (1971) 1212–1216.
[13] M.R. Hall, W. Meinke, D.A. Goldstein and R.A. Lerner, Nature New Biology 234 (1971) 227–229.
[14] E. Bell, Nature 224 (1969) 326–328.
[15] J. Koch and A. Cruceanu, Hoppe-Seyler's Z. Physiol. Chem. 352 (1971) 137–142.
[16] R.J. Britten and D.E. Kohne, Carnegie Inst. Washington Yearbook 65 (1966) 78–123.
[17] R.J. Britten and D.E. Kohne, Science 161 (1968) 529–540.
[18] J. Cairns, Cold Spring Harbour Symp. Quant. Biol. 28 (1963) 43–48.
[19] S. Ohno, Develop. Biology 27 (1972) 131–136.
[20] J. Koch, unpublished results.
[21] R.G. Krueger and B.J. McCarthy, Biochem. Biophys. Res. Commun. 41 (1970) 944–951.
[22] B.J. Benecke, P.P. Juhasz and K.H. Seifart, Hoppe-Seyler's Z. Physiol. Chem. 353 (1972) 691–693.