"CYTOPLASMIC" DNA FROM PRIMARY EMBRYONIC CELL CULTURES IS NOT INFORMATIONAL

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1. Introduction

The identification of DNA in animal cell microsomes was first reported in 1962 [1] and has been confirmed by several other reports [2–4]. Interest in this fraction increased when Bell [5] revived a suggestion first put forward by Pelc [6, 7] that this rapidly labelled (or "metabolic") DNA fraction might have an informational role. In the case of primary embryonic cell cultures, as used by Bell, one of us [8] has previously shown that this is unlikely to be the case, both because of identity with nuclear DNA in all properties measured and because of the peculiar size classes of the DNA itself. It has also been found that similar DNA fractions isolated from sea urchin embryos [9], mouse lymphoma cells [10] and pea roots [11] are nuclear in origin.

The criterion for informational character is neither rapid labelling nor polysome association, as suggested by Bell; on the other hand, the fact that the DNA resembles nuclear DNA in all physical properties does not in itself rule out an informational role. However, if the "cytoplasmic" DNA is informational in character, it must follow that it contains only a proportion, and in all probability a small proportion, of the total nucleotide sequences of the genome. It is possible to compare the sequence heterogeneity of nuclear and "cytoplasmic" DNA by comparing the rates of reannealing after melting under similar conditions. This has been done for both the intermediate and slow annealing fractions [12, 13] of the DNA, and the nuclear and "cytoplasmic" DNAs from primary cultures of embryonic mouse liver cells have very similar reannealing profiles. It can thus be concluded that the nuclear and "cytoplasmic" DNAs are similar in their sequence heterogeneity as in all other properties investigated. Our material would appear to be very similar to that studied by Bell [5] in primary cultures from chick embryos, although it is difficult to be certain as his methods have not yet been published in full, and our results rule out the possibility that this DNA has an informational role, as it contains all rather than a selection of the information in the cell genome.

2. Methods and results

"Cytoplasmic" and nuclear DNA were isolated from 16-hr primary cultures of 14-day mouse embryonic liver cells as previously described [8]. DNA isolated from cells cultured for shorter periods was

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Table 1

"Cytoplasmic"	17.6 ± 0.1
Nuclear	10.5 ± 1.5

"Satellite" DNA in nuclear and "cytoplasmic" DNA preparations: mean of 4 runs. An aliquot of DNA in 0.05 M KCl was denatured at 100° for 10 min, cooled to 60°, renatured for 3 min, and cooled rapidly to 0°. Solid CsCl was added to give a final density of 1.710 and the solution was centrifuged at 45,000 rpm for 20 hr in an MSE analytical ultracentrifuge at 25 ± 0.1°. The final DNA concentration was 25–50 μg/ml. Photographs were taken using Kodak spectroscopic safety film and the amount of satellite was estimated from the trace obtained with a Joyce–Loebl recording microdensitometer.

present in smaller amounts but had the same molecular characteristics. All DNA preparations used in these experiments were further purified by isopycnic banding in caesium chloride gradients. The DNA preparations were sonicated and the average molecular weight was similar at approx. 800,000 (double-stranded) as judged by sucrose gradient centrifugation for the nuclear and "cytoplasmic" material.

The renaturation curve can be affected by the amount of "satellite" DNA, although this component of mouse DNA does not appear to be transcribed nor to be informational in character [14]. Therefore the amount of satellite DNA after rapid reannealing was determined using an M.S.E. analytical ultracentrifuge as previously described by Flavell and Jones [15]. The amount of mouse satellite present in the "cytoplasmic" DNA was higher than that in total mouse embryo nuclear DNA (table 1). The value obtained for total nuclear DNA agrees with published values [14].

The reannealing kinetics were determined for the intermediate fraction of DNA by measuring double-strandedness by binding to hydroxylapatite. The profiles obtained for nuclear and "cytoplasmic" DNA are shown in fig. 1. It is apparent that the rates of renaturation for this fraction of DNA (from C_0t 0.1 to 100) cannot be distinguished for the two preparations.

The complete C_0t curve was determined optically using sealed cells of very short path length in the heating block of a Unicam SP800 [16]. The melting temperature of the DNA was also determined with the Unicam SP800 attached to a Servoscribe chart recorder.

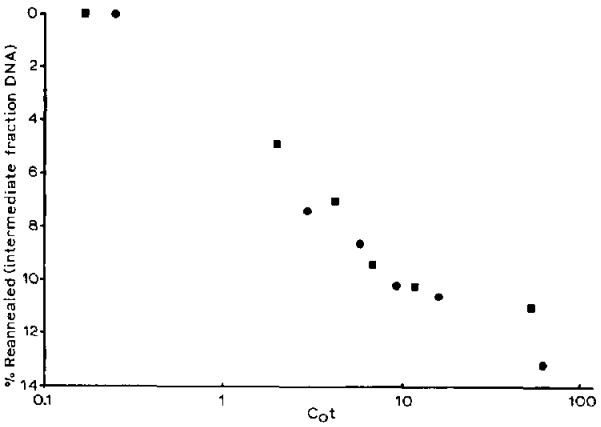

Fig. 1. Reannealing of intermediate fraction DNA. Nuclear and cytoplasmic DNA (180 μg/ml and 260 μg/ml, respectively) were denatured by addition of NaOH to 0.1 N followed by 1 M NaH_2PO_4 to give 0.12 M phosphate at neutral pH. Samples were kept at 67°. Aliquots were removed in duplicate at varying times, diluted with 5× volume of 0.12 M phosphate buffer, and mixed with hydroxylapatite; under these conditions only double-stranded DNA binds and the non-reannealed DNA remains in solution. After 3 rinses the molarity of the phosphate solution used to resuspend the HAP was increased to 0.3 M and the optical density of the eluted double-stranded DNA was determined. The proportion of reannealed DNA was the sum of the DNA eluted at 0.3 M phosphate divided by the total DNA eluted at 0.12 M and 0.3 M phosphate. The run was continued for 21 hr. The curves are constructed to show the reannealing of the intermediate fraction (between C_0t 0.1 and 100) and corrected for reannealing which has occurred at lower C_0t values. (■, cytoplasmic; ●, nuclear.)

The optical reannealing profile (which gives a direct measure of double-strandedness, unlike the hydroxylapatite method, in which attached single strand "ends" are measured with the reannealed fraction) was carried on for 14–21 days, until over 60% of the 'unique' DNA had reannealed (fig. 2). The $C_0t_{1/2}$ (the value of the C_0t at which half of the fraction had annealed) for the unique fraction of the "cytoplasmic" DNA was found to be approx. 2,000. The value obtained under identical conditions of reannealing for mouse unique fraction [17] is 1,800; these values are well within the limits of experimental error and indicate a maximum difference in sequence heterogeneity between these two fractions of 20%; that is, the "cytoplasmic" DNA contains essentially the same heterogeneity of intermediate and unique sequences as nuclear DNA in mouse.

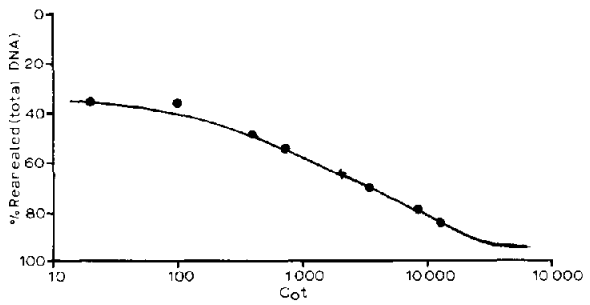

Fig. 2. Reannealing curve for the slow annealing fraction of "cytoplasmic" DNA. Conditions and data presentation are exactly as given in [16]. The T_m of the DNA was 62°, the hyperchromicity 28%. The curve shown can be compared directly with that given for total mouse nuclear DNA in [17]. 35% of the DNA reannealed in this set of experiments prior to the slow fraction, in agreement with the other data.

Any change in complexity in non-reiterated DNA is directly reflected in the C_0t required for half-reassociation [12].

The melting profiles of the "cytoplasmic" and nuclear DNAs were determined under the ionic conditions used for the reannealing experiments, and were found to be identical.

3. Discussion

If the suggestion of Bell was correct and the "cytoplasmic" DNA performed an informational role, or if this DNA were transcribed by an RNA-dependent DNA polymerase from cytoplasmic RNA, as might be suggested by certain recent data [18, 19], it is impossible that it would contain the same large range of both reiterated and, in particular, unique sequences as does total nuclear DNA. Therefore it is suggested, in keeping with the conclusion of our previous paper [8], that this DNA arises from nuclear lysis during primary cell culture, and may be derived either from intermediates in DNA biosynthesis or the action of nucleases on DNA partially protected by chromosomal proteins.

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