THE NUMBER OF GLOBIN GENE SEQUENCES IN "CYTOPLASMIC" DNA FRAGMENTS

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SUMMARY

DNA was isolated from the cytoplasm of primary cultures of mouse foetal liver cells. The proportion of globin genes was determined by two methods of cDNA-DNA hybridisation in globin complementary DNA excess. The proportion was similar in 'cytoplasmic' DNA and nuclear DNA. This argues against an informational role for this class of 'cytoplasmic' DNA, which has all of the characteristics of nuclear DNA arising from nucleosomes derived from chromatin.

INTRODUCTION

There have been a number of reports of DNA species isolated from the cytoplasm of animal and plant cells (1-10). These DNAs do not appear to be mitochondrial or viral in origin and fall into two main classes. One of these is labelled rapidly and consists of a number of discrete size classes. The other is associated with cell membranes, is labelled slowly, and may reanneal with kinetics differing from nuclear DNA. Since different tissues were used, these may represent similar DNA populations from different cell types. In both cases an informational role has been proposed for this 'communication' (11) or 'informational' (4) DNA. It has also been reported that membrane-associated cytoplasmic DNA from a human lymphoma cell line reanneals more rapidly than nuclear DNA (12, 13). Others have considered that the 'cytoplasmic' DNA is an artefact arising from contamination with nuclear DNA sequences, which might occur by nuclear breakdown either before or during cell extraction (5-8, 14).

We have previously reported the isolation of cytoplasmic DNA from primary cultures of erythroid cells from mouse foetal livers (8,14). This DNA comprises from 5-10% of the total DNA isolated from the cell culture after 18 hours. It has an average sedimentation coefficient of

approximately 7S on sucrose gradients. On gel electrophoresis it separates into a number of double-stranded components each of which is a multiple of the unit molecular weight 135,000. "Cytoplasmic" DNA reanneals with overall kinetics very similar to that of nuclear DNA. We report here data demonstrating that the proportion of globin genes is similar in nuclear and "cytoplasmic" mouse DNA.

METHODS

Mouse globin messenger RNA was isolated from mouse reticulocytes using polyU-Sepharose (Pharmacia Ltd., Uppsala) (15, 16); after two cycles no components other than 9S globin mRNA were visible on gel electrophoresis and no other detectable proteins were synthesised in a wheat germ cell free system. Complementary DNA (cDNA) was prepared with avian myeloblastosis virus reverse transcriptase (17); the complexity of reannealing of the cDNA was that of a sequence approximately 700-800 nucleotides in length, corresponding to 60% (350 nucleotides) of the length of the α+β- globin mRNAs (18). This demonstrates the absence of significant amounts of hybridising impurities in the template globin mRNA.

DNA was prepared with hydroxylapatite from nuclei isolated from mouse foetuses (19); 'cytoplasmic' DNA was prepared from primary cultures of mouse foetal livers excised at 14 days and cultured for 18 hours (5). The 'cytoplasmic' DNA had the characteristic band profile on gel electrophoresis previously reported (5).

Hybridisation of 0.8 ng cDNA to nuclear or 'cytoplasmic' DNA was performed in cDNA excess as described by Young & Paul (20). After incubation for a period twenty times that required for complete hybridisation of cDNA to complementary globin DNA sequences at the concentrations used (9 days, C_0t = 13,000; (20), the mixture was fractionated into single and double-stranded sequences on hydroxylapatite. The counts hybridised to nuclear and 'cytoplasmic' DNA annealed in parallel with the same preparation of cDNA are given in Table 1, as well as the values obtained with *E. coli* DNA (control). In each case approximately 7 globin gene sequences hybridise per haploid genome. The ratio of globin genes per haploid genome found in 'cytoplasmic' DNA to globin genes found in nuclear DNA was 0.87 for this method.

In a second set of experiments the number of globin genes per haploid genome was measured in slight cDNA excess using a method of kinetic analysis developed by Bishop & Freeman (21) and previously applied by us to determine human globin gene number (22). This technique minimises the contribution of minor components in the cDNA to the level of hybridisation.

Three separate cDNA excess experiments were performed, using three different cDNA preparations and two different 'cytoplasmic' DNA preparations. The ratio of cDNA to DNA varied from approximately 2:1 to 10:1, and the percentage cDNA hybridising from 16% to 5.8%. One set of curves is shown in Figure 1. The number of total hybridising globin gene sequences found per haploid genome was between 2-3 in each case, when determined using the equations given by Bishop & Freeman (21). The ratio of globin genes per haploid genome found in 'cytoplasmic' DNA to globin genes found in nuclear DNA was 1.23± 0.11 for this method.

	cpm reannealed per 0.3 mg DNA	sequences comp. to cDNA
Nuclear DNA	1,583; 1,647; 1,463	7.7
'Cytoplasmic' DNA	1,585; 1,205	6.7
E. coli DNA	312	-

Table 1. Annealing of Nuclear and 'cytoplasmic' DNA to excess cDNA followed by hybrid separation of hydroxylapatite:

Conditions of hybridisation were as given in Young and Paul (20). 0.3 mg total unlabelled nuclear or 'cytoplasmic' mouse DNA, and mouse globin cDNA (8,000 cpm, 0.8 ng) were dissolved in 60 µl of 0.12M sodium phosphate buffer at pH 6.8 and sealed in a capillary which was placed in boiling water for ten minutes prior to incubation at 60°C. After 9 days the contents of the capillary were diluted to 1 ml with 0.15M NaCl and passed through water-jacketed columns of hydroxylapatite which had been equilibrated to 60°C. Each column was eluted in a stepwise fashion from 0.03M sodium phosphate buffer to 0.4M sodium phosphate buffer (20). The elution of the mouse embryo total DNA was followed optically and the elution of cDNA was measured by radioactivity. The proportion of cDNA forming hybrid was taken as the proportion of counts recovered between 0.13M and 0.24M phosphate buffer; no significant radioactivity was eluted at greater than 0.24M phosphate buffer. Between 50-80% of the unlabelled DNA reannealed in each experiment. Approximately 90% of the total cpm added were recovered. The results of a control experiment in which E. coli DNA was annealed with mouse globin cDNA are also given. The two experiments with 'cytoplasmic' DNA were with different preparations.

The number of sequences complementary to cDNA were calculated assuming the mouse genome is 1.8×10^{12} daltons and the average cDNA size is 100,000 daltons (20).

DISCUSSION

In this case, 'cytoplasmic' DNA was isolated from an erythroid tissue which contains over 80% erythroid cells (23). Were the foetal liver 'cytoplasmic' DNA 'informational DNA' (4), or 'communication DNA' (24) or a selected small group of unique nuclear DNAs later found associated with cytoplasmic membranes (13), this cytoplasmic subset of DNA sequences would be expected to contain only a portion of the total number of nucleotide sequences found in the nuclear genome. If the concept of an informational role for this DNA is to be meaningful, this cannot be a random subset, but would be related to the differentiation of the tissue in question. Therefore the cytoplasmic DNA from foetal liver cells would either contain globin genes (the most likely postulate, since globin mRNA is the RNA sequence synthesised at

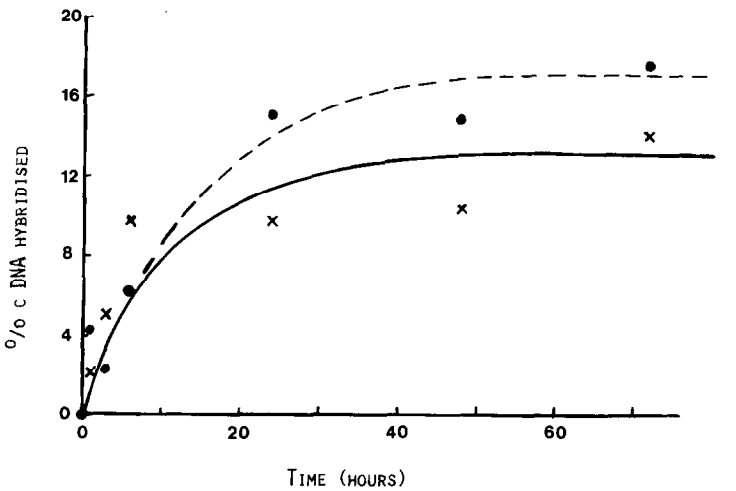

Figure 1

Rate of hybridisation of excess mouse globin cDNA to total nuclear (——— x ———) and 'cytoplasmic' (---- o ----) DNA. 50° µg of each DNA in 100 µl 0.12M sodium phosphate buffer, pH 6.8, was mixed with 5,000 counts/min mouse globin cDNA (10,000 cpm/ng) added in 1 µl. The mixture was divided into 10 capilliaries each containing 10 µl solution. These were placed at 100°C for 10 minutes and then incubated at 60°C for the indicated times, after which the capilliary was removed and the contents washed out with 250 µl nuclease assay buffer (22). 100 µl S1 nuclease was added and the solution incubated for 150 min at 37°C. 50 µl was removed for determination of total counts/min in the reaction mix. The remaining 300 µl was precipitated with carrier bovine serum albumen and perchloroacetic acid and counted. The percentage of the total counts added which are S1 nuclease resistant was determined for each time point. Zero time points and 72 hour time points were determined in duplicate and triplicate respectively. The total sequence complexity of the plus strand of DNA hybridising to cDNA is approximately 320,000 (21). As the molecular weight of the cDNA used is approximately 140,000, this corresponds to between 2-3 globin gene sequences/haploid genome.

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maximum rate and for longest time in this tissue) or not contain globin genes.

Both of the cDNA excess hybridisation techniques used for these experiments gave equal or nearly equal values for the number of globin genes hybridising per hapolid mouse genome. The values obtained with hydroxylapatite are higher than those where the hybrids are treated with S1 nuclease, as previously reported by Bishop & Freeman (21). There are many possible explanations for this difference, which are not relevant to these data. For assays using hydroxylapatite, there is a slight excess of globin

genes in nuclear over 'cytoplasmic' DNA. This is reversed for assays using S1-nuclease. In neither case is the difference greater than 25%, very much less than expected for a selected population of genes.

Since our 'cytoplasmic' DNA contains approximately the same proportion of globin genes as nuclear DNA, although it consists of only 5-10% of the total DNA, we conclude that it is a random set of genes not only overall but also with regard to a functionally active coding gene relevant to the differentiation of the tissue in question. Using hybridisation techniques similar to those employed here, Bishop & Freeman (21) have concluded that cytoplasmic DNA from duck erythrocytes contains no globin gene sequences over the proportion expected for nuclear DNA. S. Modak (Lusanne) has also reached the same conclusion for chick reticulocytes (personal communication).

It is, of course, quite impossible to state with certainty that our preparation of 'cytoplasmic' DNA is identical to that isolated by others. However, it must at least contain the cytoplasmic DNA isolated by others, even if other sequences are present as well. An examination of the size classes of cytoplasmic DNA or deoxynucleoprotein obtained by others shows at times a similarity to the regular arrays seen in our preparations (e.g. Figure 1 of Bell et al., (25); Figure 1 of Koch (11). However, in the absence of gel electrophoresis profiles of their materials, no definite conclusions can be made on this point.

It has recently been demonstrated that chromatin contains a subunit structure with 'nodal' deoxyribonuclease sensitivity (26-29), giving rise to fragments remarkably similar to those previously demonstrated by us (5).

A similar band profile is also seen after treatment of mouse satellite DNA with HinD restriction enzymes (30); the significance of this resemblance is not clear.

When studying a very small proportion of the total cellular DNA, it is possible for cells in division or (particularly in primary cultures) terminal differentiating cells to contribute what appears to be a

'cytoplasmic' deoxyribonucleoprotein composed of cleaved fragments of chromatin. We would interpret our 'cytoplasmic' DNA as arising from this source, as we previously suggested (14).

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