THE HETEROGENEITY OF MITOCHONDRIAL DNA IN DIFFERENT TISSUES FROM THE SAME ANIMAL

J. L. COOTE, Gy. SZABADOS* and T. S. WORK
National Institute for Medical Research, Mill Hill, London NW7 1AA, England

Received 18 December 1978

1. Introduction

In animal cells, mitochondrial DNA is a circular supercoiled molecule of about 16 000 base pairs (mol. wt ~1 × 10^7), but current evidence indicates that this DNA codes for only 9 peptides of the respiratory enzyme complex of the mitochondrion [1–3].

In order to maintain the capacity of the mitochondrion for protein synthesis, the nucleus of the cell must, therefore, supply information to code for the other proteins of the respiratory enzyme complex. The nucleus must also code for all the proteins of the mitoribosomes (~80), all the polymerases required for replication and transcription of mitochondrial DNA and, in addition, the amino acid activating enzymes and the initiation and elongation factors required for the maintenance of protein synthesis by the mitoribosomes.

The allocation of well over 100 nuclear genes to the maintenance of the synthesis of only 9 mitochondrial peptides seems a surprisingly inefficient way of assembling the mitochondrial respiratory complex and this investigation seeks to answer the question: can mitochondrial DNA have some unrecognised function which renders it essential that this DNA should remain in the cytoplasm?

It was established [2] that mitochondrial DNA from liver and heart would cross-hybridize to ~90%, but hybridization is a relatively crude method of assessing DNA sequence identity, moreover mitochondrial DNA has been shown [4,5] to vary between successive animals of the same species, so that hybridization studies made with DNA from pooled tissues of several animals have little significance beyond demonstrating a general similarity in structure.

In this investigation we have carefully purified the DNA from liver and from brain synaptosome mitochondria prepared from a single animal (the ox) and have used restriction endonucleases to detect whether these two types of DNA have identical or differing base sequence. Our results, obtained from 5 successive animals, showed, in confirmation of earlier work, that there were sequence differences between different animals. We also found, however, significant differences between mitochondrial DNA derived from liver and brain cells of any one animal.

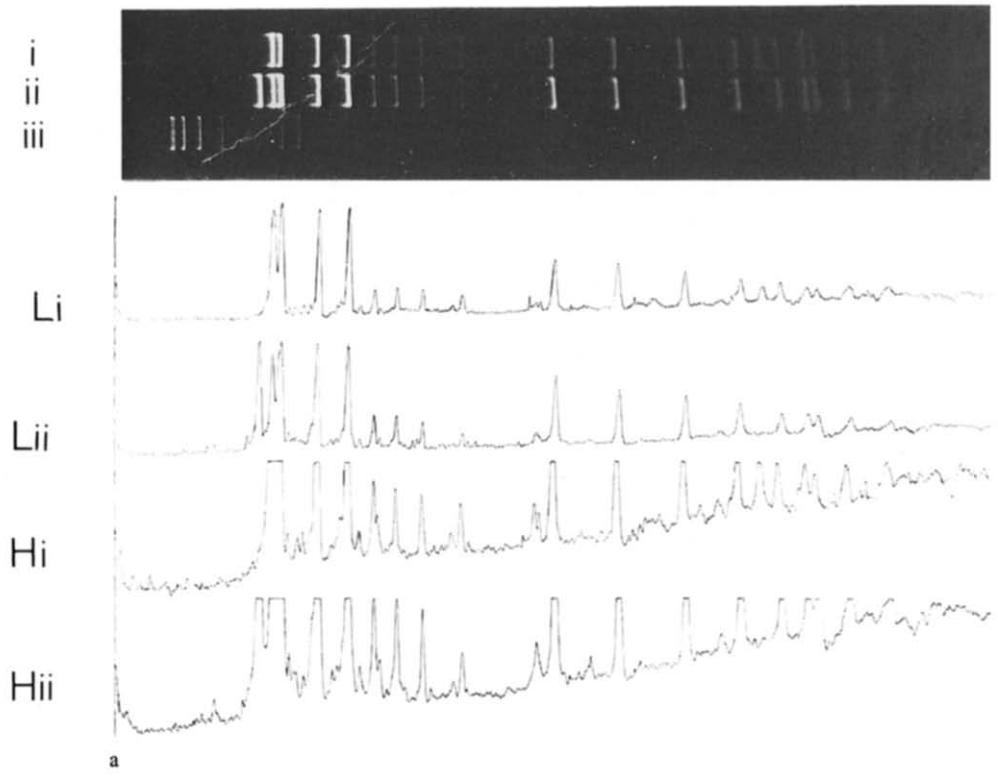
2. Experimental

Mitochondria were isolated from ox liver by standard techniques [6]. Synaptosome mitochondria were prepared from ox-brain cortex following the method in [7].

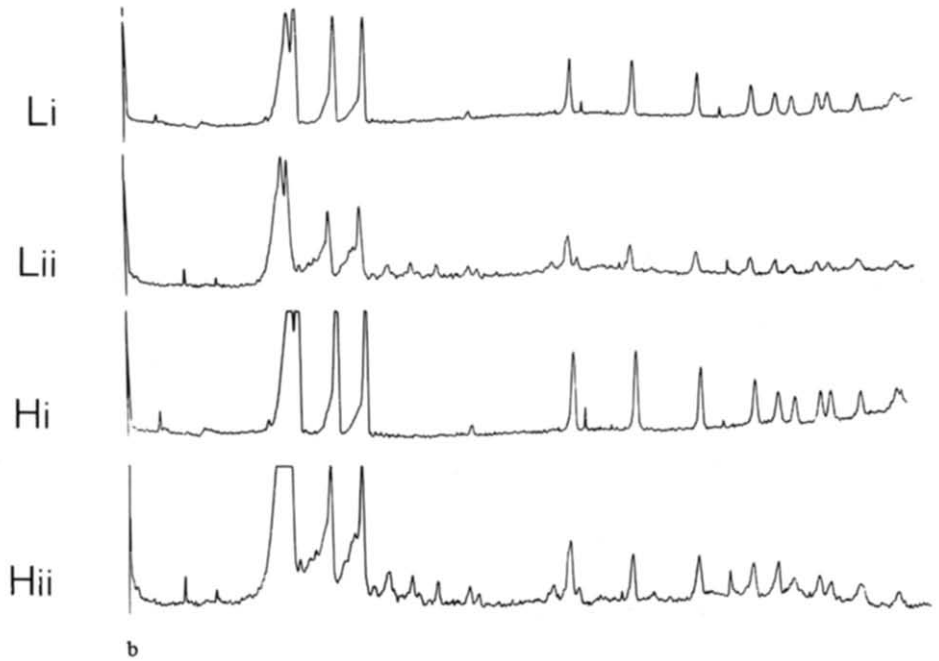
DNA was separated from the mitochondria by treatment with SDS–phenol and the mitochondrial DNA was purified by 3 successive equilibrium sedimentations in CsCl–ethidium bromide [8]. Each preparation of purified DNA gave a single symmetrical peak when examined in the analytical ultracentrifuge in CsCl without ethidium bromide.

The liver and brain DNA were initially digested

* Present address: Gy. Szabados, 2nd Institute of Biochemistry, Semmelweis University of Medicine, 1088 Budapest, Puskin u. 9, Hungary



a



b

with 3 endonucleases, *Escherichia coli* R1, *Hin*dIII and *Bam*H1. In addition, individual bands were isolated from agar gels (after a single endonuclease digestion) each redigested with a second endonuclease. The results (not shown) indicated that there was close similarity between the two types of DNA and that each contained ~16 000 base pairs. Since, however, each endonuclease digest explores only a small number of base sequences, the overall similarity between brain and liver mitochondrial DNA implies similarity of sequence but not necessarily identical base sequence. In seeking a clearer demonstration of any differences between brain and liver mitochondrial DNA another endonuclease, *Hpa*II, was tried. Single animal brain and liver DNA preparations were compared using material from several oxen in turn. The *Hpa*II patterns differed slightly from one animal to another but in each case the brain and liver from a single animal were significantly different. The results from two successive animals are shown in fig.1 and these demonstrate beyond doubt that the sequence CCGG does not occur in identical positions in brain and liver mitochondrial DNA from the same animal. This result cannot be ascribed to incomplete digestion since an equal amount of DNA treated with 3-times as much *Hpa*II added in 3 successive equal aliquots at 2 h/intervals and continuing the incubation for a further 16 h, produced the same bands in the same relative amounts.

Provided a DNA sample contains only one unique sequence, a complete digest with excess of an endonuclease, which produces a small number of chain scissions, should result in an equimolar yield of each fragment. This can be checked by measuring the fluorescence intensity of each band complexed with ethidium bromide by photographing the gel in ultraviolet light and scanning the resulting negative with a Quickscan laser densitometer. In a plot of fluorescence intensity against molecular weight the points should lie on a smooth curve. An illustration of this is provided in fig.2. The results shown in fig.1,3 indicate that, in both liver and brain, digestion with *Hpa*II gave more than one set of bands after gradient polyacrylamide gel electrophoresis (modification of method in [9]). One set of bands was 3–4-times the intensity of another set, and there were a number of minor bands of still lower intensity. In liver,

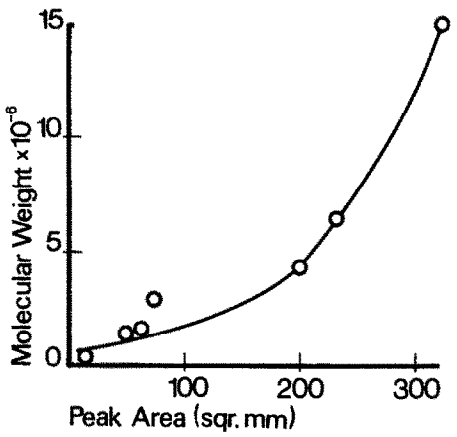


Fig.2. A plot of laser densitometer peak area against molecular weight for λ-phage/*Hin*dIII fragments separated by polyacrylamide gel electrophoresis, stained with ethidium bromide, and photographed in ultraviolet light.

Fig.1a. Mitochondrial DNA derived from brain synaptosomes and from liver of the same ox (9 μg each) were digested for 16 h at 37°C with an excess of restriction endonuclease *Hpa*II. The digests were shaken with chloroform : isoamyl alcohol (25:1) for 2 h. The aqueous phases were removed and precipitated with ethanol at −20°C. The precipitated DNA was dried in vacuo. Electrophoresis was carried out on a 20 cm long polyacrylamide gel arranged so that the top one-third consisted of 3% polyacrylamide and the bottom two-thirds had a concentration gradient from 3–7.5% polyacrylamide. λ-phage DNA digested with *Hin*dIII was used to provide molecular weight markers. Electrophoresis was for 17 h at 90 V and 40 mA. The gel was stained with ethidium bromide for 2 h, then photographed in ultraviolet light and the negative was scanned at both low (L) and high (H) gain using a Quickscan laser beam densitometer. (i) Liver mitochondrial DNA; (ii) synaptic mitochondrial DNA; (iii) λ/*Hin*dIII digest. The distances of the λ-phage/*Hin*dIII fragments from the origin (on the Quickscan chart) were plotted against their known molecular weights [13] on log–log paper. This graph was then used to calculate the molecular weights of the mitochondrial DNA/*Hpa*II fragments. The area of each peak was determined by counting squares and these results are presented in table 1.

Fig.1b shows a similar scan of mitochondrial DNA from a second animal. It is apparent that the two animals do differ but nevertheless in fig.1b the brain mitochondrial DNA again differs substantially from the liver mitochondrial DNA. Both types of DNA were purified by the same method, digested by the same batch of endonuclease and fractionated on the same gel.

such a result could be caused by contamination of parenchymal cells by other cells, but since synaptic mitochondria show the same type of 'contamination' we do not favour this as a likely explanation of the results. Alternatively, we must assume that parenchymal cell and synaptic mitochondria are heterogeneous with respect to DNA even when derived from a single animal. The results reported here imply that in fully differentiated cells of one animal (ox) the base sequence is characteristic both of the individual and of the cell type.

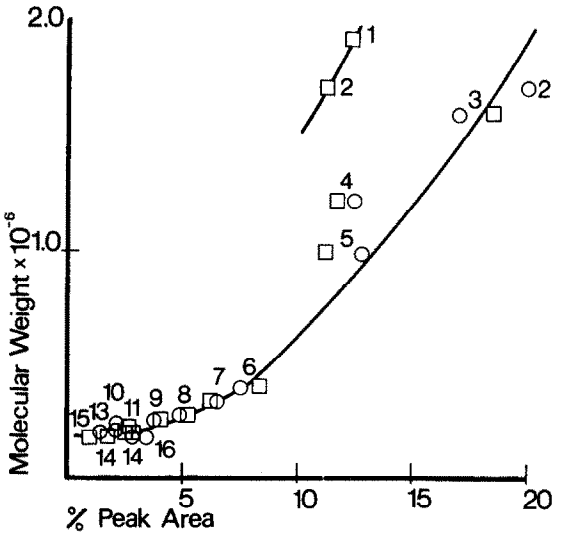


Fig.3. The relationship between the molecular weights of the DNA fragments listed in table 1 and the areas of their laser densitometer peaks. The areas are expressed as % of the sum of the areas of all the main bands. (○) Liver mitochondrial DNA/*Hpa*II. (□) Brain (synaptic) mitochondrial DNA/*Hpa*II. The continuous lines link fragments of equal molarity (c.f., fig.2). By measuring intercepts of the curves on a line parallel with the abscissa, it was calculated that in brain fragments 1 and 2 were present in only 60% of the DNA molecules, whereas in liver fragment 1 was absent and band 2 was present at >100%. From this we suggest that, in liver, fragment 1 has been cleaved into a fragment 2 [1] plus fragment 10 (which is absent from this synaptic DNA). Further, the results would indicate that one component, comprising 60% of the mitochondrial DNA, is similar (as regards *Hpa*II cleavage) in both tissues except for one CCGG sequence which is present in liver but absent from brain. Fragment 4 is present in 80% of the DNA molecules from both liver and brain and fragment 5 in 85% of brain but 100% of liver. The presence of a total of 18 brain and 15 liver minor bands of varying intensities, with total mol. wt 18.1×10^6 and 12.8×10^6, respectively would suggest that the 40% of the DNA molecules, which do not yield fragments 1 and 2, is made up of a number of components which differ in the regions occupied by fragments 1, 2, 4 and 5. This was generally true of the *Hpa*II digests of DNA from a number of individual oxen. Gels loaded with 3 μg DNA/slot showed only main component bands, and this may explain why only one type of mitochondrial DNA in any single animal was reported [13].

3. Discussion

The biological significance of our results is difficult to assess. While we are confident that in the several animals examined there were differences between the mitochondrial DNA samples from liver and brain this is not an absolute assurance that the genetic materials of the liver and brain mitochondria differ. The purification method used should segregate the fully supercoiled DNA from the replicative intermediates and from other contaminating DNA, but in all probability cloning and full sequencing of mitochondrial DNA will be necessary before a final answer can be obtained.

So far as they go, our results imply that during differentiation the mitochondria may respond to the change in their environment by altering their own genome. It is known that different cell types within the same animal differ considerably in their respiratory (mitochondrial) enzyme ratios [11], and it seems possible to us that if the mitochondrial genome played a role in differentiation then the biological advantage of the maintenance of cytoplasmic DNA at the expense of some 100 nuclear genes would become more intelligible.

Some investigations other than our own have already indicated that the mitochondrial genome may alter during differentiation. Thus, hybrid mouse cells produced by fusion of chloramphenicol (CAP)-resistant and CAP-sensitive cells of the same tissue type (homologous) were found [10] stably resistant to CAP, whereas fusion of CAP-R and CAP-S cells of different tissue origin (heterologous) produced progeny lines which rapidly lost CAP resistance. Since CAP resistance is carried by the mitochondrial genome, these results are consistent with the assumption that there are differences between the cytoplasmic genes of CAP-resistant cells of different tissue origin.

We cannot exclude at present the possibility that

the observed difference has no genetic significance and that the base sequence of mitochondrial DNA is even less defined than is implied by the results in [4,5] but we think this unlikely in view of the results in [10]. It would seem, therefore, that detailed comparison of mitochondrial DNA sequence versus cell type requires further investigation.

References

[1] Ashwell, M. and Work, T. S. (1970) Ann. Rev. Biochem. 39, 251–282.
[2] Borst, P. (1972) Ann. Rev. Biochem. 41, 334–366.
[3] Schatz, G. and Mason, T. L. (1974) Ann. Rev. Biochem. 43, 51–87.

Table 1
Ox mitochondrial DNA/*Hpa*II fragments

	Synaptic			Liver		
Band	Molecular weight	Peak area (mm^2)	% Total peak area	Molecular weight	Peak area (mm^2)	% Total peak area
1	1.92×10^6	319	12.4	Absent	–	–
2	1.71×10^6	288	11.2	1.71×10^6	422	20.0
3	1.60×10^6	476	18.5	1.60×10^6	358	17.0
4	1.22×10^6	303	11.7	1.22×10^6	261	12.4
5	0.99×10^6	291	11.2	0.99×10^6	271	12.8
6	0.40×10^6	218	8.4	0.40×10^6	159	7.6
7	0.34×10^6	163	6.3	0.34×10^6	136	6 5
8	0.28×10^6	137	5.3	0.28×10^6	103	4.9
9	0.25×10^6	105	4.1	0.28×10^6	79	3.8
10	Absent	–	–	$0 23 \times 10^6$	45	2.1
11	0.22×10^6	70	2.7	0.22×10^6	58	2.7
12	0.21×10^6	67	2.6	0.21×10^6	45	2.1
13	0.20×10^6	74	2.9	0.20×10^6	32	1.5
14	0.19×10^6	44	1.7	0.19×10^6	61	2.9
15	0.18×10^6	26	1.0	0.18×10^6	73	3.5
Minor bands						
a	3.35×10^6			Absent		
b	1.51×10^6			1.51×10^6		
c	1.45×10^6			Absent		
d	1.44×10^6		d [1]	1.42×10^6		
e	1.34×10^6			1.34×10^6		
f	1.11×10^6			1.11×10^6		
g	1.06×10^6			1.06×10^6		
h	0.93×10^6			0.93×10^6		
i	0.86×10^6			0.86×10^6		
j	0.84×10^6			0.84×10^6		
k	0.77×10^6			0.77×10^6		
l	0.68×10^6			0.68×10^6		
m	0.65×10^6			Absent		
n	0.59×10^6			0.59×10^6		
o	0.57×10^6			0.57×10^6		
p	0.44×10^6			0.44×10^6		
q	Absent			0.43×10^6		
r	0.37×10^6			Absent		
s	0.26×10^6			0.26×10^6		

Sum of major fragment molecular weights: Synaptic, 9.71×10^6; liver (treating band 2 as two fragments of the same mol. wt) 9.73×10^6

[4] Potter, S. S., Newbold, J. E., Hutchinson, III and Edgell, M. H. (1975) Proc. Natl. Acad. Sci. USA 72, 4496–4500.
[5] Francisco, J. F. and Simpson, M. V. (1977) FEBS Lett. 79, 291–294.
[6] Coote, J. L. and Work, T. S. (1971) Eur. J. Biochem. 23, 564–574.
[7] Lai, J. C. K. and Clark, J. B. (1976) Biochem. J. 154, 423–432.
[8] Bauer, W. and Vinograd, J. (1968) J. Mol. Biol. 33, 141–171.
[9] Jeppesen, P. G. N. (1974) Analyt. Biochem. 58, 195–207.
[10] Bunn, C. L., Wallace, D. C. and Eisenstadt, J. M. (1977) Somat. Cell Genet. 3, 71–92.
[11] Williams, J. N. (1968) Biochim. Biophys. Acta 162, 175–181.
[12] Murray, K. and Murray, N. E. (1975) J. Mol. Biol. 98, 551–564.
[13] Kroon, A. M., deVos, W. M. and Bakker, H. (1978) Biochim. Biophys. Acta 519, 269–273.