THE NUCLEOTIDE COMPOSITION AND PYRIMIDINE CLUSTERS IN DNA FROM BEEF HEART MITOCHONDRIA

B.F. VANYUSHIN and M.D. KIRNOS

Laboratory of Bioorganic Chemistry, Lomonosov State University, Moscow, 117234, USSR

Received 3 October 1973 *

1. Introduction

Study of primary structure of mitochondrial DNA (mtDNA) helps elucidate the general problem of DNA specificity and the numerous questions pertaining to the origin, evolution and functions of this nucleic acid. What we know so far about the composition of mtDNA have been derived from ρ and T_m determinations [1]. We have very scanty knowledge about the presence, nature and distribution pattern of minor bases in mtDNA. We have detected a small DNA-methylase activity in a mitochondrial fraction from loach embryos. It was an indication that animal mtDNA may in principle be methylated [2].

A nearest-neighbour base sequences possess unique mtDNA from slime mould [3] and determination of pyrimidine clusters frequencies in yeast mtDNA [4] showed that their nucleotide sequences possess unique specific features. This may have aroused interest to studying mtDNA; however, no such information has come to our notice, especially with respect to animal mtDNA.

This paper reports the results of a comparative study of the nucleotide composition (GC content), of the methylation character and the degree of pyrimidine clustering in DNA from beef heart mitochondria and nuclei.

2. Methods

Beef hearts were cooled in ice and homogenized in a high speed Waring-type blendor in a medium described by Borst et al. [5]. The mitochondrial fraction was isolated from the homogenate by differential centrifugation and sucrose gradient centrifugation [5]. The mitochondria were then treated with pancreatic DNA-ase (200 μg per ml) for 30 min at 30°C in the same medium containing 0.005 M $MgCl_2$ and washed at 0°C with the isolation medium supplemented with 0.01 M EDTA and then with the same medium without EDTA.

The nuclei were isolated in the same medium supplemented with 0.05 M $MgCl_2$ and 2% Triton X-100 (Schuchardt, Munich) by centrifuging the homogenate twice at 1200 *g* in a step-wise sucrose gradient (0.5, 0.7 and 1.2 M).

The mitochondria obtained were suspended in a standard saline solution (0.15 M NaCl + 0.015 M sodium citrate) containing 0.2 M EDTA, 2% sodium dodecyl sulphate (Serva) and 0.5% sodium deoxycholate (Spofa) at room temperature, pH 8.0.

5 M sodium chloride was added to the cooled mitochondrial lysate to a final concentration of 1 M; after the mixture was deproteinized by chloroform treatment, mtDNA was precipitated from the aqueous phase by adding 2.5 volumes of 96% ethanol. DNA from the nuclei was isolated as described by Marmur [6].The resulting mtDNA preparations were examined in Hitachi HS-7 electron microscope, as was done in [7].

To remove the RNA admixture, mtDNA and nuclear DNA (nDNA) were treated with 0.5 M NaOH (30°C, 18 hr), DNA was precipitated with perchloric acid in cold (0°C; $HClO_4$ final concentration: 1%, treatment with the acid, not longer than 20 min).

To determine the nucleotide composition, the DNA preparations were hydrolysed to bases (57% $HClO_4$, 100°C, 60 min); the bases were separated by thin-layer

* Delayed due to a technical fault.

chromatography [8, 9] and determined spectrophotometrically [10].

To estimate the frequencies of the different pyrimidine clusters, DNA was hydrolysed to pyrimidine sequences by the procedure of Burton [11]; the pyrimidine isopliths were separated and determined as previously [12]. The isolated isopliths were hydrolyzed to bases and after the base separation the content of 5-methylcytosine (MC) in each kind of isopliths was determined spectrophotometrically.

3. Results and discussion

An electron microscopy study has shown that our preparations of mtDNA from beef heart contain about 70% circular molecules of an average contour length of 5.45 μ (fig. 1). The shape and the length of the molecules are characteristic of animal mtDNA [1]. This means that the preparations we worked with are quite representative and contain no nDNA admixtures worth taking into consideration.

mtDNA does not differ from beef heart nDNA with respect to the GC content (table 1). This agrees with the known data that ρ and T_m values for the mtDNA of beef and other animals are similar to respective nDNA [1].

An analysis of pyrimidine isoplith frequencies has shown that mtDNA and nDNA have different nucleotide sequences (table 2). In mtDNA the quantity of monopyrimidine isopliths is almost twice as high as that in nDNA. In nDNA the content of mono- and dipyrimidine fragments amounts to 21% of the total DNA nucleotides. This is typical of nDNA of many eukaryotic organisms, especially mammals (the content of nucleotide material in mono- and dipyrimidine fragments in this DNA does not exceed 25%). In mtDNA the quantity of nucleotide material in these pyrimidine fragments is more than 33% (table 2). By this mtDNA may be distinguished not only from beef heart nDNA, but from any other total (nuclear) DNA of vertebrates studied so far [13]. As to long pyrimidine sequences ($Py_n, n \geq 4$), their frequency in mtDNA is 2–3 times lower than in nDNA (table 2). This means that mtDNA has a lower degree of pyrimidine clustering than nDNA. This feature of the primary structure makes mtDNA similar to DNA of prokaryotes (bacteria) [13].

The beef heart mtDNA is similar to the yeast mtDNA studied by the clustering degree of pyrimidines [4]. In this respect yeast mtDNA [4] also differs strongly from yeast nDNA [14, 15] and is similar to DNA of prokaryotes. Thus, in spite of the strong differences in GC content, mtDNA of one of the primitive eukaryotes (yeast) and mammals are very similar. This may be interpreted as meaning that nucleotide sequences in mtDNA in eukaryotes have some features in common and also that their mitochondria may have originated from similar sources.

MC was the minor base detected in beef heart mtDNA. MC was isolated from mtDNA hydrolysates by thin-layer chromatography and purified by rechromatography in different solvents [16]. The minor base isolated from the mtDNA has the following optical properties: (in 0.1 N HCl) λ_{max} 283 nm, λ_{min} 242 nm, A_{250}/A_{260} 0.56, A_{270}/A_{260} 1.72, A_{280}/A_{260} 235nm, A_{290}/A_{260} 2.17.

The optical properties and the chromatographic behaviour of this base allowed it to be identified as 5-methylcytosine. So, like all nuclear (total) animal DNA [10, 16], beef heart mtDNA contains MC. No other minor bases (e.g. N^6-methyladenine) were detected in beef heart mtDNA. It is noteworthy that beef heart mtDNA contains twice as much MC as nDNA. This should be taken to mean that the relative amount of methylated sequences in mtDNA is much higher than in nDNA. Such a high level of methylation may be a distinguishing feature of animal mtDNA. According to our data, in mtDNA from rat liver the MC content is also higher (by 30%) than in nDNA from the same cells and is 1.3 mole%. By the level of methylation mtDNA from beef heart seems to be unique among all animal DNA studied. Therefore in beef heart mtDNA MC is in a sense no usual minor base: its amount is quite appreciable and commensurable with that in higher plant DNA, where it is known to be very high [17]. This makes beef heart mtDNA distinctly different from all bacterial DNA, in which MC is present in very little quantities if at all [18].

Now that the presence of MC in mtDNA has been unequivocally proved, the question arises what is the specificity of DNA methylation in mitochondria. One wonders further on if DNA methylation in mitochondria and nuclei has a different specificity, if these DNAs are methylated by the same enzymes or the mito-

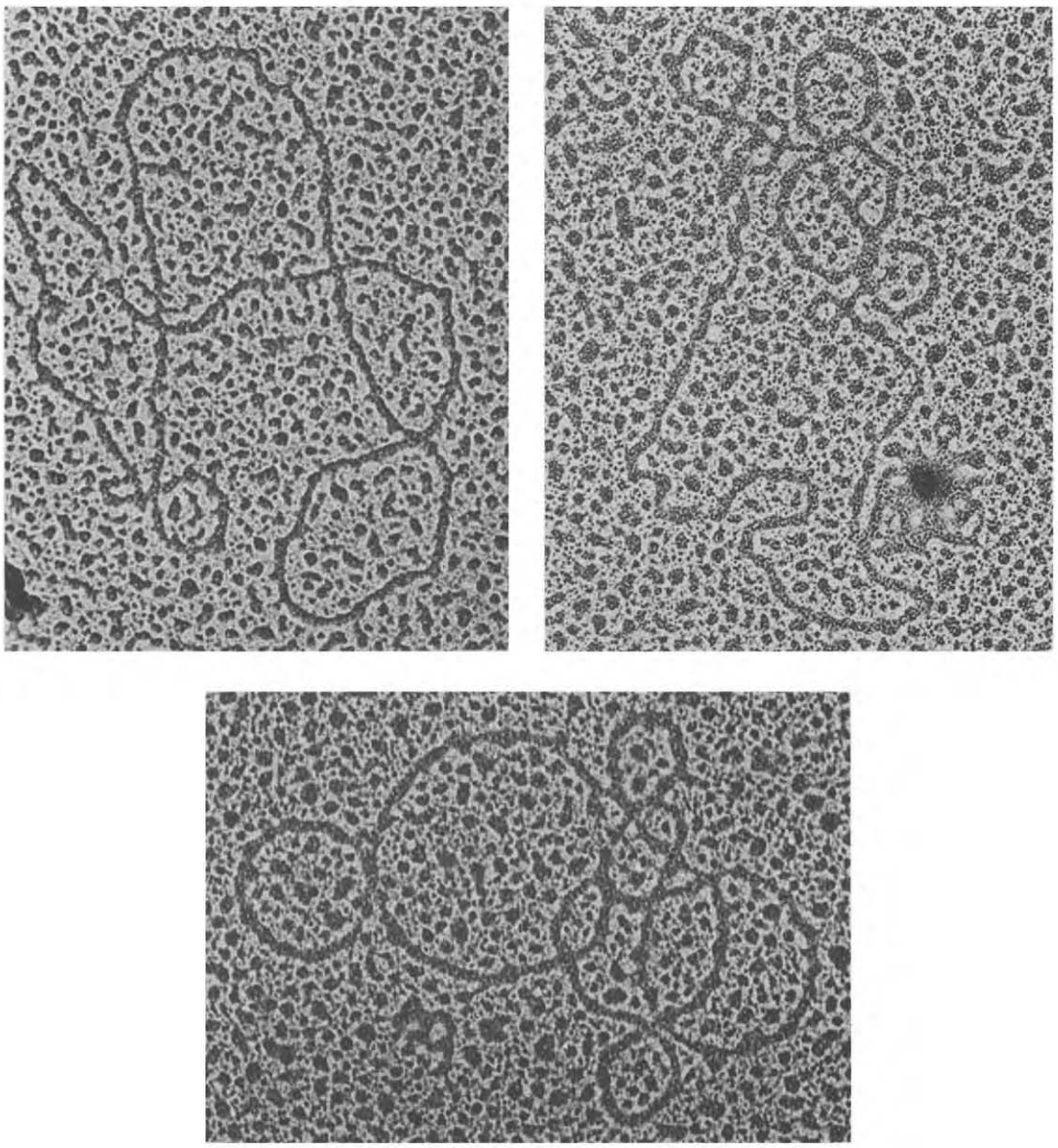

Fig. 1. Electron microscopy assay of beef heart mitochondrial DNA by Kleinschmidt's procedure [7]. × 75 000.

chondrion possesses different DNA-methylase of its own.

We have compared the pattern of distribution of MC by pyrimidine isopliths, isolated from mtDNA and nDNA of beef heart (table 3). One can see that in nDNA, similarly to total of calf thymus DNA [19], the greater part of MC (60%) is localized in the monopyrimidine fraction, i.e., in the Pu–MC–Pu sequence. In mtDNA the amount of MC in this sequence is lower (46.4%) in spite of the fact that frequency of monopyrimidine isopliths in this DNA is twice as high as that in nDNA (table 2).

Thus, the pattern of distribution of MC in the DNAs studied is quite different: in nDNA MC is predominantly localized in monopyrimidine sites, whereas in mtDNA a considerable portion of MC is also to

Table 1
The nucleotide composition of mitochondrial and nuclear DNA from beef heart.

Bases, mole %	DNA preparations	
	mtDNA	nDNA
G	23.05	22.42
C	19.45	21.31
MC	3.15 ± 0.1	1.41 ± 0.02
A	26.85	27.28
T	27.50	27.58
G+C+MC	45.65 ± 0.25	45.14 ± 0.37

be found in polypyrimidine sequences. The differences in the MC content and the character of its distribution in mtDNA and nDNA may point to the fact that methylation of cytosine residues in these DNAs apparently occurs in different sequences. In other words, it should be inferred that the specificity of DNA methylation in nuclei and mitochondria may not be the same. This is supported by our data which showed that DNA-methylases from nuclei and mitochondria of rat liver modify in vitro one and the same heterologous DNA (from *Pseudomonas aeruginosa*) in a different fashion (B.F. Vanyushin and I.B. Kudryashova, unpublished). It is not excluded that in DNA of mitochondria a DNA-methylase of their own is coded for, which may have a different specificity from that of the enzyme

Table 2
The frequencies of pyrimidine isopliths in mitochondrial and nuclear DNA from beef heart (μmoles per 100 μmoles of nucleotides, X ± σ).

Isopliths	DNA preparations	
	mtDNA	nDNA
I	18.32 ± 0.50	10.69 ± 0.15
II	15.56 ± 0.18	10.32 ± 0.12
III	9.22 ± 0.37	8.25 ± 0.20
IV	3.68 ± 0.03	6.42 ± 0.11
V	1.58 ± 0.06	4.70 ± 0.23
VI	1.64 ± 0.08*	3.96 ± 0.31
VII		3.01 ± 0.12
⩾ VIII		2.65 ± 0.06

X = mean.
σ = Standard deviation

* Total amount of isopliths (Py_n, $n \geqslant 6$).

Table 3
The pattern of distribution of 5-methylcytosine by pyrimidine isopliths from mitochondrial and nuclear DNA of beef heart.

Isopliths	Content of 5-methylcytosine (percent of total MC)	
	mtDNA	nDNA
I	46.4	60.0
II	37.5	25.8
⩾ III	16.1	14.2

coded for in the nucleus and meant for modifying nDNA.

To sum up the above said, the beef heart mtDNA studied is identical to nDNA by GC content but differs from it in that it has a lower degree of pyrimidine clustering, a higher level of methylation and peculiar MC distribution pattern. A surprisingly high level of methylation of cytosine residues seems to be a distinguishing feature of animal mtDNA. A low degree of pyrimidine clustering may be a common feature of mtDNA of eukaryotes. This feature makes mtDNA similar to prokaryotic DNA, and possibly adds support to the concept of mitochondria having prokaryotic origin.

Acknowledgements

We wish to thank Miss T.I. Kheifets for translating the paper into English and Dr. V. Polyakov for help with the electron microscopy assay.

References

[1] Borst, P. (1972) Ann. Rev. Biochem. 41, 333.
[2] Vanyushin, B.F., Kiryanov, G.I., Kudryashova, I.B. and Belozersky, A.N. (1971) FEBS Letters 15, 313.
[3] Cummins, J.E., Rusch, H.P. and Evans, T. (1967) J. Mol. Biol. 23, 281.
[4] Ehrlich, S.D., Thiery, J.-P. and Bernardi, G. (1972) J. Mol. Biol. 65, 207.
[5] Borst, P., Ruttenberg, G.J.C.M. and Kroon, A.M. (1967) Biochim. Biophys. Acta 149, 140.
[6] Marmur, J. (1961) J. Mol. Biol. 3, 208.
[7] Kleinschmidt, A. and Zahn, R.K. (1959) Z. Naturforsch. 14b, 770.

[8] Vasilyev, V.K. (1971) Nauchn. Dokl. Vyssh. Shk. Biol. Nauki 9, 118.
[9] Randerath, K. (1965) Natur 205, 908.
[10] Vanyushin, B.F., Mazin, A.L., Vasilyev, V.K. and Belozersky, A.N. (1973) Biochim. Biophys. Acta 299, 397.
[11] Burton, K. and Petersen, G.B. (1960) Biochem. J. 75, 17.
[12] Masin, A.L. and Vanyushin, B.F. (1967) Biokhimiya 32, 377.
[13] Masin, A.L. and Vanyushin, B.F. (1969) Molecularnaya Biologiya 3, 846.
[14] Symons, R.H. and Ellery, B.W. (1967) Biochim. Biophys. Acta 145, 368.
[15] Guseinov, V.A., Mazin, A.L., Vanyushin, B.F. and Belozersky, A.N. (1972) Biokhimiya 37, 381.
[16] Vanyushin, B.F., Tkacheva, S.G. and Belozersky, A.N. (1970) Nature 225, 948.
[17] Vanyushin, B.F., Kadirova, D.X., Karimov, K.K. and Belozersky, A.N. (1971) Biokhimiya 36, 1251.
[18] Vanyushin, B.F., Belozersky, A.N., Kokurina, N.A. and Kadirova, D.X. (1968) Nature 218, 1066.
[19] Doscocil, J. and Sorm, F. (1962) Biochim. Biophys. Acta 55, 953.