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Nucleic acids and protein synthesis in cancer cell mitochondria. I. Nucleic acids in rat hepatoma mitochondria

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Abstract

The contents of nucleic acids in rat liver and hepatoma mitochondria and the physico-chemical properties on DNA's isolated from these mitochondria were comparatively investigated. The results are briefly summarized as follows. 1. The contents of DNA and RNA per mg protein of the hepatoma cell mitochondria were about 10 and 2 to 4 times higher than those of rat liver mitochondria, respectively. 2. The λ max. and λ min. values of DNA isolated from the hepatoma mitochondria were 257 $m\mu$ and 231 $m\mu$, respectively and those of DNA isolated from the nuclei were 259 $m\mu$ and 233 $m\mu$, respectively, in saline-citrate, pH 7.0. 3. Three fractions of mitochondrial DNA were obtained by the sucrose density gradient and these DNA fractions corresponded, probably, to about 30 S, and 20 S and 14 S DNA's. 4. There was little difference in base compositions between nuclear and mitochondrial DNA's of the hepatoma cells. 5. The degree of hybridization between the nuclear and mitochondrial DNA's of the hepatoma cells was almost the same as that between the nuclear and nuclear DNA's of the hepatoma cells, and somewhat higher than that between the nuclear DNA of rat liver and the nuclear DNA of hepatoma cells. 6. "Highly twisted" circular, "open" circular and linear forms were observed in the DNA preparations of the hepatoma mitochondria. The average values of contour lengths of rat liver and the hepatoma DNA's observed at high frequency were 5.3 μ and 4.5 μ . 7. A discussion was made on the relation between the genetic informations of mitochondrial DNA and the formation of a mitochondrion in rat liver and the hepatoma cells.

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NUCLEIC ACIDS AND PROTEIN SYNTHESIS IN CANCER CELL MITOCHONDRIA

I. NUCLEIC ACIDS IN RAT HEPATOMA MITOCHONDRIA

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Since ALTMANN¹ proposed a hypothesis in 1890 that mitochondria might be autonomous cell organelles independent of cell nuclei, it has been more recently observed that C¹⁴-labelled amino acids are incorporated into the protein in isolated mitochondrial systems and clarified that the circular DNA occurs specifically in mitochondria.

EPHRUSSI² reported in 1950 that mutagenic agents induced respiratory deficiency in yeast, which was cytoplasmic type mutation, and since then it has been proposed that there may be a genetic information in some granules of cytoplasm³.

Actual evidences on the mitochondrial DNA have been accumulated, since Chèvremont⁴ (1959) observed by autoradiography that H³-labelled-thymidine incorporated into cytoplasmic granules, regarded as mitochondria, indicating Feulgen positivity after DNase treatment of cultured cells in certain growth stage. This point has been further substantiated by the electron microscopic observation of DNA-like fibers in mitochondria⁵, and by quantitative studies of mitochondrial DNA⁶.

The physical and chemical properties of the mitochondrial DNA's isolated from *Neurospora crassa*⁷, chicken embryo⁸ and rat liver⁹ have been investigated.

In 1966, Van BRUGGEN *et al*¹⁰. observed the molecular form of mitochondrial DNA isolated from chicken liver, and successively from mouse liver¹¹, mouse fibroma¹², and sheep heart¹³ by the method of Kleinschmidt^{14,15}. The molecular form of these mitochondrial DNA's was similar to that of polyoma viral- and boar sperm-DNA's being double stranded circular form^{16,17}, and differed from that of *Neurospora* mitochondrial-DNA¹⁸ or nuclear-DNA which exhibited double stranded linear form.

It has already been reported in our preliminaries¹⁹⁻²² that the molecular form of the mitochondrial DNA isolated from the hepatoma cells is also twisted- or open-circular and linear types. However, there was heterogeneity in the distribution of the contour length of circular DNA of the hepatoma mitochondria, and the average contour length of the circular DNA observed at the highest frequency was somewhat shorter than that of liver mitochondrial DNA.

The present communication deals with the results of comparative studies made on the physical and chemical properties of the mitochondrial DNA's isolated from rat hepatoma cell and from rat liver and a discussion was made on differences in these mitochondrial DNA's as well as the relation between the genetic informations of mitochondrial DNA and the formation of the mitochondrion.

MATERIALS AND METHODS

Isolation of mitochondria : Rat liver mitochondria were prepared by the method of HOGEBOM²³. Rat ascites hepatoma (AH 130) mitochondria were isolated by the modification of the preparation methods²⁴⁻²⁶ involving protease treatment as follows. Rat ascites hepatoma (AH 130) cells were harvested one week after the transplantation of the hepatoma cells to rat (Donryu strain). The ascites fluid was centrifuged at $800 \times g$ for one minute, and the supernatant was removed and the residue was washed with saline by centrifugation, washing repeated three times, and the last centrifugation was carried out at $800 \times g$ for 5 minutes. The washed hepatoma cells were suspended in 5 volumes of cold medium A (0.25 M sucrose containing 0.1 mM EDTA and 10 mM Tris-HCl, pH 7.4). Nagarse (bacterial protease) was added to the suspension in the final concentration of 0.01 %, and incubated for 5 minutes at 0°C before homogenization. A tightly-fitted teflon pestle of the homogenizer was rotated about 1,000 rpm and the glass tube moved up and down over the pestle. The homogenate was immediately diluted with 3 volumes of medium B (medium A plus 0.05 % bovine serum albumin) and centrifuged at $800 \times g$ for 10 minutes. The supernatant was centrifuged at $6,000 \times g$ for 10 minutes and the residue suspended in a given volume of medium B. The suspension was layered on an equal volume of 0.34 M sucrose containing 10 mM Tris-HCl, pH 7.3 and centrifuged at $900 \times g$ for 10 minutes. The upper layer was transferred to a centrifuge tube with a capillary pipette, and centrifuged at $6,000 \times g$ for 10 minutes. The supernatant was removed and the residue (mitochondrial pellet) was washed four times with medium A by centrifugation at $6,000 \times g$ for 10 minutes.

The washed mitochondria were, sometimes, further purified by the method of urografin density gradient (ρ : 1.25—1.10)⁶, and for the quantitative studies of nucleic acids in mitochondria, the purified mitochondria were treated with DNase (EC 3. 1. 4. 5) and RNase (EC 2. 7. 7. 16).

Isolation of nuclei : Nuclei were isolated from rat liver and rat ascites hepatoma (AH 130) cells by the method of CHAUVEAU²⁷.

Isolation of mitochondrial DNA : Mitochondrial DNA was isolated by a modification of Marmur's method²⁸ as follows. Purified mitochondria were suspended in 20 volumes of saline, and centrifuged at $7,000 \times g$ for 10 minutes.

The mitochondria were suspended in 5 volumes of the buffer⁸ at pH 8.0, containing 1 % sodium deoxycholate, 0.15 M NaCl, and 0.1 M EDTA, and shaken for 20 minutes at 0 to 4°C (or alternatively, mitochondria were suspended in 0.1 M Tris-HCl buffer, pH 8.0, containing 1 % sodium dodecyl sulfate, and heated for 10 minutes at 60°C). Sodium perchlorate solution (5 M) was added to the mixture in the final concentration of 1 M and the mixture was then shaken for 20 minutes at 0 to 4°C with an equal volume of chloroform containing isoamyl alcohol in a volume ratio of 24 : 1 (medium C), and centrifuged for 20 minutes at 5,000 × g. The aqueous phase was repeatedly treated with equal volume of medium C until deproteinization was complete as indicated by the absence of precipitate at the interface. The aqueous phase was separated and nucleic acids were precipitated by adding 2 volumes of chilled ethanol gently and stored the mixture at -10°C overnight.

The nucleate was sedimented, redissolved in saline-citrate (0.15 M NaCl-0.015 M sodium citrate, pH 7.0), and incubated for 30 minutes at 37°C with heat treated-RNase 1 A (50 μg per ml) and -RNase T₁ (20 μg per ml). After cooling to 0°C, the mixture was shaken for 15 minutes with an equal volume of medium C at 0 to 4°C, and centrifuged for 10 minutes at 5,000 × g, and the aqueous phase was separated. The treatment with medium C was repeated until deproteinization was complete (usually this treatment was repeated 3 times). The aqueous phase was separated and the DNA was precipitated by adding 2 volumes of chilled ethanol and stored at -10°C overnight. The DNA precipitate was sedimented and redissolved in saline-citrate, and the solution was then dialyzed against saline-citrate at 4°C overnight. This DNA solution represented the total mitochondrial DNA and was usually stored in 2 volumes of ethanol at -10°C.

Density gradient centrifugation of mitochondrial DNA: Linear density gradients of 20—5 % sucrose in 0.15 M NaCl, 0.015 M sodium citrate, pH 7.5, were performed in a Beckman Spinco Model L₂ preparative ultracentrifuge with aid of a simple mixing device. The DNA solution (0.2 ml) was layered on top of the gradients. Centrifugation was carried out for 20 hours at 0°C and 24,000 rpm in the SW 39 rotor of a Spinco Model L₂ preparative ultracentrifuge. Four drops of fraction were collected after puncturing the bottom of the tube.

Isolation of nuclear DNA : Nuclear DNA was isolated and purified by the method of Marmur²⁸ or Saito and Miura²⁹.

Chemical analysis : DNA and RNA were extracted by the method of Volkin and Cohn³⁰, and deoxyribose and ribose were estimated by the method of Burton³¹ and of Brown³², respectively. Proteins were determined by the method of Lowry *et al*³³.

Base analysis of DNA : Base analysis of the DNA was carried out by the procedure of Schneider and Kuff⁹, and of WYATT³⁴ as follows. DNA's were isolated by the modification of Marmur's method and after precipitation with ethanol these were dissolved in 0.3 N NaOH and incubated for 1 hour at 37°C to hydrolyze RNA which contaminated in the DNA preparation. Then, the DNA's were precipitated in the cold with 0.6 N perchloric acid. The alkaline hydrolysis and precipitation was repeated twice more. After removing contaminated RNA from the DNA by centrifugation, the precipitate was dried *in vacuo* hydrolyzed by hydrochloric or perchloric acid. The hydrolyzate were chromatographed using the solvent of Wyatt³⁴.

Hybridization between nuclear and mitochondrial DNA : The DNA-filter technique of Gillespie and Spiegelman³⁵, as modified by Denhardt³⁶, and Du Buy *et al*³⁷. was employed to detect complementary DNA. Solutions of rat liver and rat hepatoma nuclear and mitochondrial DNA's in double-strength saline-citrate were adjusted to the final concentration of about 50 μg per ml each. The solutions were then heated to 100°C for 15 minutes and quickly chilled in ice for 10 minutes. Aliquots (1 ml) were then added to filters presoaked in double-strength saline-citrate. The filters were dried overnight in a vacuum desiccator at room temperature, then for 2 hours at 80°C in a vacuum oven. The filters, containing either nuclear or mitochondrial DNA, were incubated in separate vials, containing 1.0 ml preincubation medium, for 6 hours at 60°C. This medium contained 0.02 % each of Ficoll, polyvinylpyrrolidone, and bovine serum albumin (fraction V) in double-strength saline-citrate. The preincubation was followed by an additional 12-hour incubation at 60°C and with denatured nuclear P³²-labelled DNA, isolated from rat ascites hepatoma (AH 130) cells in the same medium. The filters were then washed in double-strength saline-citrate at 60°C, dried and counted with a Packard liquid scintillation counter.

Electron microscopy : The modified KLEINSCHMIDT technique¹⁵ of Freifelder and Kleinschmidt was used for spreading mitochondrial DNA on the monolayer of cytochrome c. The DNA was suspended at a concentration of 2 μg per ml in 1 M ammonium acetate containing 0.01 per cent cytochrome c. Formalin was added to a concentration of 0.5 per cent just prior to allowing the solution to flow down a clean glass slide onto a hypophase of 0.3 M ammonium acetate also containing 0.5 per cent formalin. DNA was picked up on carbon coated-collodion support films mounted on copper grids. Grids were shadowed on a rotary turntable. The center of the turntable was 7 cm from a tungsten basket, at an angle of 6°. About 40 mg of platinum-paradium (80 : 20) were evaporated in a tungsten basket over a period of several minutes in a vacuum of less than 10⁻⁴mm Hg.

Electron micrographs were taken with a Hitachi-11 C type, operated usually at 75 KV, at a magnification of 10,000. The photographic films of the DNA molecules were enlarged at a magnification of 5 times and traced in duplicate with a map-measuring device.

Reagents : RNase A (type I A), bovine serum albumin (Fraction V), urografin were products of Sigma Chemical Co., Armour Pharmaceutical Co. and Schering A G Berlin, respectively. RNase T₁ was the gift from Egami Laboratory, Department of Biophysics and Biochemistry, Tokyo University. All other reagents used were of analytical grade.

Abbreviations : EDTA, ethylenediaminetetraacetate ; Tris, tris (hydroxymethyl) amino methane ; DNase, deoxyribonuclease ; RNase, ribonuclease.

RESULTS

Nucleic acid contents of rat liver and hepatoma mitochondria : Isolated mitochondria were further purified by the urografin density gradient and by the treatment of DNase or RNase to remove the contamination of nuclear DNA or cytoplasmic RNA.

Table 1. Concentration of DNA and RNA in rat liver and ascites hepatoma (AH 130) cells

Mitochondria	DNA ($\mu\text{g}/\text{mg}$ protein)		RNA ($\mu\text{g}/\text{mg}$ protein)	
	without DNase	with DNase*	without RNase	with RNase*
Rat liver Mt.		0.5		4.8
AH 130 cell Mt.	4.7	5.3	23.7	9.2

* DNA and RNA were estimated by the method of Volkin-Cohn after DNase or RNase-treatment of mitochondria purified by urografin density gradient centrifugation.

As shown in Table 1, the DNA and RNA contents in rat liver were 0.5 μg and 4.8 μg per 1 mg protein, respectively, on the other hands, those in ascites hepatoma mitochondria, 5 μg and 9 to 24 μg per 1 mg protein, respectively. Namely, both contents of DNA and RNA in hepatoma mitochondria are considerably higher than those in rat liver mitochondria.

Absorption spectrum of nuclear and mitochondrial DNA : The maximum and minimum absorptions of rat hepatoma mitochondrial DNA dissolved in saline-citrate (pH 7.0) were 257 $\text{m}\mu$ and 231 $\text{m}\mu$, respectively, and the absorption ratios at 260 $\text{m}\mu$: 230 $\text{m}\mu$: 280 $\text{m}\mu$ were 1.00 : 0.49 : 0.51, respectively, (Fig. 1a). In the case of rat liver mitochondrial DNA, the maximum and minimum

absorptions were 258 $m\mu$ and 233 $m\mu$ respectively, and the absorption ratios at 260 $m\mu$: 230 $m\mu$: 280 $m\mu$ were 1.00 : 0.52 : 0.55, respectively (Fig. 1 b).

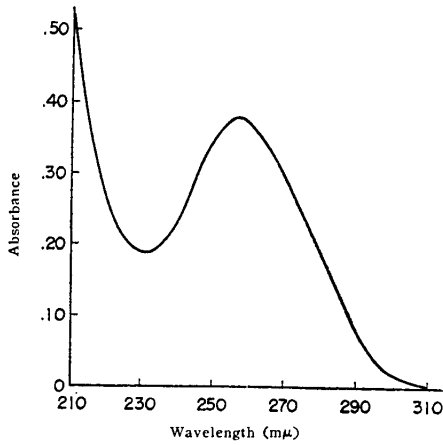


Fig. 1a Absorption spectrum of mitochondrial DNA isolated from rat hepatoma cells

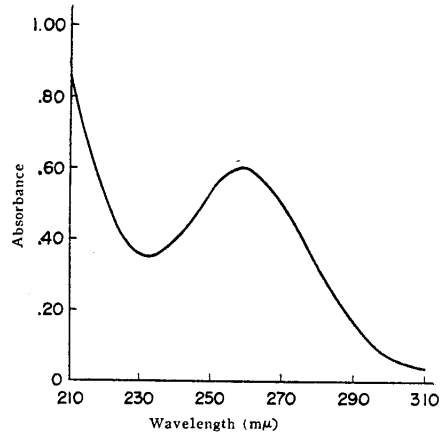


Fig. 1b Absorption spectrum of mitochondrial DNA isolated from rat liver

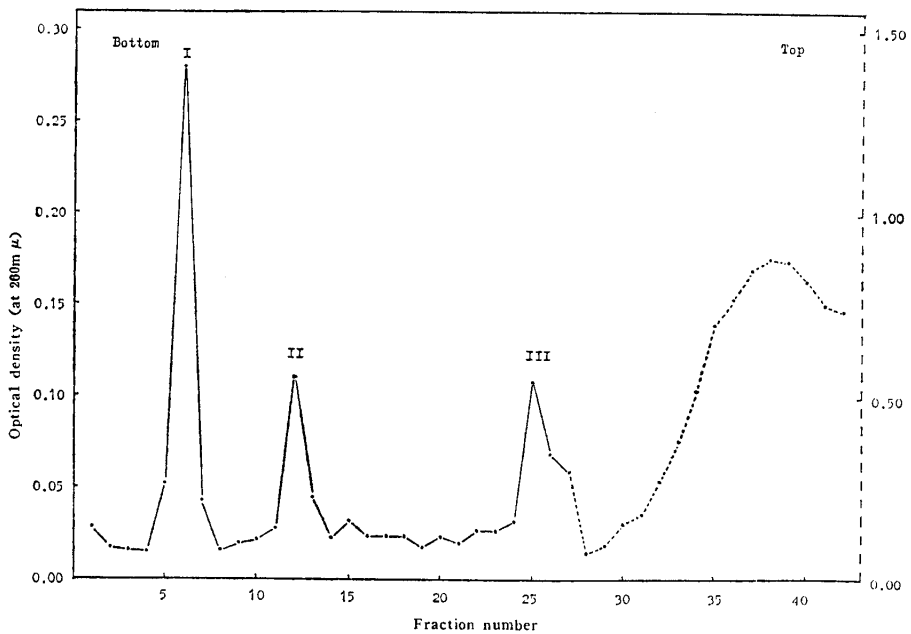


Fig. 2 Fractionation of mitochondrial DNA from ascites hepatoma (AH 130) cells on sucrose density gradient.

Condition of fractionation : Linear gradients of 20—5% sucrose in 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0. Centrifugation was carried out at 24,000 rpm for 19.5 hours at 1°C.

It was also confirmed that these mitochondrial DNA's were double stranded DNA, since the ultra-violet absorption of the mitochondrial DNA increased about 20 % with heat denaturation. The maximum and minimum absorptions of nuclear DNA of rat hepatoma (AH 130) cell were 259 $m\mu$ and 233 $m\mu$, respectively.

Sucrose density gradient of mitochondrial DNA : As shown in Fig. 2, the total mitochondrial DNA isolated from rat ascites hepatoma cells was further fractionated with 20—5 % sucrose density gradients. Three peaks were observed and each peak might correspond to about 30 $S_{20,w}$, 20 $S_{20,w}$, and 14 $S_{20,w}$ by boundary centrifugation of the hepatoma mitochondrial DNA (Table 2).

Table 2. $S_{20,w}$ values of nuclear and mitochondrial DNA from rat ascites hepatoma (AH 130) cells*

Cell fraction	DNA fraction	Value of $S_{20,w}$
Nuclei	DNA	18
Mitochondria	DNA I	~ 30
Mitochondria	DNA II	20
Mitochondria	DNA III	14

* Condition of boundary sedimentation ; 30 μ g DNA in 0.8 ml of a bulk solution containing 0.15 M sodium chloride and 0.015 M sodium citrate, at 42,040 rpm in 2°C, by a Spinco Model E analytical ultracentrifuge. Photographs were taken at each 8 minutes interval after 12 minutes run.

Base composition of rat hepatoma DNA : The result of preliminary experiments on the base composition of DNA's is shown in Table 3. G-C con-

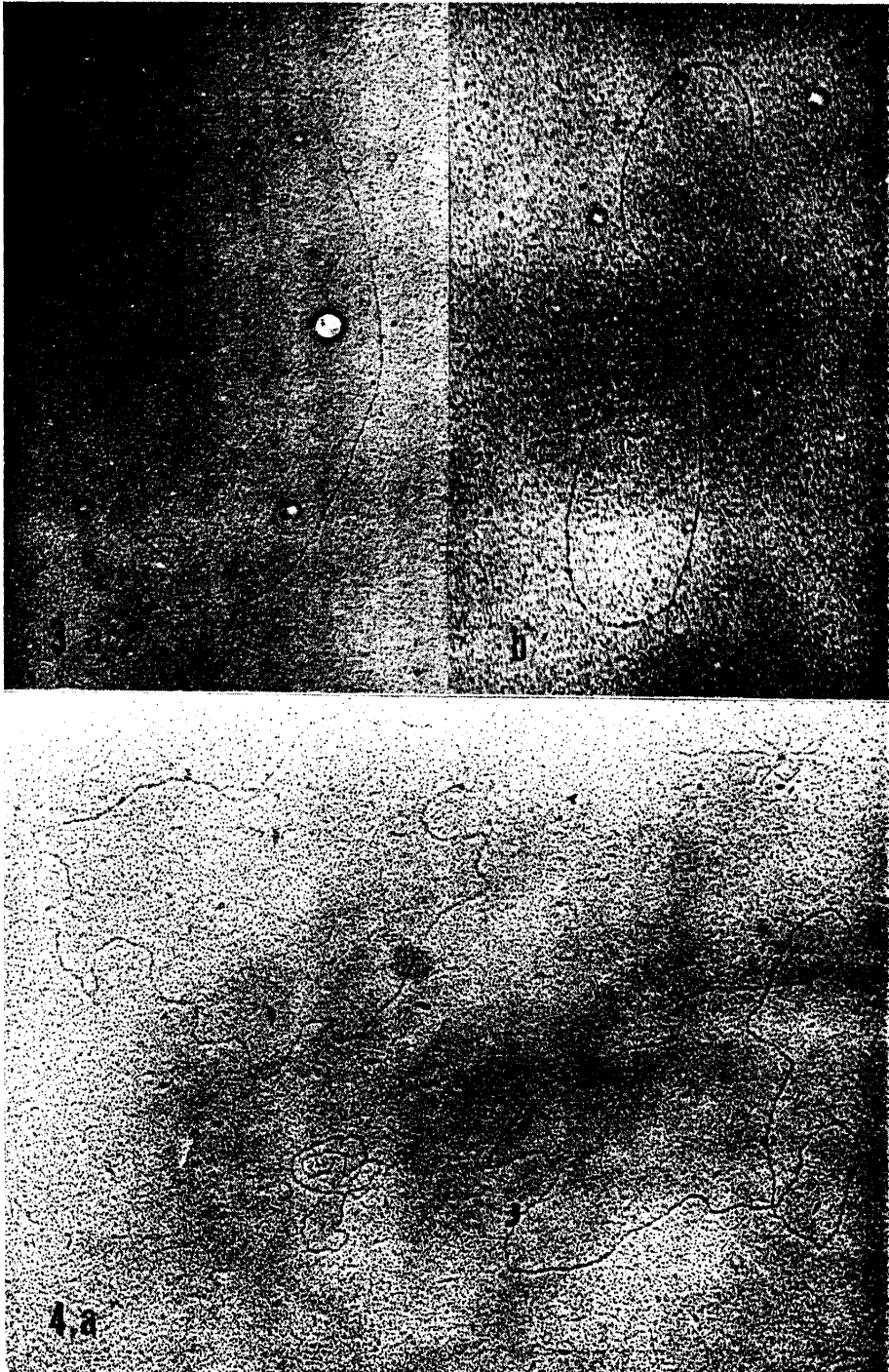
Table 3. Base composition of nuclear and mitochondrial DNA's from rat ascites hepatoma (AH 130) cells*

DNA sample	Adenine	Gunine	Cytosine	Thymine	$\frac{(A+T)}{(G+C)}$	(G+C)
Mitochondria	28.4	23.1	23.1	25.4	1.16	46.2
Nuclei	26.7	23.8	23.2	26.3	1.13	47.0

* Molar proportions of bases per 100 moles of total bases

tent of nuclear and mitochondrial DNA's which were isolated by the modification of the Marmur's method was 47.0 and 46.2 %, respectively. Therefore, there was little difference in G-C content between nuclear and mitochondrial DNA.

Hybridization of nuclear DNA to mitochondrial DNA : A result of hybridization of P^{32} -labelled nuclear DNA isolated from rat ascites hepatoma



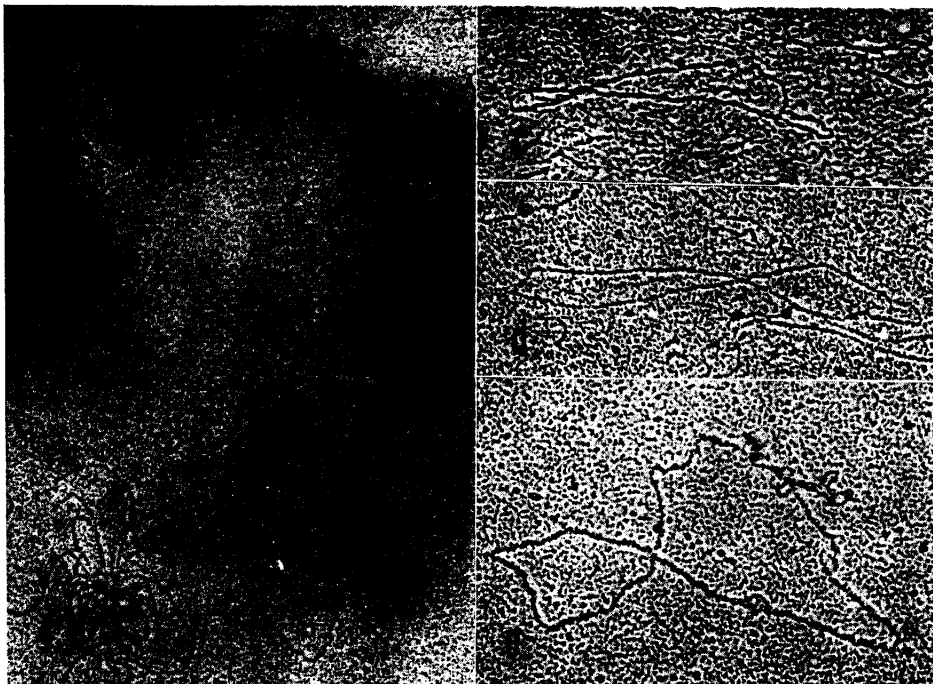


Fig. 3 Electron micrographs of circular DNA from rat liver mitochondria. Magnification :
 $\times 40,000$.

(a) A "open" circular form contour length is 5.5μ . (b) A "closed" circular form, contour length is 5.5μ .

Fig. 4 Electron micrographs of circular DNA from rat hepatoma mitochondria. Magnification :
 $\times 50,000$.

(a) "open" circular DNA's. Contour length of the DNA molecules at the left and right are 4.1μ and 4.4μ , respectively. (b) "Highly twisted" or "super coiled" form. Contour length of the DNA molecule at the left is about 4μ . (c)–(e) Various examples of small circular DNA's. Contour length of the DNA molecules in (c), (d) and (e) is 1.8μ , 2.2μ and 3.1μ , respectively.

cells to the hepatoma mitochondrial DNA (fraction 2) or to rat liver nuclear DNA is shown in Table 4. The percentage of the binding between the hepatoma nuclear DNA and the hepatoma mitochondrial DNA was somewhat higher than that between the hepatoma nuclear DNA and rat liver nuclear DNA.

Molecular form of mitochondrial DNA : The electron micrographs of monolayer preparation of mitochondrial DNA isolated from rat liver and from rat hepatoma (AH 130) cells, are shown in Figures 3 and 4. Three kinds of molecular form were observed in the mitochondrial DNA. The first molecular form was "highly twisted" circular, the second "open" circular and the third

Table 4. Hybridization of P^{32} -labelled rat ascites hepatoma (AH 130) nuclear DNA to AH-nuclear and mitochondrial DNA's, and normal rat liver nuclear DNA, immobilized on the filter membranes*.

Type of DNA on filter	Total counts recovered	Total counts on filter	Binding %
AH cell-nuclear DNA	23290	3845	16.6
AH cell-mitochondrial DNA	23234	3655	15.7
Rat liver-nuclear DNA	23747	3251	13.7
Blank	23184	700	3.0

* About 30 μg of AH-nuclear, mitochondrial and rat liver nuclear DNA per filter, incubated with 4.37 μg AH-nuclear P^{32} -labelled DNA (ca. 300 cpm per μg). All samples were counted for 20 min.

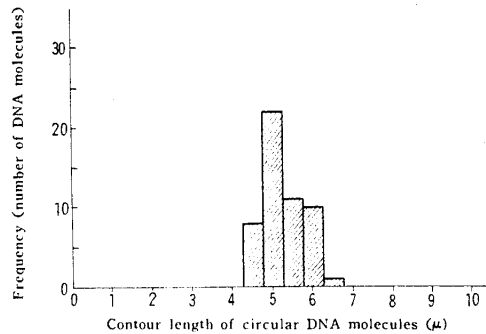


Fig. 5 A histogram of the distribution of contour lengths of circular DNA molecules isolated from rat liver mitochondria.

Total number of DNA molecules : 52

Mean value of the length : 5.32 μ (standard deviation 0.46)

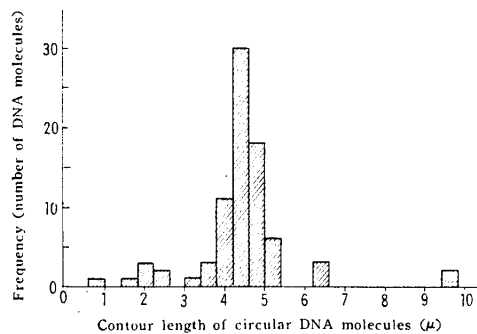


Fig. 6 A histogram of the distribution of contour lengths of circular DNA molecules isolated from rat ascites hepatoma (AH 130) mitochondria.

Total number of DNA molecules : 81

Mean value of the length of the highest frequency group : 4.46 μ (standard deviation 0.40)

linear. The histograms of the distribution of the mitochondrial circular DNA by electron microscopy are shown in Figures 5 and 6. It is obvious by these histograms that the contour lengths of the hepatoma mitochondrial DNA are widely distributed in contrast to that of the liver mitochondrial DNA distributed in a relatively narrow area (4.5—6.5 μ). The mean value of the contour length of the former DNA in the high frequency group (3.0—5.4 μ) was 4.5 μ and that of the latter DNA 5.3 μ .

Although DNA like small circular filaments (the contour length was more or less than 1 μ) were observed, further investigation is necessary to elucidate the properties of these filaments.

DISCUSSION

The amount of DNA per mg protein of rat liver mitochondria accorded well with the value reported by NASS *et al*³⁸. In the case of rat ascites hepatoma mitochondria, the amount of DNA and of RNA per mg protein was 10 times and several times higher than those of rat liver mitochondria, respectively. It is considered that the content of DNA and RNA per mitochondrion is of somewhat similar order as it was previously calculated that the number of the mitochondrion per mg protein of rat hepatoma mitochondria was 4 times higher than that of rat liver mitochondria³⁹ (Table 5). Molecular weights (daltons) of total

Table 5. DNA and RNA content per mitochondrion from rat liver and ascites hepatoma (AH 130) cells

Mitochondrion	RNA g/Mt.	DNA g/Mt.	Daltons of total DNA/Mt.	Length of a Mt. DNA molecule	M. W. of a Mt. DNA molecule	Equivalent number of DNA molecules per each Mt.
Rat liver Mt.	1.0×10^{-15}	1.0×10^{-16}	6.0×10^7	5.32 μ	10.2×10^6	6
AH 130 Mt.	5.0×10^{-16}	2.5×10^{-16}	15.0×10^7	4.46 μ	8.6×10^6	18

mitochondrial DNA of rat liver and of hepatoma cells were calculated as 6×10^7 and 15×10^7 , respectively. Assuming that mitochondrial DNA occurs as a double stranded circular DNA observed at high frequency by electron microscopy, it was calculated that mitochondrial DNA of rat liver and of the hepatoma cells were composed of 6 molecules of DNA (10.2×10^6 daltons) and 18 molecules of DNA (8.6×10^6 daltons). One molecule of mitochondrial DNA of rat liver contains about 5000 cords for amino acids by calculation from the triplet cordon theory. Assuming that average molecular weight of amino acids and of mitochondrial proteins is about 100 and 25,000, respectively, it is equivalent to about

twenty kinds of the protein informations. On the assumption that circular DNA's in mitochondrion contain double genetic informations, it is suggested that the hepatoma cell mitochondrion might lack somewhat of genetic informations in rat liver mitochondrion.

Recently, it has been reported that circular DNA is observed in DNA preparation from HeLa cells by electron microscopy by means of a dye-bouyant density⁴⁰. Although the circular DNA cannot be identified as the mitochondrial DNA, it may be similar to mitochondrial DNA judging from its molecular form.

KROON *et al*¹³. have proposed a model of molecular forms in mitochondrial DNA which was modified from a model of polyoma viral DNA⁴¹. They consider that the molecular form of mitochondrial DNA is "highly twisted" circular in the physiological state and the highly twisted circular form is altered to "open" circular and linear forms in the process of the preparation of mitochondrial DNA as a result of mechanical damages. However, considering the replication mechanism of circular DNA, it appears possible that the changes in the molecular form of mitochondrial DNA are reversible in the natural state (Fig. 7). Several

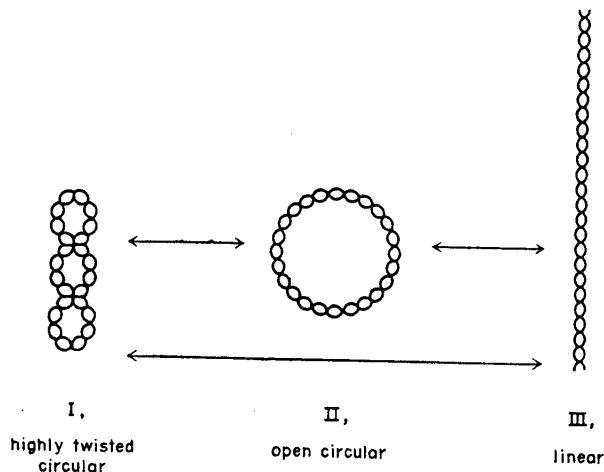


Fig. 7 Diagrammatic representation of the several forms of mitochondrial DNA from rat hepatoma cells, modified from a paper by Vinograd *et al.* (1965) on polyoma viral DNA and from a paper by Kroon *et al.* (1966) on chick liver or sheep heart mitochondrial DNA.

models for the mechanism of DNA replication have been proposed⁴²⁻⁴⁴.

Buoyant density (ρ) of nuclear and mitochondrial DNA's has been reported on the various organisms. Differences of buoyant density ($\Delta\rho$) between nuclear DNA and mitochondrial DNA in fungi^{7,45} and avian cells^{8,46} are about 0.014 and 0.009, but the difference is not so significant in the case of mammalian cells^{9,11,13}.

Guanine-cytosine contents between the nuclear and mitochondrial DNA's of rat hepatoma cells did not differ significantly as observed by the base analysis and this result coincided with the finding that the degree of hybridization between the nuclear and mitochondrial DNA's of the hepatoma cells was almost the same as that between the nuclear DNA's of the hepatoma cells.

Little is yet known of the role of mitochondrial DNA in the protein synthesis, to say nothing of the role in the formation of mitochondrion. Many investigations on the mitochondrial RNA have been recently accumulated. Namely, it is reported that mitochondria contain transfer RNA-like 4S-RNA⁴⁷, messenger RNA-like RNA⁴⁷ and ribosomal RNA-like 5S-RNA^{48,49}. It also appears possible that mitochondrial DNA replicates semiconservatively⁵⁰ and mitochondria contain DNA-and RNA-polymerases^{7,9,51,52}.

However, considering the fact that C¹⁴-amino acids are mainly incorporated into insoluble proteins^{53,54} of mitochondria, especially inner membraneous proteins⁵⁵, and the possibility that the genetic informations in a circular mitochondrial DNA might be several times shorter in comparison with the kinds of proteins by which mitochondrion may be formed, it is suggested that some genetic informations from nuclear DNA might also participate in the complete formation of a mitochondrion.

Recently, a close relation of endoplasmic reticulum to the mitochondrial formation is suggested in the process of the formation of mitochondrial sheath during a spermatogenesis by electron microscopy⁵⁶. The outer membranes of the mitochondria have been recently isolated, and it is demonstrated that NADH₂-cytochrome c reductase (EC 1. 6. 2. 1) or cytochrome b₅ is contained in the outer membrane^{57,58}. Since similar enzyme or cytochrome is also observed in the endoplasmic reticulum, this supports the view that they have an origin common with other subcellular elements.

The molecular forms of the three fractions of mitochondrial DNA by the sucrose density gradient can be deduced from the results in the polyoma viral DNA⁴¹ and in the mitochondrial DNA of normal cells¹³. However further investigations are necessary to clarify the relation between these molecular forms of mitochondrial DNA and the function in the process of replication.

SUMMARY

The contents of nucleic acids in rat liver and hepatoma mitochondria and the physico-chemical properties on DNA's isolated from these mitochondria were comparatively investigated. The results are briefly summarized as follows.

1. The contents of DNA and RNA per mg protein of the hepatoma cell mitochondria were about 10 and 2 to 4 times higher than those of rat liver

mitochondria, respectively.

2. The λ max. and λ min. values of DNA isolated from the hepatoma mitochondria were 257 m μ and 231 m μ , respectively and those of DNA isolated from the nuclei were 259 m μ and 233 m μ , respectively, in saline-citrate, pH 7.0.

3. Three fractions of mitochondrial DNA were obtained by the sucrose density gradient and these DNA fractions corresponded, probably, to about 30 S, and 20 S and 14 S DNA's.

4. There was little difference in base compositions between nuclear and mitochondrial DNA's of the hepatoma cells.

5. The degree of hybridization between the nuclear and mitochondrial DNA's of the hepatoma cells was almost the same as that between the nuclear and nuclear DNA's of the hepatoma cells, and somewhat higher than that between the nuclear DNA of rat liver and the nuclear DNA of hepatoma cells.

6. "Highly twisted" circular, "open" circular and linear forms were observed in the DNA preparations of the hepatoma mitochondria. The average values of contour lengths of rat liver and the hepatoma DNA's observed at high frequency were 5.3 μ and 4.5 μ .

7. A discussion was made on the relation between the genetic informations of mitochondrial DNA and the formation of a mitochondrion in rat liver and the hepatoma cells.

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