ISOLATION AND CHARACTERIZATION OF MITOCHONDRIAL DNA FROM *DROSOPHILA MELANOGASTER*

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ABSTRACT

Mitochondrial DNA (MtDNA) with a neutral buoyant density of 1.681 g/cm^3 has been isolated from unfertilized eggs of *Drosophila melanogaster*. This DNA is a circular molecule with an average length of 5.3 μm; it reassociates with a low $C_0t_{1/2}$ after denaturation, and in alkaline isopycnic centrifugation it separates into strands differing in density by 0.005 g/cm^3. MtDNA isolated from purified mitochondria of unfertilized eggs or from total larval DNA melts with three distinct thermal transitions. The three melting temperature values suggest that the molecule may have three regions differing in average base composition. DNA isolated from unfertilized eggs of *D. melanogaster* contains approximately equal amounts of MtDNA and another DNA with a buoyant density of 1.697 g/cm^3, slightly less dense than main peak DNA. The possibility that the heavier DNA fraction consists of amplified ribosomal DNA was excluded by hybridization experiments, but otherwise nothing is known of its origin or function.

INTRODUCTION

Mitochondrial DNA (MtDNA)[1] from several higher organisms renatures rapidly (Corneo et al., 1966; Borst and Ruttenberg, 1966; Nass, 1966; Kroon et al., 1966; Dawid and Wolstenholme, 1968), is circular (Van Bruggen et al., 1966; Sinclair and Stevens, 1966; Wolstenholme and Dawid, 1967), and shows strand separation in alkaline isopycnic centrifugation (Dawid and Wolstenholme, 1967; Leffler et al., 1970; Corneo et al., 1968). We report here the isolation of MtDNA from *Drosophila melanogaster* and its characterization by isopycnic centrifugation, thermal denaturation, and electron microscopy.

MtDNA from *D. melanogaster* is a circular molecule, renatures rapidly, and separates into two strands in alkaline CsCl. However, it has unusual thermal denaturation properties. MtDNA from *Rana pipiens*, *Xenopus laevis* (Dawid, 1965), and sheep heart (Kroon et al., 1966) shows only one transition on thermal denaturation. Leffler et al. (1970) have reported a biphasic melting curve for rat liver MtDNA and correlated the transitions with sequential denaturation of nicked and intact circles. Renatured rat liver MtDNA melted with a single transition. Wolstenholme et al. (1972) have observed the same phenomenon and, using the electron microscope, have identified at least three specific regions of rat liver MtDNA which melt 1°–2°C below the rest of the circle. We have ob-

[1] *Abbreviations used in this paper:* EBr, ethidium bromide; EDTA, ethylenediaminetetraacetate; MtDNA, mitochondrial DNA; rDNA, the DNA coding for ribosomal RNA; rRNA, ribosomal RNA; SSC, 0.15 M NaCl, 0.015 M Na-citrate, pH 7.0; T_m, melting temperature.

served three major transitions during thermal denaturation of both native and renatured MtDNA from *D. melanogaster* which appear to be due to real differences in base composition in different portions of the molecule.

MATERIALS AND METHODS

Mitochondria were prepared from unfertilized eggs of the $1(1)E12^{ts}$/ClB stock of *D. melanogaster*. This stock bears a recessive temperature-sensitive lethal mutation on the X chromosome which allows only heterozygous females to develop at the restrictive temperature. From such virgin females unfertilized eggs were collected in a large population cage. Mitochondria were purified essentially as described by Dawid (1966). All operations were carried out at 5°C. Approximately 7.7 ml frozen, unfertilized eggs (6.5×10^5 eggs) were homogenized in 6 vol of 0.25 M sucrose, 1 mM ethylenediaminetetraacetate (EDTA), 0.03 M Tris, pH 7.4. The homogenate was centrifuged three times at 480 *g* for 15 min and the pellets discarded. After the last 480 *g* centrifugation, the supernatant was removed and centrifuged for 20 min at 12,100 *g*, The resulting pellet was resuspended and again centrifuged for 20 min at 12,100 *g*. The crude mitochondrial pellet, resuspended in 1 ml homogenization medium, was layered onto linear sucrose gradients, 0.9–2.1 M, containing 0.07 M EDTA, 0.03 M Tris, pH 7.4, and was centrifuged at 22,000 rpm in the Spinco SW25.1 rotor for 75 min. Fractions were collected and assayed polarographically for succinoxidase activity (King, 1967) using a Clark-type oxygen electrode. Fractions containing the peak activity were pooled, diluted 1:4 with 0.07 M EDTA, 0.03 M Tris, pH 7.4, and centrifuged at 27,000 *g* for 15 min to obtain a purified mitochondrial pellet.

Mitochondrial pellets were lysed by gentle stirring for 1–2 h in 0.05 M Tris, 0.1 M EDTA, 0.5% Sarkosyl (Na-lauroyl-sarcosinate, Geigy Chemical Corp., Ardsley, N. Y.). MtDNA was purified either by phenol extraction of the lysate followed by precipitation of the DNA with 95% ethanol or by preparative CsCl centrifugation of the lysate. Saturated CsCl was added to the lysate to give a final density of 1.69 g/cm^3 and the solution was centrifuged in the Spinco 50 rotor at 18°C and 42,000 rpm for 20 h. 10-drop fractions were collected from the bottom of the centrifuge tube and the absorbancy at 260 nm determined with a Gilford model 2000 spectrophotometer. Samples taken from the fraction with the highest absorbancy were examined by electron microscopy. Fractions containing DNA were pooled, precipitated with 70% ethanol, and redissolved in 0.1 × 0.15 M NaCl, 0.015 M Na-citrate, pH 7.0 (SSC). DNA in 0.1 × SSC, either extracted with phenol or recovered from CsCl gradients, was treated with pancreatic RNase (100 μg/ml), T_1 RNase (330 U/ml), and α-amylase (100 μg/ml). After 1 h at 37°C, pronase (100 μg/ml) was added and the solution incubated an additional hour. MtDNA was then precipitated with 95% ethanol and redissolved in 0.1 × SSC.

Crude MtDNA was prepared from unfertilized eggs laid by females with the genetic constitution $X\widehat{XY}$. These females were obtained by crossing $\widehat{XY}$ males with wild-type females ($Y^SX \cdot Y^LIn(1)EN$, Y^SB f v $y \cdot Y^Ly+$/C(1)RM, y v bb/0 ♂ X Oregon R ♀) and their sibling brothers were therefore sterile XO males. Eggs were collected, dechorionated with 2% Na-hypochlorite for 2 min, washed, and frozen. Egg DNA was prepared by homogenizing 2 g of eggs in 0.05 M Tris, 0.1 M EDTA, 0.35 M sucrose, pH 7.8. The homogenate was centrifuged at 2000 *g* for 10 min and the pellet discarded. The supernatant fraction was centrifuged at 13,000 *g* for 20 min and the resulting pellet was resuspended in 0.05 M Tris, 0.1 M EDTA, pH 7.8. The suspension was resedimented at 13,000 *g* for 20 min. The pellet was lysed and the crude MtDNA was purified by phenol extraction and enzymatic digestion as described for MtDNA.

D. melanogaster DNA from larvae was prepared with slight modification of the method of Laird and McCarthy (1968). From this DNA a fraction having the same properties as MtDNA was isolated by repeated isopycnic centrifugation.

Analytical isopycnic centrifugation was carried out by standard methods (Szybalski, 1968). Samples of DNA in either SSC or 0.1 × SSC were boiled for 10 min to denature the DNA. Samples were either rapidly cooled in ice to maintain denaturation or adjusted to 2 × SSC and the DNA allowed to reassociate at 66°C for 0.5 h. Neutral buoyant densities of denatured and renatured DNA were obtained by adding saturated CsCl directly to the samples to a final density of 1.69 g/cm^3 before centrifugation. Analytical CsCl gradients, ρ = 1.63 g/cm^3, containing ethidium bromide (EBr) at a concentration of 33.3 μg/ml and 0.5–1 μg DNA were centrifuged at 44,770 rpm for 25–30 h at 20°C. During all analytical runs, samples were photographed on Kodak Commercial film using UV optics and subsequently traced with a Joyce-Loebl recording microdensitometer.

Band sedimentation analyses (Vinograd et al., 1963) were performed at 25°C in 30 mm, 4° sector, Kel-F cells with 20 μl of lamellar solution containing approximately 0.2 μg DNA in SSC. DNA was centrifuged at 36,500 rpm in either 0.9 M NaCl containing 0.1 M NaOH or in 1 M NaCl containing 0.01 M Tris, pH 7.5. Corrections of sedimentation coefficients and calculations of molecular weights were made according to Studier (1965).

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Thermal denaturation curves were determined in SSC (Mandel and Marmur, 1968) using *Escherichia coli* DNA as an internal standard. DNA was sheared before reassociation using a Branson Sonifier model W140D equipped with a microtip. The extent of shearing was determined by alkaline band centrifugation.

MtDNA was spread for electron microscopy using a modification of the Freifelder and Kleinschmidt (1965) technique. Approximately 5-μl samples of MtDNA taken directly from the peak fraction of preparative CsCl gradients were added to 95 μl of 2 M KCl containing 0.02% cytochrome *c*. This solution was spread on a trough containing 0.15 M KCl. DNA was picked up on carbon-coated Formvar films on 200-mesh copper grids and was shadowed with 80% Pt − 20% Pd wire at an angle of 6°. Electron micrographs were taken with a Philips 200 electron microscope at a magnification of 23,000. No internal length standard was used. Molecules were measured with a map measurer on positive prints at a magnification of approximately 60,000.

Filter hybridizations were carried out by the procedures of Gillespie and Spiegelman (1965). All filters were hybridized in the same vessel in 2 × SSC. Tritium-labeled 18S and 28S ribosomal RNA (rRNA) was isolated from Schneider's (1972) cultured *D. melanogaster* embryonic cell line no. 2. Cells were grown in Schneider's *Drosophila* Medium (Gibco #172, Grand Island Biological Co., Grand Island, N. Y.) to a concentration of about 10^6 cells/ml. [5-^{3}H]uridine (27.8 Ci/mM) was added to a final concentration of 10 μCi/ml. After 48 h of incorporation at 25°C in shaker flasks, cells were harvested by centrifugation at 755 *g* for 10 min. RNA was extracted and purified by the method of Greenberg (1969) modified to include polyvinyl sulfate, 4 μg/ml, in all buffer solutions. Extracted total [^{3}H]RNA was layered on 10–30% wt/vol sucrose gradients and centrifuged in the Spinco SW41 rotor for 5 h at 40,000 rpmt and 15°C. Gradient fractions were collected from the bottom of the tube and samples of each fraction were spotted on nitrocellulose filters and counted with a Nuclear-Chicago Mark I scintillation counter. Both 18S and 28S rRNA peaks were combined and precipitated with 2 vol of 95% ethanol. The supernatant was decanted after centrifugation at 12,000 *g* for 15 min and the rRNA stored in 70% ethanol in the freezer. The tritiated rRNA had a specific activity of 278,000 cpm/μg and was used at a final concentration of 2.7 μg/ml. Filters were incubated with labeled rRNA overnight at 66°C.

RESULTS

Analytical centrifugation of the mitochondrial lysate showed two bands: a heavy band with a density of 1.681 g/cm^3 and a much larger light band. Since the material in the large light peak formed a sharp band after 3 h of centrifugation, it was assumed to be contaminating carbohydrate. After purification by phenol extraction and enzyme treatment, MtDNA banded as a single peak in neutral CsCl at a density of 1.681 g/cm^3 (Fig. 1 *a*). Two bands of approximately equal size were observed in alkaline CsCl (Fig. 1 *d*) at 1.740 g/cm^3 and 1.735 g/cm^3. It is likely that these bands are the separated heavy and light strands of MtDNA. Denatured MtDNA centrifuged in neutral CsCl had a density of 1.696 g/cm^3 (Fig. 1 *b*). After renaturation the density decreased to a value of 1.685 g/cm^3 (Fig. 1 *c*), close to that of native MtDNA. In analytical EBr gradients, purified MtDNA banded at a lighter density than did *E. coli*, *Micrococcus lysodeikticus*, or *D. melanogaster* main peak DNA. Since covalently closed circles, form I, would have banded at a heavier density than the linear marker DNAs (Bauer and Vinograd, 1968), the EBr gradients indicated that purified MtDNA was present as either nicked circles, form II, or linear molecules. Attempts to demonstrate intact circles in the original mitochondrial lysate using analytical EBr gradients were unsuccessful because the large amount of contaminating carbohydrate banded at the same position in the gradient as MtDNA.

Electron microscopy of MtDNA taken directly from preparative CsCl gradients of lysed mitochondria demonstrated the presence of twisted, intact circles (form I) and open nicked circles (form II) (Vinograd et al., 1965) (Fig. 2). A very few short pieces of linear DNA were also seen. The mean length of the open circles was 5.3 ± 0.03 μm (Fig. 3).

Before thermal denaturation, samples of purified MtDNA were centrifuged to determine neutral and alkaline sedimentation coefficients. Neutral sedimentation yielded a single peak with an s_{obs} = 29.2 and $s_{20,w}$ = 33.4 (average of three determinations). Although this value fell between those reported by Dawid and Wolstenholme (1967) for form I and form II circles of the same size, the absence of a peak in the 39S range and the low molecular weight calculated from alkaline sedimentation experiments implied that no intact, form I circles were present. Alkaline sedimentation of MtDNA produced a single broad peak with an s_{obs} = 10.7 and an $s_{20,w}$ = 12.5. The single-stranded molecular weight was calculated

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to be 8.6 × 10^5 daltons, indicating a number of nicks in the original circles.

The thermal denaturation curve of MtDNA is shown in Fig. 4 and represents a 33% hyperchromic increase during the course of the melt. Approximately 24% of the native DNA melted with a melting temperature (T_m) = 72.6°C, 13% melted slightly higher with a T_m = 76°C, and 55% melted with a T_m = 79.6°C. The remaining hyperchromic increase of 8% was accounted for by a gradual rise in optical density at the beginning and end of the melt. MtDNA was allowed to reassociate at 60°C. The remelt was almost identical with the original melt curve and showed the same three transitions (Fig. 4).

After reassociation, MtDNA was sheared to a single-stranded molecular weight of 2 × 10^5 daltons as measured by alkaline band sedimentation. The sheared DNA was thermally denatured and reannealed at 60°C. Reassociation was very rapid and about 50% complete in the 15 min taken by our melting apparatus to bring the temperature to 60°C. The $C_{0t1/2}$ was estimated to be no greater than 0.08.

A DNA having the characteristics described above for MtDNA was isolated from total larval DNA by repeated isopycnic centrifugation. This DNA had a neutral buoyant density of 1.683 g/cm³. Denatured larval MtDNA centrifuged in neutral CsCl banded at a density of 1.697 g/cm³ and, after renaturation, its density decreased to 1.684 g/cm³. Its purity was estimated as greater than 90% by analytical centrifugation.

Thermal denaturation curves for larval MtDNA (Fig. 5) indicated that the original melt and remelt were again almost identical. Both demonstrated the three major transitions found in MtDNA isolated from eggs. In this case, 21% of the DNA melted with a T_m = 71°C, 12% with a T_m = 74.7°C, and 65% with a T_m = 78.3°C. The differences between T_m values for larval and egg MtDNA may have been due to small amounts of other DNAs contaminating the larval DNA. However, MtDNA from both sources demonstrated essentially the same complex melt curve.

Crude MtDNA isolated from the 13,000 *g* pellet of unfertilized eggs was resolved into two bands isopycnic centrifugation in CsCl (Fig. 6 *a*). The lighter band, ρ = 1.681 g/cm³, separated into heavy and light strands with respective densities of 1.740 g/cm³ and 1.735 g/cm³ in alkaline gradients (Fig. 6 *c*). After denaturation and reassocia-

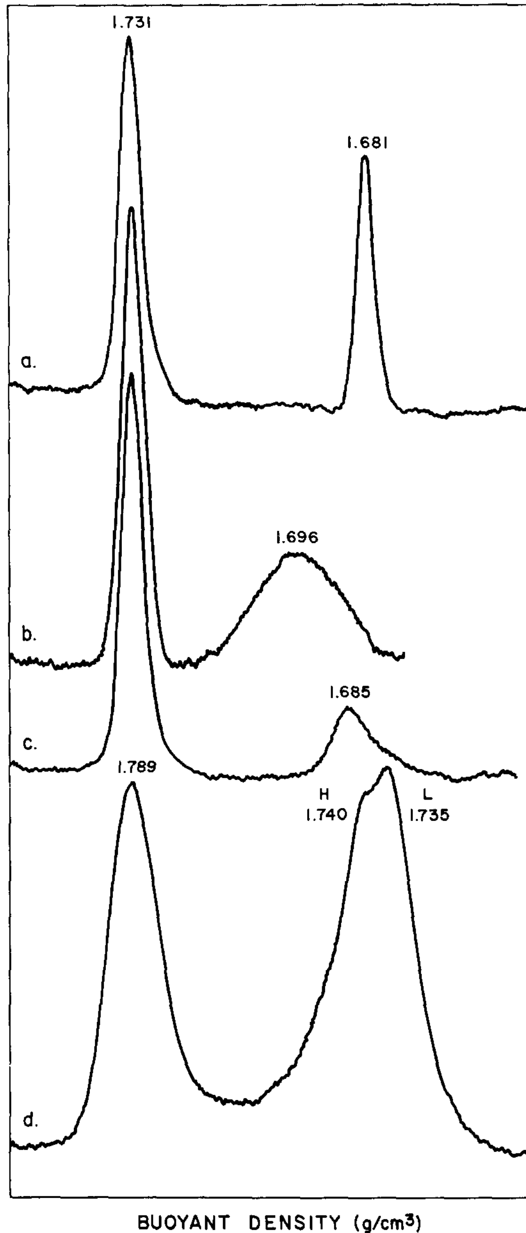

FIGURE 1 Buoyant density in CsCl of MtDNA isolated from purified mitochondria of unfertilized *D. melanogaster* eggs. *a*. Neutral buoyant density of native MtDNA. *b*. Neutral buoyant density of heat denatured MtDNA. *c*. Neutral buoyant density of MtDNA denatured with heat and allowed to renature for 0.5 h in 2 × SSC at 66°C. *d*. Alkaline buoyant density of MtDNA. *M. lysodeikticus* DNA was used as a density standard, neutral ρ = 1.731 g/cm³ and alkaline ρ = 1.789 g/cm³.

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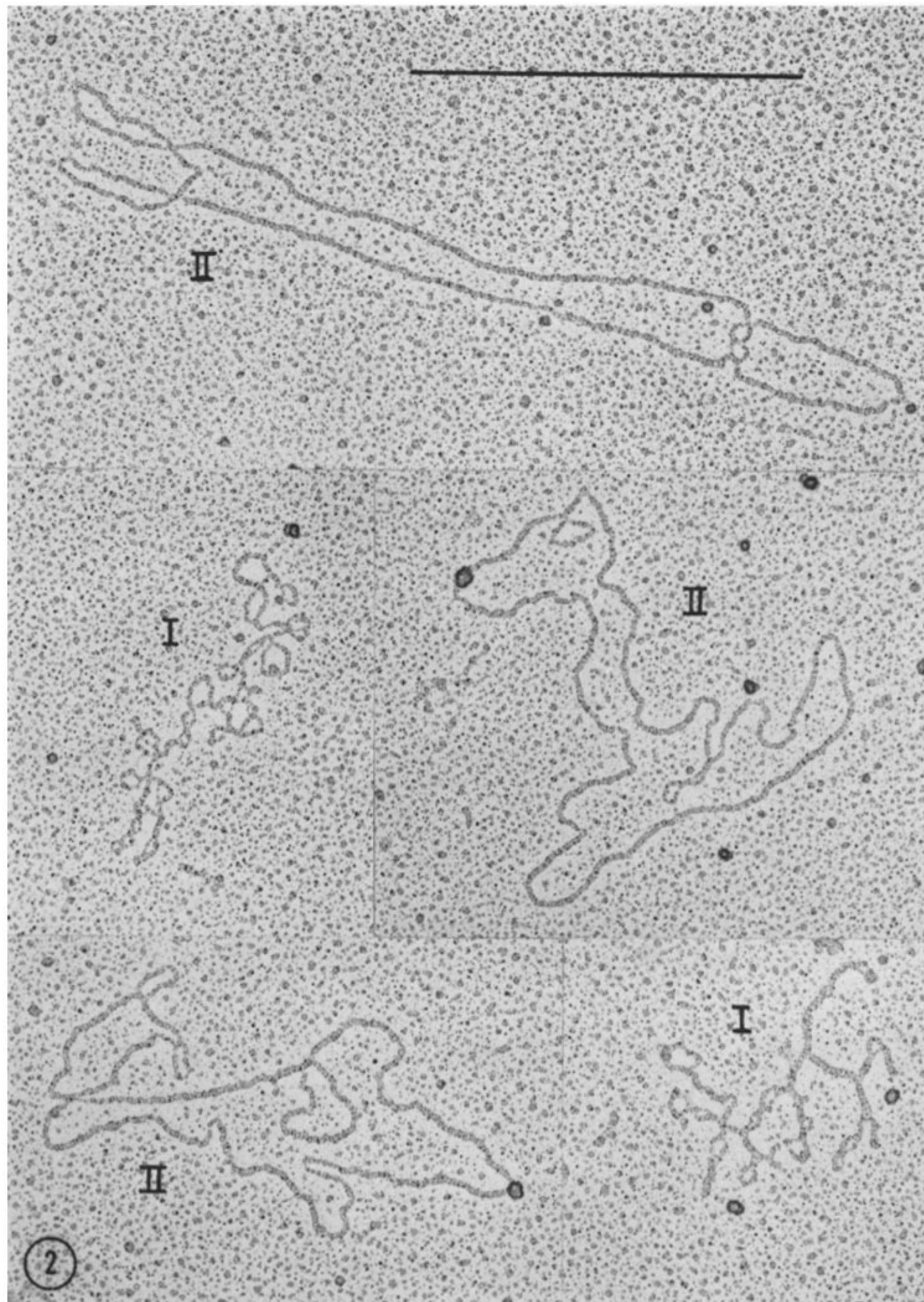

FIGURE 2 Electron micrographs of circular MtDNA isolated from *D. melanogaster*. Open, nicked circles are designated as II; twisted, intact circles as I. × 53,000.

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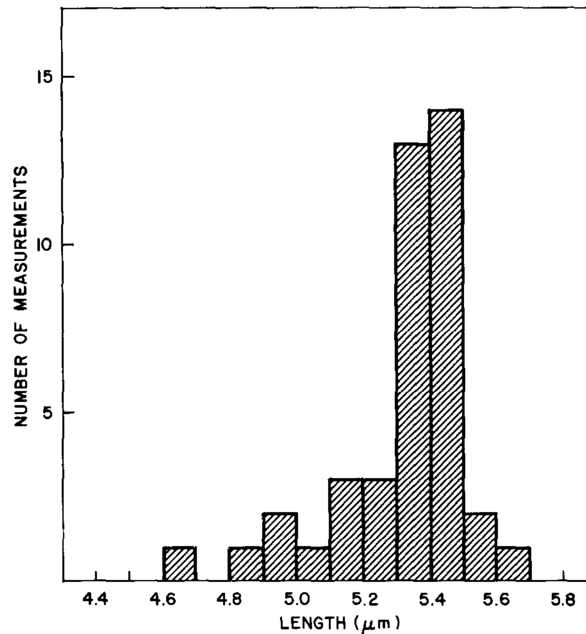

FIGURE 3 Distribution of lengths of MtDNA circles isolated from *D. melanogaster*. Only open, form II circles were measured. $n = 41$.

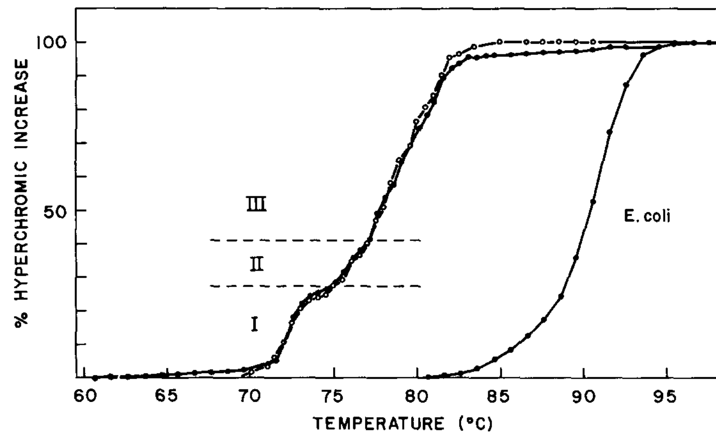

FIGURE 4 Thermal denaturation curve of MtDNA isolated from purified mitochondria of unfertilized *D. melanogaster* eggs. ●——●, original melt; ○——○, remelt. Transitions I, II, and III are indicated in the figure. *E. coli* DNA, $T_m = 90.5°C$, was denatured simultaneously as an internal standard.

tion it returned to a buoyant density of 1.684 g/cm^3. From this behavior the light band was identified as MtDNA. The heavier band at 1.697 g/cm^3 was somewhat more puzzling. It showed only a single peak in alkaline CsCl and maintained its denatured density of 1.713 g/cm^3 in neutral CsCl after reassociation under conditions leading to almost complete reassociation of MtDNA (Fig. 6 *b*). However, it is not simply main peak nuclear DNA since its neutral and alkaline buoyant densities (Figs. 6 *a* and 6 *c*) were 0.005 g/cm^3 lighter than those of *D. melanogaster* main peak DNA, which has a neutral buoyant density of 1.702 g/cm^3 and an alkaline buoyant density of 1.760 g/cm^3 (Gall et al., 1971).

In the toad *Xenopus* and in many other organisms the genes which code for rRNA undergo an extrachromosomal amplification in oocytes to

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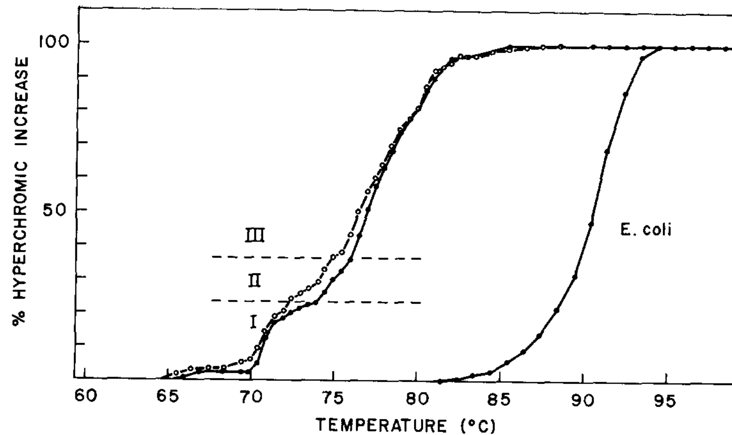

FIGURE 5 Thermal denaturation curve of MtDNA isolated by repeated isopycnic centrifugation of total larval DNA. ●——●, original melt; ○——○, remelt. Transitions I, II, and III are indicated in the figure. *E. coli* DNA, T_m = 90.5°C, was denatured simultaneously as an internal standard.

such an extent that they constitute the majority of the nuclear DNA (Brown and Dawid, 1968; Gall, 1968). In *D. melanogaster* the rDNA sequences have a density slightly less than that of the main peak (Sinclair and Brown, 1971). We examined the possibility that the egg DNA banding at 1.697 g/cm^3 might be amplified rDNA. Tritiated-rRNA of *D. melanogaster* was hybridized to CsCl gradient fractions of two DNA samples: one was DNA from embryonic nuclei (Fig. 7) and the other was the crude mitochondrial DNA from the 13,000 *g* pellet (Fig. 8). In the embryonic nuclear DNA the rDNA sequences were localized on the light side of the main peak (Fig. 7). Approximately 10 μg of DNA was included in this gradient and this amount of DNA should contain at least 0.06 μg of rDNA (Ritossa and Spiegelman, 1965). If the rDNA contained spacer regions, the total amount of rDNA would be somewhat higher. A total of 10,141 cpm of rRNA was bound by the fractions of this gradient. The gradient of crude mitochondrial DNA (Fig. 8) contained about 0.6 μg of the DNA having a buoyant density of 1.697 g/cm^3 and a like amount of MtDNA. A small amount (less than 0.5 μg) of unfractionated ^{14}C-labeled DNA was included as a density marker. The fractions from this gradient failed to show any significant binding of rRNA above the background level (Fig. 8). If the 0.6 μg of DNA at density 1.697 g/cm^3 had been rDNA, it would have bound approximately 100,000 cpm under the conditions used (the gradients shown in Figs. 7 and 8 were hybridized together). We conclude, therefore, that the crude MtDNA is not detectably enriched with respect to rDNA.

DISCUSSION

The DNA of *D. melanogaster* is known to consist of a main peak, a major satellite (Laird and McCarthy, 1968; Rae, 1970) which is actually composed of two species of repetitive DNA (Gall et al., 1971), and a light dAT satellite (Fansler et al., 1970; Travaglini et al., 1968). These DNAs are found at densities of 1.702 g/cm^3, 1.689 g/cm^3, and 1.675 g/cm^3, respectively (Blumenfeld and Forrest, 1971; Gall et al., 1971). Fansler et al. (1970) using DNA isolated from prelarval embryos also identified three bands; main peak DNA at 1.699 g/cm^3, a major satellite at 1.685 g/cm^3, which they termed mitochondrial, and a smaller poly-dAT satellite at 1.669 g/cm^3. These densities are consistently about 0.004 g/cm^3 lighter than the values we have used. Thus, it is likely that the DNA found at 1.685 g/cm^3 and called MtDNA (Fansler et al., 1970; Travaglini et al., 1968) was in fact nuclear satellite DNA, and that true MtDNA was not identified.

D. melanogaster MtDNA has a neutral buoyant density of 1.681 g/cm^3, and, in common with most other MtDNAs, is circular, renatures rapidly, and separates into strands of differing buoyant density in alkaline CsCl. Recently Kram et al. (1972) have reported the density of MtDNA isolated from *D. melanogaster* as 1.680 g/cm^3. Bultmann and Laird (1972) have also observed the same neutral buoyant density, unusual melting characteristics, and circular lengths of MtDNA from *D. melanogaster* as we have.

In contrast to other MtDNAs, that of *Drosophila*

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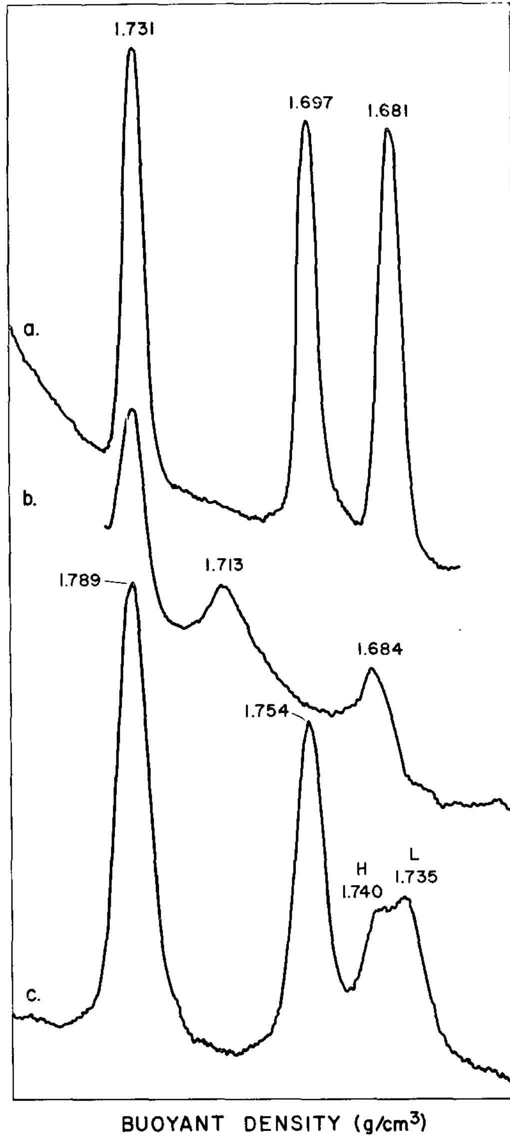

FIGURE 6 Buoyant density in CsCl of DNA isolated from the 13,000 *g*, crude mitochondrial pellet of unfertilized *D. melanogaster* eggs. *M. lysodeikticus* DNA was used as a density standard, neutral $\rho = 1.731$ g/cm^3 and alkaline $\rho = 1.789$ g/cm^3. *a.* Neutral buoyant density of crude MtDNA. *b.* Neutral buoyant density of heat-denatured crude MtDNA after renaturation in 2 × SSC for 0.5 h at 66°C. *c.* Alkaline buoyant density of crude MtDNA. The band at $\rho = 1.681$ g/cm^3 in *a* behaves like MtDNA, including strand separation in alkaline CsCl. The heavier band at $\rho = 1.697$ g/cm^3 fails to renature after 0.5 h at 66°C and does not show strand separation in alkaline CsCl. Its density is significantly lower than main peak DNA.

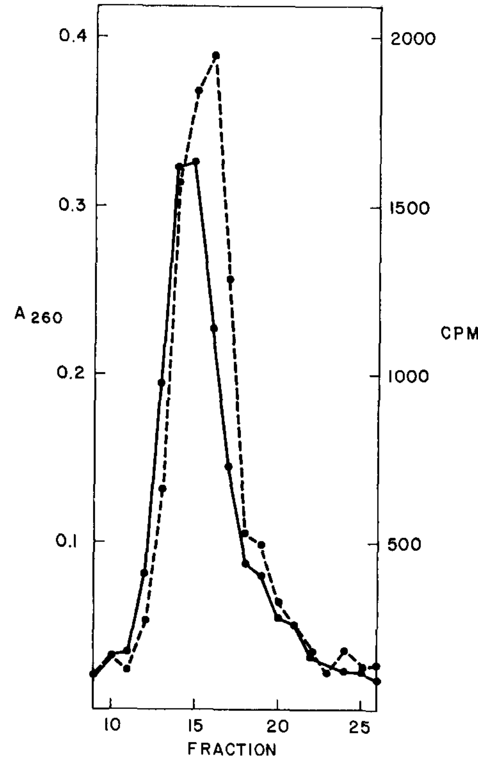

FIGURE 7 Hybridization of ^{3}H-labeled rRNA to DNA isolated from embryonic nuclei of *D. melanogaster*. 10 μg of embryonic nuclear DNA was centrifuged to equilibrium in CsCl. Fractions were collected, denatured, and loaded onto nitrocellulose filters. The filters were then incubated with rRNA (278,000 cpm/μg) isolated from cultured *D. melanogaster* cells. ●——●, A_{260}; ●--●, ^{3}H counts bound to DNA fractions. The fractions which contain rDNA are slightly less dense than the main peak.

melts in several distinct stages. Leffler et al. (1970) have reported a biphasic thermal denaturation curve for rat liver MtDNA and ascribed the lower transition at 86°C to nicked circles in form II and the higher transition above 88°C to intact circles in form I. The remelting transition of this material was sharp and monotonic, occurring at 85.5°C. However, *D. melanogaster* MtDNA isolated from either total larval DNA or purified egg mitochondria displays the same polyphasic melt curve for both native and reassociated DNA and in both cases no hyperchromic increase is observed above 87°C. Thus, the multitransitional behavior of *D. melanogaster* MtDNA appears to be due to real differences in base composition in various portions of the molecule.

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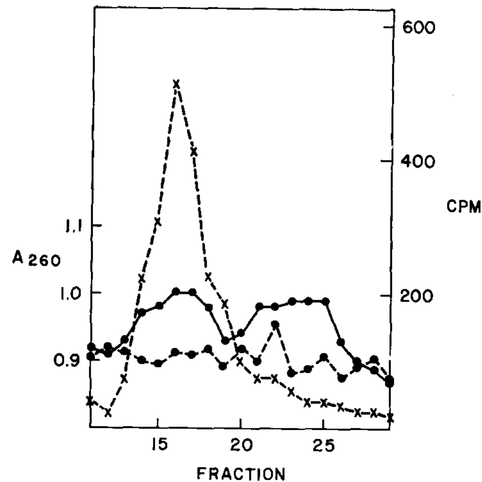

FIGURE 8 Hybridization of ^{3}H-labeled rRNA to DNA isolated from the 13,000 *g*, crude mitochondrial pellet of unfertilized *D. melanogaster* eggs. Crude MtDNA containing 0.6 μg of the DNA having a density of 1.697 g/cm^3 and an approximately equal amount of MtDNA of density 1.681 g/cm^3 was centrifuged to equilibrium in CsCl. Approximately 0.5 μg of unfractionated ^{14}C-labeled DNA isolated from cultured *D. melanogaster* cells was included in the gradient as a density marker. Fractions were collected, denatured, loaded onto nitrocellulose filters, and hybridized with ^{3}H-labeled rRNA (278,000 cmp/μg) isolated from cultured *D. melanogaster* cells. ●——●, A_{260}; ●--●, ^{3}H counts bound by DNA; x--x, ^{14}C counts from unfractionated *D. melanogaster* DNA. None of the gradient fractions bound rRNA above background levels. If the egg DNA at ρ = 1.697 g/cm^3 were rDNA, it would have bound approximately 100,000 cpm.

MtDNA isolated from yeast "petite" mutants by Bernardi et al. (1970) has also been shown to melt with several transitions which occur at the same temperatures for both native and reassociated DNA. Interestingly, the T_m of the initial transition of these yeast MtDNAs occurred between 71°C and 73°C which corresponds to the T_m of the first transition of *D. melanogaster* MtDNA. As suggested by Bernardi et al. (1970) this portion of the Mt-DNA is probably composed of stretches of (dAT: dAT) interspersed with a few G:C base pairs and/or nonalternating (dA:dT). The two higher transitions of *D. melanogaster* MtDNA at 76°C and 79.6°C probably represent areas of the molecule containing higher proportions of G:C base pairs.

A DNA isolated from the 13,000 *g* pellet of unfertilized eggs was observed at a neutral buoyant density of 1.697 g/cm^3, slightly lighter than the density of main peak nuclear DNA. The possibility that this peak consisted of amplified rDNA sequences was excluded, but otherwise nothing is known of its origin or function.

We wish to thank Dr. Marvin Kalt for help with electron microscopy, Dr. D. Suzuki for the gift of strain 1(1)E12^{ts} flies, and Dr. John Schenkman for help with the succinoxidase assays.

S. Friedman is a Fellow of the Jane Coffin Childs Memorial Fund for Medical Research.

This work was supported by funds from United States Public Health Service Grants GM 51227-01 and GM 12427-08 from the National Institute of General Medical Sciences, from National Science Foundation Grant GB-17267X2, and from the Jane Coffin Childs Memorial Fund for Medical Research Grant 61-250.

Received for publication 18 May 1972, and in revised form 11 September 1972.

REFERENCES

BAUER, W., and J. VINOGRAD. 1968. *J. Mol. Biol.* **33**:141.

BERNARDI, G., M. FAURES, G. PIPERNO, and P. P. SLONIMSKI. 1970. *J. Mol. Biol.* **48**:23.

BLUMENFEID, M., and H. S. FORREST. 1971. *Proc. Natl. Acad. Sci. U. S. A.* **68**:3145.

BORST, P., and G. J. C. M. RUTTENBERG. 1966. *Biochim. Biophys. Acta.* **114**:645.

BROWN, D. D., and I. B. DAWID. 1968. *Science (Wash. D. C.).* **160**:272.

BULTMANN, A., and C. LAIRD. 1973. *Biochim. Biophys. Acta.* In press.

CORNEO, G., C. MOORE, D. R. SANADI, L. I. GROSSMAN, and J. MARMUR. 1966. *Science (Wash. D. C.).* **151**:687.

CORNEO, G., L. ZARDI, and E. POLLI. 1968. *J. Mol. Biol.* **36**:419.

DAWID, I. B. 1965. *J. Mol. Biol.* **12**:581.

DAWID, I. B. 1966. *Proc. Natl. Acad. Sci. U. S. A.* **56**:269.

DAWID, I. B., and D. R. WOLSTENHOLME. 1967. *J. Mol. Biol.* **28**:233.

DAWID, I. B., and D. R. WOLSTENHOLME. 1968. *Biophys. J.* **8**:65.

FANSLER, B. S., E. C. TRAVAGLINI, L. A. LOEB, and J. SCHULTZ. 1970. *Biochem. Biophys. Res. Commun.* **40**:1266.

FREIFELDER, D., and A. K. KLEINSCHMIDT. 1965. *J. Mol. Biol.* **14**:271.

GALL, J. G. 1968. *Proc. Natl. Acad. Sci. U. S. A.* **60**:553.

GALL, J. G., E. H. COHEN, and M. L. POLAN. 1971. *Chromosoma.* **33**:319.

GILLESPIE, D., and S. SPIEGELMAN. 1965. *J. Mol. Biol.* **12**:829.

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Greenberg, J. R. 1969. *J. Mol. Biol.* **46**:85.
King, T. E. 1967. *Methods Enzymol.* **10**:202.
Kram, R., M. Botchan, and J. E. Hearst. 1972. *J. Mol. Biol.* **64**:103.
Kroon, A. M., P. Borst, E. F. J. Van Bruggen, and G. J. C. M. Ruttenberg. 1966. *Proc. Natl. Acad. Sci. U. S. A.* **56**:1836.
Laird, C., and B. McCarthy. 1968. *Genetics.* **60**:303.
Leffler, A. T., E. Creskoff, S. W. Luborsky, V. McFarland, and P. T. Mora. 1970. *J. Mol. Biol.* **48**:455.
Mandel, M., and J. Marmur. 1968. *Methods Enzymol.* **12B**:195.
Nass, M. M. K. 1966. *Proc. Natl. Acad. Sci. U. S. A.* **56**:1215.
Rae, P. 1970. *Proc. Natl. Acad. Sci. U. S. A.* **67**:1018.
Ritossa, F. M., and S. Spiegelman. 1965. *Proc. Natl. Acad. Sci. U. S. A.* **53**:735.
Schneider, I. 1972. *J. Embryol. Exp. Morphol.* **27**: 353.
Sinclair, J. H., and D. D. Brown. 1971. *Biochemistry.* **10**:2761.
Sinclair, J. H., and B. J. Stevens. 1966. *Proc. Natl. Acad. Sci. U. S. A.* **56**:508.
Studier, F. W. 1965. *J. Mol. Biol.* **11**:373.
Szybalski, W. 1968. *Methods Enzymol.* **12B**:330.
Travaglini, E. C., J. Petrovic, and J. Schultz. 1968. *J. Cell Biol.* **39** (2, Pt. 2): 136 a. (Abstr.)
Van Bruggen, E. F. J., P. Borst, G. J. C. M. Ruttenberg, M. Gruber, and A. M. Kroon. 1966. *Biochim. Biophys. Acta.* **119**:437.
Vinograd, J., R. Bruner, R. Kent, and J. Weigle. 1963. *Proc. Natl. Acad. Sci. U. S. A.* **49**:902.
Vinograd, J., J. Lebowitz, R. Radloff, R. Watson, and P. Laipis. 1965. *Proc. Natl. Acad. Sci. U. S. A.* **53**:1104.
Wolstenholme, D. R., and I. B. Dawid. 1967. *Chromosoma.* **20**:445.
Wolstenholme, D. R., R. G. Kirschner, and N. J. Gross. 1972. *J. Cell Biol.* **53**:393.

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