MOLECULAR WEIGHT OF MITOCHONDRIAL DNA IN PHYSARUM POLYCEPHALUM

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The mitochondrial DNA's from a wide variety of organisms have a common molecular weight of approximately 10 x 10^6 daltons. Most of the molecules are isolated in a circular form (Pikó et al., 1968; Sinclair et al., 1967; Dawid and Wolstenholme, 1967; Van Bruggen et al., 1966) except in the case of yeast where only a small fraction is circular and the remainder linear (Shapiro et al., 1968). In contrast, Suyama and Miura (1968) have reported that mitochondrial DNA's from Tetrahymena, Paramecium and mung bean hypocotyl are up to 4 times larger than those of the first class of organisms. The molecules were in each case linear and no circles were observed. It thus appears that there is a second class of mitochondrial DNA, found so far in two protozoa and one plant. We report here that the mitochondrial DNA of the acellular slime mold, Physarum polycephalum, also falls in the higher molecular weight category. It is heterogeneous, and no closed circles could be detected.

This study was facilitated by the ability to label specifically Physarum mitochondrial DNA (density 1.686 g/cc) by exposing a culture to radioactive thymidine during the G2 portion of the cell cycle (Evans, 1966; Guttes, Hanawalt and Guttes, 1967; Holt and Gurney, 1968). Sedimentation of labeled, crude lysates provided a rapid assay of particular lytic conditions and led to the procedure described under Materials and Methods.

Materials and Methods

Axenic cultures of Physarum polycephalum (strain M3A) were grown at 22°C using modifications of methods developed by Rusch and collaborators (Daniel

and Rusch, 1961) as described previously (Holt and Gurney, 1968). In addition, the tryptone in the medium was replaced by N-Z-Case (Sheffield Chemical, Norwich, N. Y.). Surface cultures were labeled by placing the filter paper bearing the mold in the regular medium containing 6 μc/ml ^{3}H-thymidine (New England Nuclear, methyl label, 16.5 μC/μmole). After labeling, the culture was scraped from the filter paper, washed by centrifugation at 0°C in either carbonate buffer (0.1 M sodium carbonate, 0.1 M EDTA pH 10) or saline-EDTA (0.1 M sodium chloride, 0.1 M EDTA pH 8.1) and resuspended such that 1.5 ml fresh buffer or saline-EDTA was added to a culture of diameter approximately 4.5 cm. An equal volume of 15% sodium lauroyl sarcosinate (Geigy Chemical Corp., Ardsley, N. Y.) was added and the mixture was rolled at 60 rpm for 40 min at 0°C. Except where mentioned, the resulting crude lysate was used for sedimentation analysis without further purification.

P22 bacteriophage were labeled with thymine-2-^{14}C, purified and lysed in 2% sodium lauroyl sarcosinate as described by Botstein (1968). T7 bacteriophage were grown on Escherichia coli B3 with thymine-2-^{14}C (0.45 μC/μg) added at the time of infection. After purification of the phage in CsCl (Crothers and Zimm, 1965), DNA was extracted with phenol (MacHattie et al., 1967).

Samples containing less than 0.4 μg DNA were layered on gradients of 5-20% sucrose dissolved in 0.1 M NaCl plus 0.05 M potassium phosphate, pH 6.7, and centrifuged at 20°C in a Spinco SW39 rotor. Three drop fractions were collected from the bottom of the tube onto discs of filter paper. The papers were dried, washed in cold 5% trichloroacetic acid, washed in 95% ethanol, dried again, and counted in a toluene based scintillation fluid. Alkaline gradients were made with fresh solutions of 5 and 20% sucrose which had been adjusted to pH 12.2 (standard glass electrode) with concentrated NaOH. One tenth milliliter samples of the Physarum lysate were made alkaline by layering them onto 0.1 ml of 0.35 M NaOH on top of the gradient. The gradients were allowed to stand for 15-30 min before centrifugation. CsCl centrifugation was carried out as described previously (Holt and Gurney, 1968). Ethidium

bromide was kindly supplied by Boots Pure Drug Co., Ltd. (Nottingham, England).

Results and Discussion

A surface culture of Physarum in G2 phase was labeled with ^{3}H-thymidine and lysed as described above. Separate aliquots of the lysate were sedimented in neutral sucrose (Fig. 1a) and banded in CsCl (Fig. 2U). The CsCl gradient shows that about 80% of the incorporated radioactivity is in the mitochondrial (light) DNA. About 15% of the radioactivity is in the previously observed (Holt and Gurney, 1968) heavy component (ca. 1.706 g/cc), and a slight shoulder on the heavy side of the light peak suggests some labeling of the principal component (1.700 g/cc). The sucrose gradient shows essentially all of the ^{3}H-labeled DNA sedimenting more rapidly than the marker DNA, which has a sedimentation coefficient ($s^{o}_{w,20}$) of 32S. About 20% of the labeled DNA

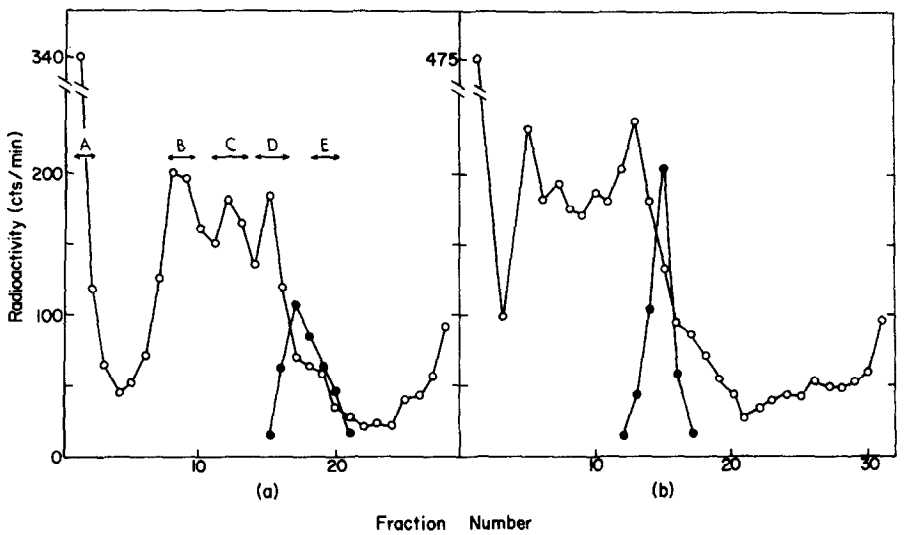

Fig. 1. Sedimentation of crude lysates labeled with ^{3}H-thymidine.
(a) A culture was labeled for 2 1/4 hours beginning 6 1/2 hours after metaphase, lysed, mixed with ^{14}C-T7 DNA and sedimented for 95 min at 35,000 rpm as described under Materials and Methods. The lysate applied to the gradient corresponded to about 0.04 cm^{2} of surface culture.
(b) A culture was labeled for 4 hours beginning 5 hours after metaphase, lysed, mixed with T7 DNA, heated at 60°C for 12 min and sedimented for 95 min at 38,500 rpm.
All gradient fractions are shown.
o-o-o, ^{3}H from Physarum lysate
●-●-●, ^{14}C marker DNA

sedimented to the bottom of the tube and may be an artifact (Burgi and Hershey, 1963). The labeled forms we observe are not replicative intermediates since increasing the time of exposure to ^{3}H-thymidine does not alter the sedimentation pattern (Compare Fig. 1a and 1b).

The rapid sedimentation of mitochondrial DNA was obtained only when the slime mold was washed with EDTA and lysed at 0°C. If lysis was instead carried out at 60°C, the bulk of the mitochondrial DNA sedimented at about 6S. This may explain the lower sedimentation rates reported by others (Evans, 1967; Guttes et al., 1967) for Physarum mitochondrial DNA. However, lysates

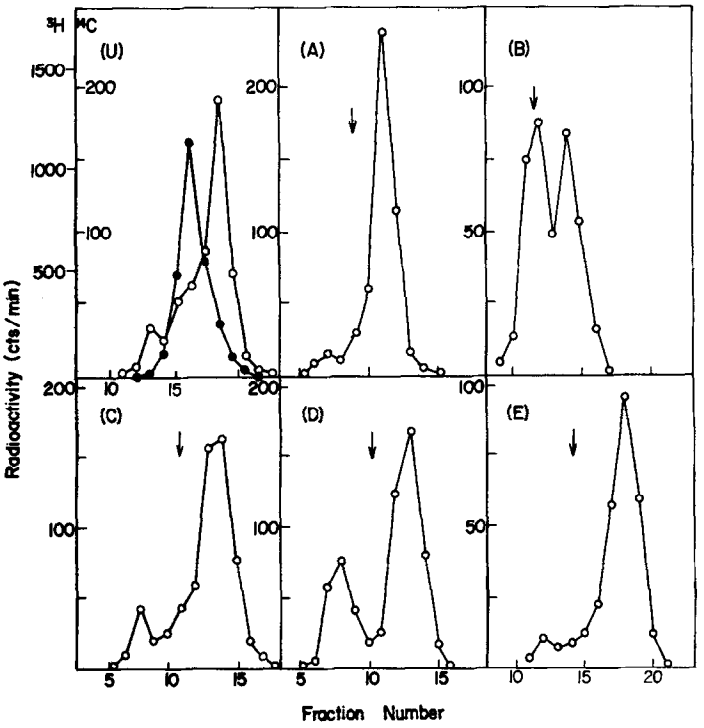

Fig. 2. CsCl analysis of unfractionated lysate and sucrose gradient fractions.
(U) A sample of the ^{3}H-labeled lysate applied to the gradient in Fig. 1a was mixed with fully labeled ^{14}C-DNA from Physarum and centrifuged in CsCl. The ^{14}C-DNA was prepared from a culture incubated for several doublings with thymidine-2-^{14}C.
(A-E) Fractions from the sucrose gradient in Fig. 1a were combined as shown in Fig. 1a, dialyzed against saline-EDTA, mixed with ^{14}C-DNA as above and centrifuged in CsCl. The arrows indicate the position of the ^{14}C maxima.
o-o-o, ^{3}H; •-•-•, ^{14}C.

may be heated to 60°C, after lysis is complete, without affecting the sedimentation pattern (compare Fig. 1a and 1b). The relative homogeneity of the marker DNA (Fig. 1b) shows that aggregation of small pieces of DNA was not significant and would not explain the high sedimentation rate of the mitochondrial DNA.

When fractions from the sucrose gradient of Fig. 1a were further analyzed on CsCl gradients, the patterns shown in Fig. 2 were obtained. Mitochondrial DNA was present in significant quantities in all five fractions examined. The heavy, nuclear component appears primarily in sucrose fraction (D), suggesting that it is a homogeneous material sedimenting somewhat more slowly than the bulk of the other labeled components. The main component is present primarily in fraction (B), suggesting that it too is homogeneous as isolated but some-

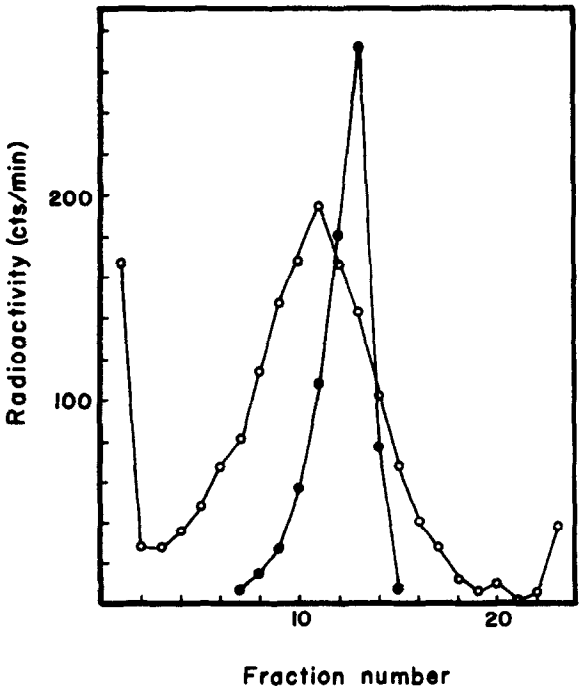

Fig. 3. Sedimentation of purified DNA.
A ^{3}H-labeled lysate was prepared essentially as described under Fig. 1 and centrifuged in CsCl. The mitochondrial DNA region of the gradient was dialyzed, mixed with ^{14}C-P22 DNA as a marker, and sedimented for 85 min at 35,000 rpm.
o-o-o, ^{3}H; •-•-•, ^{14}C.

what faster sedimenting than the bulk of the mitochondrial DNA.

Mitochondrial DNA purified by extensive pronase digestion (Massie and Zimm, 1965), phenol extraction (MacHattie et al., 1967), or CsCl centrifugation also sediments more rapidly than T7 DNA in neutral sucrose. The CsCl-purified material, which is essentially free of the other DNA components, still demonstrates considerable heterogeneity and sediments with a peak at 36S (Fig. 3). This sedimentation value corresponds to a molecular weight of 37×10^6 daltons for linear molecules (Studier, 1965) and 26×10^6 daltons for open circles (Ogawa and Tomizawa, 1967).

Both ethidium bromide (Radloff, Bauer and Vinograd, 1967) and alkaline sucrose gradients have been used to study the shape of the mitochondrial DNA. Closed circles were not detected by either method. Thus the mitochondrial DNA is either linear or in the form of open circles. The width of the mitochondrial DNA peak is sufficiently great to eliminate the possibility that the heterogeneity arises solely from a mixture of linear and open circle forms of equal size.

On alkaline sucrose gradients, the peak of radioactive DNA sometimes sedimented faster than T7 DNA, lending further support to the conclusion that the rapid sedimentation is not due to binding of the DNA to another cellular component, or to aggregation. The same result was obtained when P22 phage DNA ($s^{o}_{w,20}$ = 33S) was used as a marker. However, in several preparations the mitochondrial DNA moved more slowly than the phage markers. This variability observed on alkaline gradients did not arise from varying ^{3}H-decay in the preparations. Thus there appears to be a variable number of single strand nicks produced prior to sedimentation of the lysate. A real question then remains as to whether the DNA as extracted, i.e. heterogeneous with no detectable closed circles, is a true representation of the in vivo situation. A lower limit on the size of the DNA can be set, however, while further attempts are made to improve the isolation procedure.

Acknowledgment

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References

Botstein, D. (1968). J. Mol. Biol. 34, 621.
Burgi, E. and Hershey, A. D. (1963). Biophys. J. 3, 309.
Crothers, D. M. and Zimm, B. H. (1965). J. Mol. Biol. 12, 525.
Daniel, J. W. and Rusch, H. P. (1961). J. Gen. Microbiol. 25, 47.
Dawid, I. B. and Wolstenholme, D. R. (1967). J. Mol. Biol. 28, 233.
Evans, T. E. (1967). Fed. Proc. 26, 867.
Guttes, E. and Guttes, S. (1964). Science 145, 1057.
Guttes, E., Hanawalt, P. C., and Guttes, S. (1967). Biochim. Biophys. Acta 142, 181.
Holt, C. E. and Gurney, T. (1968). J. Cell. Biol., in press.
MacHattie, L. A., Ritchie, D. A., Thomas, C. A. Jr. and Richardson, C. C. (1967). J. Mol. Biol. 23, 355.
Massie, H. R. and Zimm, B. H. (1965). Proc. Nat. Acad. Sci. U.S.A. 54, 1636.
Ogawa, H. and Tomizawa, J.-I. (1967). J. Mol. Biol. 23, 265.
Pikó, L., Blair, D. G., Tyler, A. and Vinograd, J. (1968). Proc. Nat. Acad. Sci. U.S.A. 59, 838.
Radloff, R., Bauer, W. and Vinograd, J. (1967). Proc. Nat. Acad. Sci. U.S.A. 57, 1514.
Shapiro, L., Grossman, L. I., Marmur, J. and Kleinschmidt, A. K. (1967). J. Mol. Biol. 33, 907.
Sinclair, J. H., Stevens, J., Gross, N. and Rabinowitz, M. (1967). Biochim. Biophys. Acta 145, 528.
Studier, F. W. (1965). J. Mol. Biol. 11, 373.
Suyama, Y. and Miura, K. (1968). Proc. Nat. Acad. Sci. U.S.A. 60, 235.
Van Bruggen, E. F. J., Borst, P., Ruttenberg, G. J. C. M., Gruber, M. and Kroon, A. M. (1966). Biochim. Biophys. Acta 119, 437.