freezing level of the atmosphere during a thunderstorm, the produced free charges may be very large. Calculations show that the charges are, indeed, more than adequate to describe commonly observed active thunderstorms. Quantitative estimates of the established charge distributions inside active thunderstorms are in preparation.

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17 November 1965

Mitochondrial DNA in Yeast and Some Mammalian Species

Abstract. *Yeast DNA, in a cesium chloride density gradient, shows a minor or satellite band with a density lower than that of the main nuclear component. The DNA isolated from purified mitochondria of yeasts corresponds in density to this satellite band. In solution, this DNA more easily undergoes renaturation as compared to DNA from cell nuclei. The ease of this renaturation is presumably due to a homogeneity greater than that of nuclear DNA. Mitochondrial DNA isolated from several mammalian species has the same or higher density than nuclear DNA, but differs in its ready renaturability.*

Extranuclear DNA has been found in two kinds of cytoplasmic organelles, the plastids of plants and the mitochondria (*1*). Mitochondrial DNA has been demonstrated in unicellular organisms such as molds (*2*), yeast (*3*), algae (*4*), and protozoa (*5*), as well as in mammalian species (*6*). The evidence for the presence of DNA is based on chemical analysis of isolated and purified mitochondria, electron microscopy, and cesium chloride density-gradient centrifugation. However, although the general occurrence of mitochondrial DNA seems definitely established, many of its properties remain to be described. We now report on the buoyant density and the renaturation properties of mitochondrial DNA from two different species of yeast, as well as from beef and sheep hearts, and from the livers of mouse, guinea pig, rat, and chicken.

Saccharomyces cerevisiae, Harden and Young strain (*7*) and *Saccharomyces carlsbergensis,* strains N.C.Y.C. 74 (*8*) and S-74 (*9*) were grown in an enriched medium (*10*) under aerobic conditions and were harvested at different times during the growth cycle. Yeast protoplasts and mitochondria were prepared essentially as described (*10*). The yeast protoplasts were prepared with helicase or glusulase, commercially available (*11*) digestive enzyme mixtures obtained from the snail *Helix pomatia.*

Mouse, guinea pig, and rat liver mitochondria were isolated essentially as described by Schneider (*12*). Beef-heart mitochondria were prepared by a modification of this method, by first mincing the beef-heart tissue into very small pieces and then dispersing in a glass homogenizer. The crude mitochondrial preparations were highly purified by banding in a discontinuous gradient of Ficoll (Pharmacia, Uppsala, Sweden) (*13*) and carefully isolating the visible mitochondrial regions with a pipette. The integrity of the mitochondrial preparations was ascertained from the respiratory-control index, determined with a Clark electrode for measuring dissolved oxygen. The homogeneity of the mitochondrial preparations was monitored by phase-contrast microscopy.

DNA was isolated from the mitochondria by a modification of the method of Marmur (*14*). Because of the small amount of DNA present in mitochondria, alcohol precipitations were eliminated. After removal of protein, the preparation was treated with T_1 and pancreatic ribonucleases and then dialyzed exhaustively against SSC (0.15*M* NaCl, 0.015*M* sodium citrate, *p*H 7). The DNA was denatured by heating for 10 minutes at 100°C in SSC, followed by rapid cooling in an ice bath; the DNA was renatured by warming denatured DNA (10 μg/ml) at 65°C in double-strength SSC for 5 hours and then slowly cooling to room temperature.

The DNA samples were analyzed by CsCl density-gradient centrifugation at 25°C at 44,770 rev/min in the Spinco model E analytical ultracentrifuge (*15*). The density marker was DNA (density 1.742 g/cm^3) from the virulent *Bacillus subtillis* bacteriophage 2C. The ultraviolet photographs were scanned with a Joyce-Loebl microdensitometer. The densities of the DNA samples were determined (*16*) and are expressed relative to the density of *Escherichia coli* DNA, taken as 1.710 g/cm^3.

The results from the study of DNA are shown in Fig. 1. The DNA isolated from the whole yeast cell, when it is banded in a CsCl gradient, shows a major band of density 1.700 g/cm^3, a shoulder of density 1.704 g/cm^3, and a minor or satellite band at a density of 1.685 g/cm^3 (Fig. 1*A*). The shoulder at 1.704 g/cm^3 is of un-

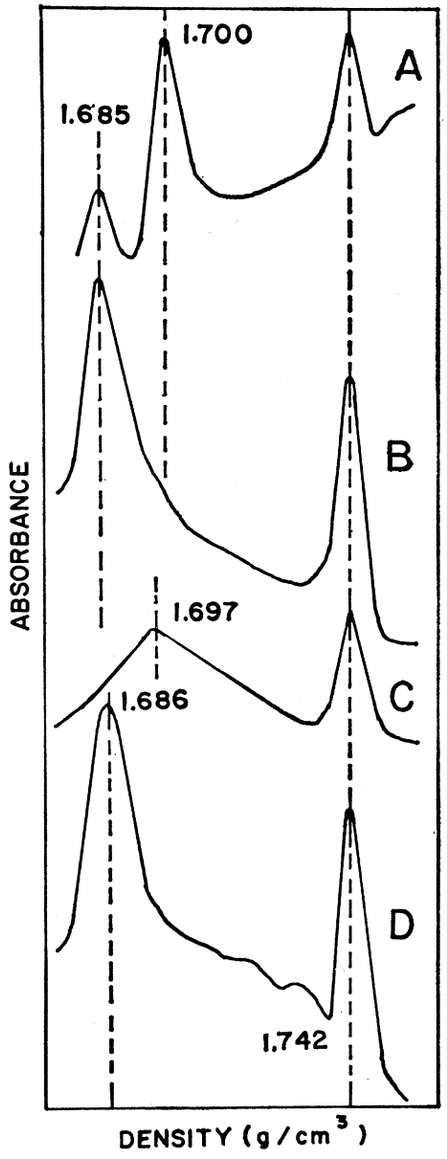

Fig. 1. Microdensitometer tracings of DNA from whole yeast cells (*A*), mitochondrial DNA (*B*), heat-denatured mitochondrial DNA (*C*), and renatured mitochondrial DNA (*D*) centrifuged to equilibrium in a CsCl density gradient. A peak at 1.704 g/cm^3, clearly visible on the exposed film, but not seen in this tracing, appears reproducibly as a shoulder in whole cell DNA from *Saccharomyces carlsbergensis.*

known origin, but increases in density by about 0.015 g/cm^3 upon denaturation. The satellite band is present in DNA preparations from *Saccharomyces cerevisiae* and *S. carlsbergensis*, and it is present in the logarithmic and stationary phases of growth. The amount of satellite DNA is approximately 10 percent of the total. After incubating the samples of yeast DNA with deoxyribonuclease (Worthington, 10 μg/ml, 37°C, 30 minutes), the main band as well as the satellite band disappeared. On the other hand, when yeast DNA was treated with α-amylase (Worthington, 250 μg/ml, 37°C, 45 minutes), which was active in degrading glycogen to low-molecular-weight compounds, both the main and satellite bands remained unaffected. The thermal-denaturation profile of the yeast DNA in SSC, obtained according to Marmur and Doty (*17*), shows a sharp transition temperature (T_m = 85.0°C) and a hyperchromicity of 40 percent. The initial part of the heating curve has a small shoulder, presumably due to the melting of satellite DNA. From the buoyant density in CsCl and the melting temperature the main component of the yeast DNA has a guanine-plus-cytosine (G+C) content of 40 percent and the satellite DNA a G+C content of 25 percent.

Figure 1*B* shows the CsCl pattern of the DNA extracted from the yeast mitochondria; the band of the mitochondrial DNA corresponds in density to the satellite band of Fig. 1*A*. In other preparations of yeast mitochondria, some DNA corresponding in density to the nuclear DNA band was still present. However, the satellite DNA was enriched to contain at least 50 percent of the total DNA present. Upon heat denaturation the satellite band, as well as the main band, increased in density by approximately 0.015 g/cm^3 (Fig. 1*C*).

In contrast to the nuclear DNA, when the denatured mitochondrial DNA is allowed to renature in solution under the experimental conditions described, its density in CsCl returns to the value of native DNA (Fig. 1*B*).

Mitochondrial DNA in *S. carlsbergensis* has now been shown by N^{15} transfer experiments to replicate semiconservatively, and under the growth conditions used, heavy (N^{15}–N^{15}) mitochondrial DNA can still be seen after nuclear DNA has undergone one replication to completely hybrid (N^{15}–N^{14}) form; thereafter unique hybrid mitochondrial DNA can still be readily seen after three nuclear divisions, when hybrid nuclear DNA is no longer visible. The observed shoulder at 1.704 g/cm^3 also indicates semiconservative replication (*18*).

Whole cell DNA from a cytoplasmic (petite) mutant of *S. carlsbergensis* N.C.Y.C. 74, and from two petite mutants of *S. cerevisiae*, all supplied by Dr. F. Sherman, show no trace of mitochondrial DNA when banded in CsCl; however, all contain a nuclear peak at 1.700 g/cm^3 and a heavy shoulder at 1.704 g/cm^3, both of which increase in density by about 0.015 g/cm^3 upon denaturation (*18*).

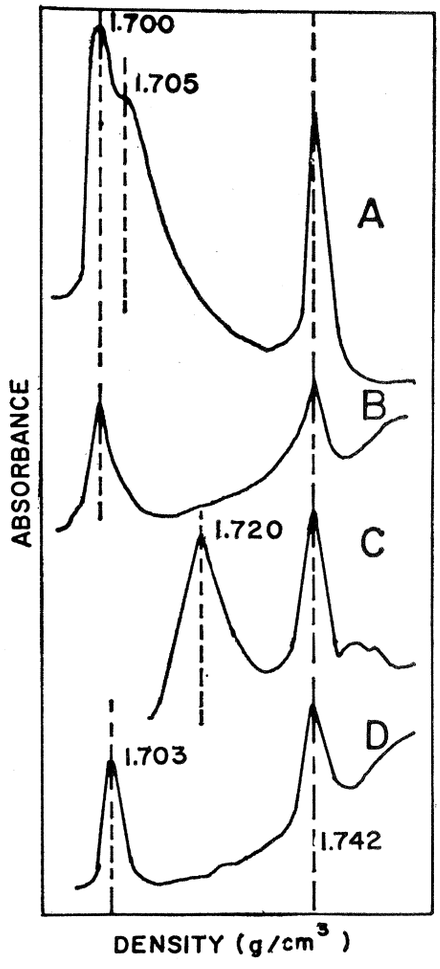

Fig. 2. Microdensitometer tracings of DNA isolated from whole cells of guinea-pig-liver tissue (*A*), mitochondrial DNA (*B*), heat-denatured mitochondrial DNA (*C*), and renatured mitochondrial DNA (*D*) centrifuged to equilibrium in a CsCl density gradient. The peak on the right in each tracing corresponds to the density marker, bacteriophage 2C DNA.

Mitochondrial DNA from animal sources is identical in four of the six mammalian species we investigated. The results obtained with guinea-pig-liver DNA are presented in Fig. 2. When DNA isolated from whole tissue homogenates is run in a CsCl gradient, a broad main band with a mean buoyant density of 1.700 g/cm^3 ± 0.001 g/cm^3 and a satellite band with a density of 1.705 g/cm^3 (Fig. 2*A*) are obtained (*19*). The nature of this satellite band is thus far unknown; the buoyant density of guinea pig mitochondrial DNA corresponds to the density of the main, nuclear DNA component; however, mitochondrial DNA displays a very narrow band in CsCl, and it does not contain any DNA corresponding in density to the satellite band (Fig. 2*B*). After heat denaturation, mitochondrial DNA from guinea pig liver increases in density, showing a double-stranded nature (Fig. 2*C*). Like yeast mitochondrial DNA, heat-denatured mitochondrial DNA can renature under our experimental conditions (Fig. 2*B*).

Effects of denaturation and renaturation of mitochondrial DNA of beef-heart and livers from mouse and rat were identical. These mammalian mitochondrial DNA's correspond in density to the main nuclear DNA, but are more readily renaturable after heating; sheep-heart mitochondrial DNA, however, shows a density of 1.714 g/cm^3, compared with 1.704 g/cm^3 for the nuclear fraction, and chicken-liver mitochondrial DNA has a density of 1.707 g/cm^3 compared with 1.701 g/cm^3 for nuclear DNA [see Rabinowitz *et al.* (*6*)]. In neither of the last two cases is there any visible contamination of the fractions in a CsCl gradient.

Experiments on mammalian nuclear DNA showed that it is not able to renature under the foregoing conditions as judged by the lowering of its density.

The DNA from the purified mouse, rat, guinea pig, and beef nuclei contained satellite DNA in the same proportion to the main component as in DNA extracted from the whole cell. Furthermore, the content of satellite DNA was, by CsCl density-gradient centrifugation, the same in DNA from bull sperm (*20*), where the number of mitochondria per cell is very low (*21*), as in beef-heart DNA. The DNA isolated from purified mitochondria does not yield the satellite DNA band, and its origin remains unknown.

The same density of mitochondrial

DNA was observed when mitochondria were isolated from brain and heart of sheep, and liver and heart of chicken; furthermore, mitochondria from guinea pig liver, which banded at two discrete densities in a Ficoll gradient, each showed DNA of density 1.701 g/cm^3.

Some experiments were also carried out to calculate the amount of DNA present in mitochondria of mammalian tissues in relation to mitochondrial proteins; the diphenylamine reaction (*22*) was used to evaluate the amount of DNA, and the method of Lowry *et al.* (*23*) was used to estimate mitochondrial proteins. The results obtained in the mitochondrial fractions of mammalian tissues were in the range of 4 to 5 μg of DNA per milligram of mitochondrial proteins. The amount of DNA present in a beef-heart mitochondrion was estimated as approximately 5×10^{-11} μg based on its reported protein content of 1.1×10^{-13} g by Green and Oda (*24*). This should correspond to a molecule of DNA with a molecular weight of 3×10^7 if all the DNA in a mitochondrion is present as a single molecule.

In the light of previously reported results and those reported here, we can conclude that the unique density in CsCl gradients (of mitochondrial DNA in simpler organisms) and the ability to renature in solution are two useful criteria to identify mitochondrial DNA. The density of mitochondrial DNA is different from the density of nuclear DNA in simpler unicellular organisms, molds, yeasts, algae, and protozoa, while it is apparently the same or higher in mammalian species. It is not known whether the unique density of mitochondrial DNA corresponds to the presence of a unique base, nor is it known whether the extent of methylation of adenine and cytosine is the same as in nuclear DNA. It was shown by Ray and Hanawalt (*25*) that the methylcytosine content of DNA from the chloroplasts of *Euglena gracilis* was lower than that of nuclear DNA. The most likely explanation for the ability of mitochondrial DNA to renature is its much greater homogeneity, compared with the nuclear DNA.

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11 FEBRUARY 1966

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26. Supported by a grant from AEC. G.C. was supported by an International Fellowship (FO5-833-01) from NIH. C.M. was aided by a grant from NIH. D.R.S. was supported by a grant-in-aid from the American Heart Association. Support for J.M. was from the Health Research Council of the City of New York (I-322).
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27 December 1965

Organization of DNA in Dipteran Polytene Chromosomes as Indicated by Polarized Fluorescence Microscopy

Abstract. *Analysis of the polarization of fluorescence from* Drosophila virilis *polytene chromosomes stained with acridine orange suggests that the DNA in the interband regions of these chromosomes cannot be in a supercoiled configuration, but must lie parallel to the chromosome axis.*

Recent studies have shown that the mode of binding of aminoacridine dyes to DNA in vitro, when the ratio of dye to nucleotide is about one to four or less, is by intercalation: that is, the planar dye molecule is sandwiched between the two adjacent base pairs inside the DNA helix (*1–3*). For higher ratios, a new mode of binding appears; the dye molecules are "stacked" along the outside of the helix with their planes perpendicular to the helix axis (*3, 4*). In both of these binding modes, the planes of the fused rings of the dye molecules are parallel to the planes of the purine-pyrimidine rings. The acridines are well-known fluorescent dyes, and in such planar molecules appreciable absorption will occur only if the direction of the electric field associated with the impinging light (that is, the *E*-vector) is not perpendicular to the plane of the rings. Similarly, the emitted fluorescence will have maximum intensity where the *E*-vector is parallel to the plane of the dye molecule. Therefore, if an acridine dye is bound to DNA and the complex is oriented, there will be one direction of polarization for which relatively little light will be absorbed or emitted. Lerman utilized this fact to support the intercalation model by orienting acridine-stained DNA in a flow gradient (*2*).

Using the dye acridine orange, which has a high specificity for nucleic acids in cellular preparations (*5*), we are taking advantage of this polarization phenomenon to analyze the orientation of DNA in biological material. This report deals with our preliminary results with the salivary gland chromosomes of *Drosophila virilis*.

The instrument is built around a fluorescence microscope equipped with a Zeiss oil-immersion objective of numerical aperture 1.32 (magnifying power, × 100) and a Spencer dry lens of numerical aperture 0.5 (magnifying power, ×20) inverted as a condenser. In front of the Hg-arc light source are two glass plates, tilted to act as reflection polarizers that correct for the slight inherent polarization of the light source. The plates are adjusted so that the light striking the specimen is completely unpolarized for those wave-