DNA SATELLITES FROM CELLS OF GREEN AND APLASTIDIC ALGAE[1]

Judith Leff, Manley Mandel[2]
H. T. Epstein and Jerome A. Schiff

Departments of Biology and Biochemistry
Brandeis University
Waltham 54, Massachusetts

Received August 15, 1963

The ability of Euglena cells to transmit chloroplasts to their progeny can be eliminated using ultraviolet light (Lyman, et al, 1961). Since this process shows peaks of effectiveness at 260 and at 280 mu, is photoreactivable (Schiff, et al, 1961) and has been localized to cytoplasmic structures (Lyman, et al, 1961, Gibor and Granick, 1962) the hypothesis has been advanced that a plastid-localized genome of a nucleoprotein nature exists in these cells and this finding has provoked a search for this nucleoprotein species. Chun, et al (1963) have described a species of DNA from Chlamydomonas, Chlorella and higher plants which appears as a satellite to the main DNA band in density gradient centrifugation. They also showed that this satellite is enriched in the chloroplast fractions of higher plants. This communication describes work of a similar nature on Euglena, Chlamydomonas and Polytoma which compares the DNA content of chloroplast-containing cells with that of aplastidic mutants

[1]Supported by Research Grant RG-6344 from the U. S. Public Health Service.
[2]Present address: Section of Molecular Biology
Department of Biology, M.D.
Anderson Hospital and Tumor Institute
University of Texas
Houston, Texas

to provide a correlation between presence of the satellite and the ability to form chloroplasts.

Euglena gracilis var bacillaris and a UV-induced aplastidic mutant W_3BUL were cultivated as described previously (Lyman, et al, 1961). Chalmydomonas Reinhardi (Y_1 strain obtained through R. P. Levine from R. Sager) was cultured as described by Levine and Ebersold (1959). Polytoma obtusum, obtained from L. Provasoli, Haskins Laboratories, was grown on a medium recommended by him (Personal Communication). DNA was isolated from washed cells lysed by the addition of sodium lauryl sulfate to a final concentration of 1.0%. Marmur's method (1961) was followed except that the lysed mixture was immediately chilled rather than being heated. For the isolation from Chlamydomonas, it proved essential to avoid the heating step. This modification has been necessary in the isolation of DNA from other organisms (Mandel and Honigberg, 1963). Purified DNA was dissolved in 0.15 M sodium chloride containing 0.015 M citrate at pH 7.0 (SSC). Cesium chloride density gradient ultracentrifugation was carried out in the model E Spinco Analytical Ultracentrifuge as described by Schildkraut et al (1962). Fully deuterated Pseudomonas aeruginosa DNA of density 1.763 g/cm^3 at 0.5 µg/centrifuge cell served as the reference standard. Ultra-violet photographs were scanned with a Joyce-Loebl double beam recording microdensitometer.

Chloroplast-containing light-grown cells of Euglena gracilis var bacillaris yielded DNA which, on density gradient centrifugation, gave the pattern shown in fig. 1A. In addition to the main band at a density of 1.708 a satellite appears at density 1.688 suggesting a mean base composition 20% lower in guanine plus cytosine in the satellite. That these bands are indeed due to DNA is shown by the expected increase in density of both bands on

heat denaturation, accompanied by a broadening of the bands (fig. 1B).

The association of the satellite DNA with chloroplast-forming ability is likely from a comparison with the DNA from whole cells of W_3BUL a white, non-photosynthetic mutant of bacillaris which lacks plastid structures as judged by electron and fluorescence microscopy. Fig. 1C shows that this mutant yields only the main DNA band at 1.708 but lacks the satellite completely. To be certain of this the centrifuge cells received up to $2\frac{1}{2}$ times as much material in some cases as was used for the preparations from the wild-type so that the amount of satellite material would have to be less than 0.3% of the total DNA to escape detection, since we estimate we can detect a satellite concentration as low as 1% of the main band. Since the satellite in the DNA from wild-type cells represents about 4% of the total, the aplastidic mutant could not contain more than 8% of the satellite material found in the wild-type cells. The absence of the satellite from the aplastidic mutant suggests that this DNA may be uniquely associated with the ability to produce chloroplasts.

In view of the report of Chun, et al (1963) that Chlamydomonas Reinhardi yields DNA containing a main band at a density of 1.723 and a single satellite at 1.695, it seemed advisable to check a closely related aplastidic strain as in the case of Euglena. Polytoma obtusum, a naturally occuring aplastidic chlamydomonad, was selected for this study. Fig. 2A shows our centrifugation of Chlamydomonas Y_1 DNA. The main band is at density 1.721 and a satellite 1.694 both in good agreement with Chun, et al (1963). We find an additional satellite, not hitherto reported,

at density 1.712. On heat denaturation all three bands show the expected increase in density (fig. 2B).

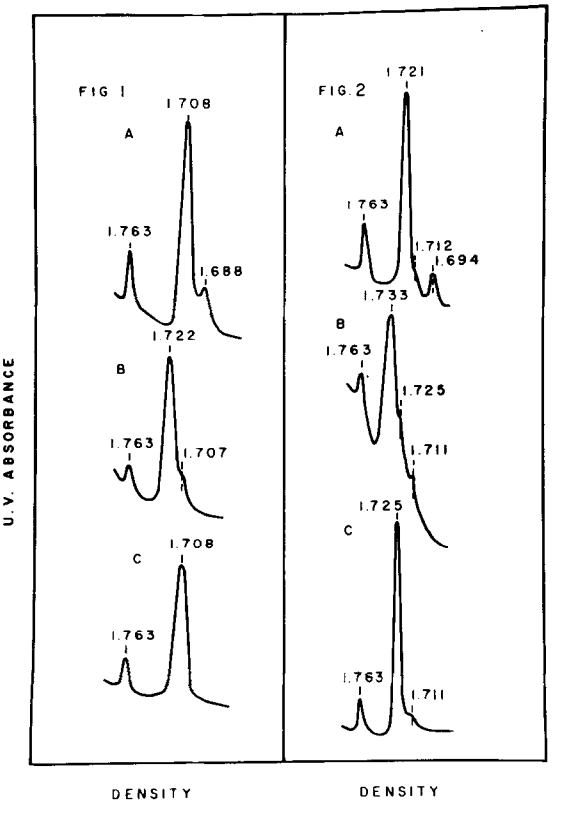

Fig. 1: Microdensitometer tracings of UV photographs of DNA from Euglena gracilis var. bacillaris banded in Cesium Chloride density gradient.
A: Wild-type, native DNA (13 µg);
B: Wild type, DNA denatured in 0.1 SSC, 100 C, 10 min. fast-cooled in ice (13 µg);
C: W_3BUL, native DNA (11 µg).

Fig. 2: Microdensitometer tracings of UV photographs of DNA banded in Cesium Chloride density gradient.
A: Chlamydomonas Reinhardi Y_1, native DNA (6.4 µg);
B: Chlamydomonas Reinhardi Y_1, DNA denatured as in fig. 1B (6.4 µg);
C: Polytoma obtusum, native DNA (7 µg).

DNA from Polytoma lacks the satellite at density 1.695 but retains the main band (possibly at a slightly greater density, 1.725) and the minor satellite at 1.711.

Taken together, the results with Chlamydomonas obtained by Chun, et al, the additional data from Polytoma mentioned above, and the work thus far completed with DNA from Euglena suggest strongly that there is a DNA associated with the ability to form chloroplasts. Previous results on plastid inheritance in Euglena are consistent with this interpretation.

References

Chun, E. H. L., Vaughan, M. H. Jr., and Rich, A. J. Mol. Biol. In press (1963)
Gibor, A. and Granick, S. J. Cell Biol. 15: 599 (1962)
Levine, R. P. and Ebersold, W. T. Z. Vererb.-Lehre 90: 74 (1959)
Lyman, H., Epstein, H. T. and Schiff, J. A. Biochem. Biophys. Acta 50: 301 (1961)
Mandel, M. and Honigberg, B. M. J. Protozool. In press (1963)
Marmur, J. J. Mol. Biol. 3: 208 (1961)
Schiff, J. A., Lyman, H. and Epstein, H. T. Biochem. Biophys. Acta 50: 310 (1961)
Schildkraut, C. L., Marmur, J. and Doty, P. M. J. Mol. Biol. 4: 430 (1962)