SATELLITE DNA IN PHOTOSYNTHETIC BACTERIA

Y. Suyama and Jane Gibson

Department of Biology, University of Pennsylvania
Philadelphia, Pennsylvania

and

Division of Biological Sciences
Section of Microbiology, Cornell University
Ithaca, New York

Received July 11, 1966

DNA from a single bacterial species generally shows a unimodal distribution and relatively homogeneous composition throughout its length (Sueoka, Marmur and Doty, 1959; Rolfe and Meselson, 1959). An exception to this rule has been reported in Halobacteria, some of which contained satellite DNA (Joshi, Guild and Handler, 1963). In recent years, evidence has been accumulating (Preer, in press) that in higher organisms satellite DNA is associated with subcellular structures such as plastids, mitochondria, kinetoplasts, kinetosomes, etc. The photosynthetic bacteria present an interesting problem in this connection. The photosynthetic apparatus is restricted to subcellular structures named chromatophores (Schachman, Pardee and Stanier, 1952), but although these take the functional place of plastids, there is some question as to their origin. In Rhodospirillum rubrum they appear to form a continuum throughout the cell, and to be invaginations of the cell membrane (Holt and Marr, 1965), while in Rhodopseudomonas sheroides the high degree of homogeneity of the isolated particles has led to the conclusion that they are discrete structures within the cell (Gibson, 1965a). Although isolated chromatophores have been reported to contain very little DNA (Schachman, Pardee and Stanier, 1952;

Supported by an AEC grant [AT(30-1)-3588] to Y. Suyama and a PHS grant [GM 12202] to Dr. Britton Chance.

Gibson, 1965b) it seemed desirable to reinvestigate carefully the possibility that a unique species of DNA, analogous to the plastid DNA of higher plants, might be found in these organisms.

We have examined the DNA of the following bacteria: Chromatium, Rhodopseudomonas spheroides, Rhodospirillum rubrum and Rhosopseudomonas palustris. Evidence was obtained that the first two organisms possessed satellite DNA's, but at least in Chromatium, the satellite DNA was not physically associated with isolated chromatophores.

The Chromatium D culture obtained from a single cell isolate was grown in the medium of Morita et al. (1965) at 30°. Rps. spheroides (ATCC no. 14690), R. rubrum (ATCC no. 11170), Rps. palustris (a gift of Dr. S. Taniguchi) were grown in the medium containing 0.3°/o Bacto-yeast extract (Difco), 0.2°/o Bacto Casamino acid (Difco) semi-aerobically with illumination for 3 days. Cells were harvested by centrifugation and washed once in 3°/o NaCl, 0.02 M potassium phosphate buffer, pH 7.8, or 0.02 M potassium phosphate buffer, pH 7.4. For preparation of a chromatophore fraction from Chromatium, the washed cells were resuspended in 3°/o NaCl, 0.02 M potassium phosphate buffer, pH 7.8 and crushed by a French pressure cell at 9,000 psi. The disrupted cells were centrifuged at 7,000 rpm in a Servall refrigerated centrifuge for 10 min and passed through a 0.45 μ millipore filter to remove whole cells and large cell fragments. The filtrate was centrifuged at 25,000 rpm in a Spinco Model L preparative ultracentrifuge for 30 min. The pellet was washed once with the saline-phosphate buffer and finally resuspended in approximately 5 ml saline-EDTA (0.15 M NaCl, 0.1 M EDTA, pH 8.0) to give a concentration of approximately 5 mg protein/ml.

For isolating DNA from whole cells, the washed cells were suspended in saline-EDTA and lysed by addition of 10°/o sodium lauryl sulfate to a final concentration of 0.5°/o and subsequent heating at 60° for 10 min as described by Marmur (1961). The lysate was then treated with phenol at pH 9.0 by shaking on a rotary shaker in the cold for 5 min and DNA fibers formed upon addition of two volumes of ethanol were collected on a glass rod and dissolved in BPES (0.006 M Na_2HPO_4, 0.002 M HaH_2PO_4, 0.001 M Na_2EDTA, and 0.179 M NaCl) (Crothers, 1964). This was then treated with RNase (10 μg/ml), and the phenol and ethanol treatments were repeated. For obtaining DNA from the chromatophore fraction, extensive dialysis was substituted for the ethanol step, and the final content of the dialysis tubing concentrated by pervaporation and again dialyzed against BPES.

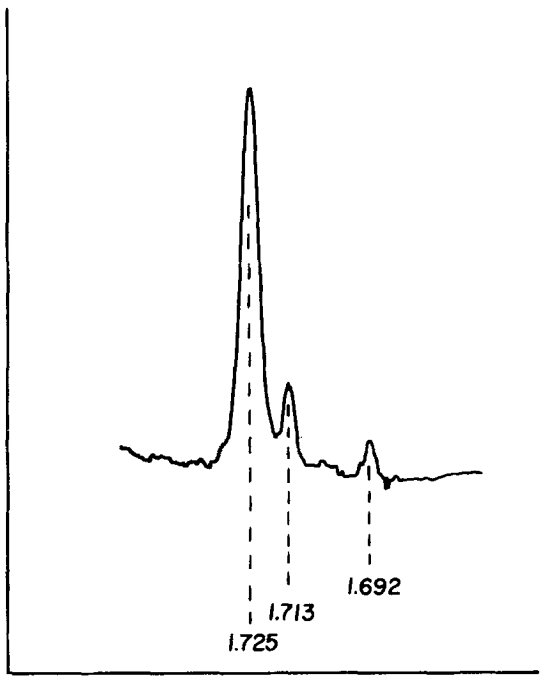

Fig. 1. Microdensitometer tracing of the ultraviolet absorption photograph of Chromatium whole-cell DNA (4.5 μg) in equilibrated CsCl gradient formed by centrifugation at 44,770 rpm for 24 hrs. DNA (ρ = 1.692 g·cm^{-3}) isolated from Tetrahymena pyriformis, whose density was calibrated against B. subtilis DNA (ρ = 1.703 g·cm^{-3}) (Schildkraut, Marmur and Doty, 1962) was used as a density reference. The ratio of the amounts of DNA corresponding to the minor and to the major band is estimated to be approximately 1/5.

Figure 1 shows a microdensitometer tracing of Chromatium bulk whole cell DNA banded in an equilibrated CsCl density gradient. The main band corresponds to the density, ρ = 1.725 g·cm^{-3} and the satellite band, ρ = 1.713 g·cm^{-3}. Both bands are sensitive to DNase digestion (10 μg/ml, 5 mM $MgCl_2$, 20 min at room temperature), and heating in a boiling water bath for 3 min and fast cooling resulted in appearance of two additional peaks at the densities, 1.740 g·cm^{-3} and 1.728 g·cm^{-3}, suggesting that both original bands represent native double stranded DNA. The amount of DNA corresponding to the minor band was estimated to be approximately 10 to 20% of that of the main band. The ratio of the

amounts of the two DNA's did not vary in preparations made from a number of single cell isolates from Chromatium D cultures, and was also the same in cells harvested at different stages of growth. Two bands were also found in a freshly isolated strain of Chromatium. Further studies with the chromatophore fraction isolated from Chromatium showed that the satellite DNA was not contained within the structure. Although the final preparation of chromatophores contained a small but variable amount of DNA (approximately 10 μg DNA/mg protein of chromatophores), this DNA forms only a diffused band in CsCl gradient during a 48 hour centrifugation at 44,770 rpm, the mean density of the DNA corresponding to that of the whole-cell main DNA.* This means that the satellite DNA is not enriched specifically in the chromatophore fraction.

Table 1. DNA of photosynthetic bacteria as determined by CsCl density gradient centrifugation analysis.

Organisms	Number of bands	Densities ($g \cdot cm^{-3}$)
Chromatium D	2	1.725 (Major); 1.713 (Minor)
Rps. spheroides*	2	1.730 (Major); 1.724 (Minor)
Rps. palustris+	1	1.725
R. rubrum	1	1.725

CsCl density gradient centrifugations were made at 44,770 rpm for periods over 20 hrs at 20°. Density calibration was based on B. subtilis DNA ($\rho = 1.703$) (Schildkraut, Marmur and Doty, 1962). The density of R. rubrum was previously reported to be 1.726 $g \cdot cm^{-3}$ (Schildkraut, Marmur and Doty, 1962).

* The density of the minor band was estimated directly from the film, where the band was clearly visible; but in microdensitometer tracings the presence of the minor band is detectable only as a shoulder.

+ No satellite band was resolved in CsCl gradient containing more than 8.3 μg DNA/ml.

* A sharp band was occasionally formed in a CsCl gradient made with the chromatophore DNA preparation. However, this band is not of DNA nature, since it was resistant to DNase and RNase treatments, and heating for 10 min in a boiling water bath and fast cooling did not change the band density ($\rho = 1.682$ $g \cdot cm^{-3}$).

Furthermore, the DNA isolated from the chromatophore fraction by the present method is relatively small in molecular size (less than 1 million daltons), supporting the notion of Gibson (1965b) that the trace of DNA found with the isolated chromatophores of Rps. spheroides represent contaminating, degraded small molecular-weight DNA. However, further studies are necessary to answer the question of whether or not the DNA isolated from our chromatophore fraction is indeed a contaminating DNA.

Results of density gradient centrifugation analyses of DNA isolated from three other bacteria are presented in Table 1. Rps. spheroides consistently exhibited a satellite DNA, but the other two organisms have failed to show the presence of an extra band of DNA in these experiments.

Although the present studies revealed the presence of an extra species of DNA in some of the photosynthetic bacteria studied here, the origin and specific function of this DNA are yet to be determined. A possibility exists that this DNA is of episomal origin and involved in a function which may be unique to this group of bacteria.

REFERENCES

Crothers, D. M., J. Mol. Biol. 9, 712 (1964).
Gibson, K. D., Biochemistry, 4, 2027 (1965a).
Gibson, K. D., Biochemistry, 4, 2042 (1965b).
Holt, S. C., and Marr, A. C., J. Bacteriol. 89, 1402 (1965).
Joshi, J. G., Guild, W. R., and Handler, P., J. Mol. Biol. 6, 34 (1963).
Marmur, J., J. Mol. Biol. 3, 208 (1961).
Morita, S., Edwards, M. L., and Gibson, J., Biochim. Biophys. Acta 109, 45 (1965).
Preer, J. R., Jr., Prog. in Protozool. III (in press).
Rolfe, R., and Meselson, M., Proc. Natl. Acad. Sci. U.S., 43, 1039 (1959).
Schachman, H. K., Pardee, A. B., and Stanier, R. Y., Arch. Biochem. Biophys. 38, 245 (1952).
Schildkraut, C. L., Marmur, J., and Doty, P., J. Mol. Biol. 4, 430 (1962).
Sueoka, N., Marmur, J., and Doty, P., Nature 183, 1427 (1959).