OCCURENCE OF THE G+C RICH SATELLITE DNA IN UNICELLULAR ALGAE.

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SUMMARY

A G+C rich satellite DNA has been evidenced in the unicellular algae Chlorella and Porphyridium. This satellite DNA bands at 1.722-4 in CsCl gradient, corresponding to a base composition of 64% G+C. Preliminary time-course studies indicate that this satellite DNA exhibits metabolic properties and may be concerned with gene amplification. In many respects this satellite DNA appears closely related to the G+C rich nuclear satellite DNA of plants.

INTRODUCTION

The heterogeneity of plant and animal DNA's is now well documented; for plants, this heterogeneity results partly from the presence of small amounts of DNA within mitochondria and chloroplasts, and indeed from the nucleus itself. Besides the classical chromosomal DNA, MATSUDA and SIEGEL (1) reported the occurence of a nucleolar DNA with a buoyant density of 1.706 and more recently, QUETIER GUILLE and VEDEL (2) isolated a G+C rich satellite DNA with a buoyant density of 1.722-4. This later satellite DNA has been evidenced in the nucleus of numerous dicots, is seemingly lacking among monocots and is concerned with the r-DNA amplification. Many properties exhibited by this satellite DNA and by the satellite DNA present in amphibian oocytes (BROWN and DAWID(3), BIRNSTIEL et al(4)) are quite similar.

It was thus of interest to check for the presence of such a satellite in unicellular algae. IWAMURA (5) had already reported an alteration of the DNA base composition during the synchronous growth of Chlorella ellipsoidea: the G+C decreases when DNA duplicates and reincreases after the cellular division. These alterations remained unaffected whatever the method of ana-

lysis (6). These results have been reinvestigated, using DNA extracted by the chloroform-octanol procedure and analytical ultracentrifugations in equilibrium CsCl density gradients

MATERIAL and METHODS

Chlorella pyrenoidosa and Porphyridium sp. are synchronously cultured, as previously described (7,8). The cycles are respectively 14^h light- 10^h darkness and 10^h- 10^h. Algae are harvested by centrifugation and the DNA extracted according to the procedure of QUETIER and GUILLE (9) with a modified SDS step (up to 30mn, 1.5 higher concentration). DNA usually undergoes an RNase treatment (bovine pancreatic RNase).

Analytical ultracentrifugations are performed on a Spinco model E supplied with UV optic. The DNA solution (3 to 10 µg in 0.67 ml of Tris-HCl buffer pH 8.5) is mixed with 0.8821g of optical grade CsCl and 0.03 ml of a marker DNA (1 to 3 µg of phage 2C DNA in the Tris-HCl buffer, ρ=1.742). Centrifugations are achieved at 44,000 rpm, 25°C during 21^h. UV photographs are analysed by a Joyce-Loebl double-beam recording microdensitometer and buoyant densities are determined according to HEARST and VINOGRAD (10) with a small computer program (11).

RESULTS

Most of the results reported here have been obtained on Porphyridium sp. Tracings of equilibrium CsCl density gradients of DNA extracted from the two algae exhibit two bands (fig.1 a-b). Schlieren optic does not detect any peak nor diffraction band, as it could be the case with some carbohydrate polymers (12). Both bands are RNase-resistant and DNase-sensitive.

The first kind of DNA -the light band- occurs in all preparations, whatever the growth conditions, with a buoyant density of 1.714 (56% G+C) for Porphyridium sp. and of 1.709-10 for Chlorella pyrenoidosa. These DNA's are the classical DNA of these organisms. The satellite DNA bands at 1.722-4 (about 64% G+C) for the two algae; this value corresponds well to the G+C rich nuclear satellite DNA of plants (2). A second satellite DNA has been sometimes observed at 1.694 for Porphyridium sp. and could be of chloroplastic origin.

The G+C rich satellite DNA does not result from non-axe-

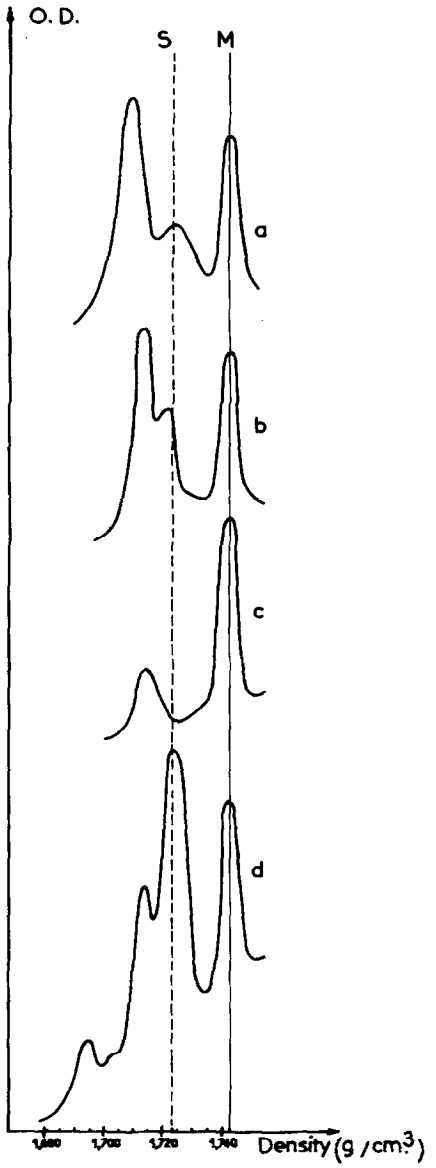

Fig. 1: Banding pattern of DNA extracted from:
a- Chlorella, 10 hours of darkness(normal cycle).
b- Porphyridium, 5 hours of darkness(normal cycle).
c- Porphyridium, 3 hours of illumination(normal cycle).
d- Porphyridium, 8 days of darkness following normal cycles.
See material and methods for details. M= marker DNA, S= G+C rich satellite DNA.

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mic culture conditions: the Porphyridium sp. suspension, when plated on nutrient agar, reveals the presence of bacteria, but

DNA extracted from growing cultures of these bacteria bands at 1.696 and no satellite DNA is detectable over the critical buoyant density range.

Biological properties.

In these two algae the G+C rich satellite DNA appears only during given physiological states; for usual cycles, it becomes detectable at the beginning of the dark phase and disappears within the first hours of illumination (fig.1-c). The Porphyridium sp. satellite DNA accumulates when these algae are kept in darkness for more than the usual period (8 days); in these conditions, the relative amount of satellite DNA appears quite enormous (fig.1-d); however an intensively selective extraction cannot be ruled out.This dramatic amount of satellite DNA then disappears with the first hours of illumination. The appearance of the satellite DNA seems to not require the whole cycle since it appears at the expected time in algae kept in light after several cycles, or kept at reduced light (500 lux). A low temperature (4°C) does not prevent its occurence.

In the case of Chlorella and Porphyridium, duplication of chromosomal DNA takes place just at the end of the light period and mitosis occurs at the beginning of the dark period. Any further division requires another light exposure, which implies an active resumption of the metabolism. The G+C rich satellite DNA thus seems to occur during the physiological state in which cellular division is known to never take place, even if the restrictive conditions are maintained. This point has been checked by cell counting.

DISCUSSION

Chlorella pyrenoidosa and Porphyridium sp. do contain a G+C rich satellite DNA, likely very similar to the one described in higher plants .The study of complete similarity requires further experiments, including the nuclear localisation and the hybridisation with ribosomal RNA. Its synthesis appears desynchronised from that of the classical nuclear DNA, as it has been demonstrated by labelling time-course experiments for the G+C rich nuclear satellite DNA of plants (13). In a similar work achieved on Euglena gracilis (another -but quite different in many regards- unicellular algae), RODE (14) never detected such a satellite DNA, in spite of numerous different culture conditions.

Occurence of such unusual satellite DNA's has been recently reviewed for numerous organisms, unicellular ones included (15). IWAMURA (16) recently reported the presence of a satellite DNA in Chlorella ellipsoidea (L_2 cells, cf. TAMIYA, 17) which bands at 1.722. This DNA has been claimed of mitochondrial origin, without convincing evidence in our opinion. SUYAMA and GIBSON (18) described the presence of two bands of DNA in Rhodospirillum spheroides (1.725 and 1.713) and in Chromatium (1.730 and 1.724). A chromatophore origin was ruled out, but which of the two bands corresponds to the classical DNA was not indicated. A satellite DNA has been likewise reported in axenic cultures of Halobacterium salinirum and Halobacterium cutirubrum (19); the two DNA's band at 1.718 and 1.726; which is the classical DNA was not evidenced. The last two examples deal with Blastocladiella emmersonnii with a nucleolar satellite DNA at 1.725 (20) and Chlamydomonas reinhardii with a satellite DNA at 1.715. This later DNA exhibits metabolic properties which make possible the relation with the Nh satellite DNA of plants, since it appears during zygote maturation and disappears with zygote germination (21). What could be the biological significance of such satellite DNA's? Looking for a possible r-DNA nature, as demonstrated for the Nh satellite DNA of plants (2, 23) may be worthy.

It becomes obvious, according to the litterature, that such r-DNA occur in many different organisms (15) and are associated with gene amplification. Indeed, this later phenomenon may be looked on as a wide-spread informational mechanism, if related, for instance, to the "working DNA" (22, 23). Moreover, the disappearance of this G+C rich satellite DNA, as demonstrated for plants, cannot be completely explained by the "dilution" resulting from cellular multiplication; this phenomenon could involve such mechanisms as the chromatin elimination (see 25, 24, 23). The regulation of this amplification remains until presently far from understood (23). In any case, it obviously appears that much more information is required upon our extraction of DNA, especially regarding quantitative yields and selectiveness. Such a work is under investigation in our lab (26).

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