HYBRIDIZATION STUDIES OF BLUE-GREEN ALGAL AND HIGHER PLANT CHLOROPLAST DNA

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1. Introduction

The endosymbiotic origin of chloroplasts has recently become a very popular hypothesis. There is considerable evidence from some comparative studies of RNA [1] and proteins [2] which supports the hypothesis that chloroplasts originated from blue-green algae. Surprisingly, there have only been a few studies reported on the direct comparison of these two types of DNA [3]. Kung et al. [4] reported that the physicochemical properties of the DNA of blue-green algae and bacteria were more alike than those of blue-green algal and chloroplast DNA. Thus, although this result offered little support to the endosymbiotic origin of chloroplasts, it did not disprove it. Pigott and Carr [5] demonstrated by the membrane filter procedure that rRNA from several blue-green algae hybridized to the DNA of *Euglena gracilis* chloroplasts, thus indicating a genetic homology between blue-green algae and chloroplasts from *Euglena gracilis.* As far as I am aware, there is no conclusive evidence from DNA-DNA hybridization studies made between blue-green algae and chloroplasts. In this report, I wish to present evidence obtained by DNA–DNA hybridization studies between a blue-green alga and higher plant chloroplasts, which indicates some degree of base sequence homology.

2. Methods

Chloroplasts from young, broad bean leaves were prepared as described by Kung and Williams [6], applying their criteria of purity for preparations. Axenic cultures of blue-green algae, *Lyngbya* sp., were obtained from Carolina Biological Supply Co. (Indiana University Culture Collection No. 487) and grown at 25–30° in 1000 ml Erlenmeyer flasks containing 400 ml liquid media with a constant supply of air. The cultures were harvested by filtration and checked for bacterial contamination before extracting DNA.

DNA's from broad bean chloroplasts and *Lyngbya* sp. were prepared and characterized as previously described [6–7]. The DNA's at a concentration of 20 μg/ml in double-strength 0.15 M sodium chloride and 0.015 M trisodium citrate (SSC), pH 7.2, were denatured by heating for 10 min at 100° and renatured for 2–20 hr at 60° [8]. In the hybridization experiments, an equal amount (10–15 μg/ml) of chloroplast and *Lyngbya* sp. DNA, and *Lyngbya* sp. and *Bacillus subtilis* DNA were mixed and annealed together. The incubation time used for annealing was 16 hr. The hybrid formed was detected by its position in the analytical ultracentrifuge after banding in a CsCl density gradient [10]. CsCl (optical grade) density-gradient centrifugation was carried out in a Spino Model E analytical ultracentrifuge at 44,77 rpm, and U.V. absorption photographs scanned with an Analytrol.

3. Results

Fig. 1 is the densitometer tracings obtained from the U.V. photograph of density gradient centrifugation. Renatured DNA from broad bean chloroplasts, *Bacillus subtilis* and *Lyngbya* sp. banded at their corresponding density (table 1) of 1.698, 1.706 and

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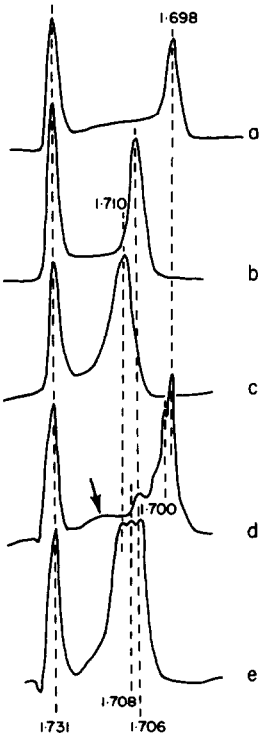

Fig. 1. Hybridization experiments of DNA's from broad bean chloroplasts, *Lyngbya* sp. and *Bacillus subtilis*. Densitometer tracings of U.V. absorption photographs of DNA banded in CsCl density gradients. a, renatured chloroplast DNA; b, renatured *Bacillus subtilis* DNA; c, renatured *Lyngbya* sp. DNA; d, hybrid formed between chloroplast and *Lyngbya* sp. DNA's; e, hybrid formed between *Bacillus subtilis* and *Lyngbya* sp. DNA's. The CsCl density gradients were centrifuged for 24 hr at 44,770 rpm at 25° with *Micrococcus lysodeikticus* DNA (p = 1.731 g/cm^3) as marker.

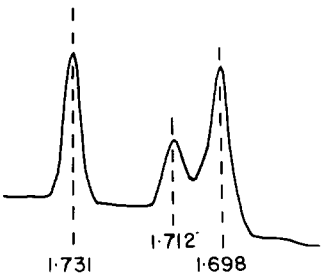

Fig. 2. The absence of sequence homology between broad bean chloroplast DNA (1.698 g/cm^3) and calf thymus DNA (1.712 g/cm^3).

1.710 g/cm^3, respectively (fig. 1a–c). When chloroplast and *Lyngbya* sp DNA's were mixed and annealed together, two bands and a shoulder of intermediate density were formed in addition to the renatured chloroplast DNA (1.698 g/cm^3) and some unpaired (denatured) *Lyngbya* sp. DNA (arrow) (fig. 1d). The bands at 1.700 and 1.706 g/cm^3 and the shoulder were intermediate between the renatured chloroplast (1.698 g/cm^3) and *Lyngbya* sp. (1.710 g/cm^3) DNA bands and, therefore, represent hybrids formed by annealing these two types of DNA's. The formation of hybrids with different densities suggests that these hybrids either have different proportions of double and single stranded regions, and/or have different lengths of chloroplast and blue-green algal DNA. Nevertheless, the formation of such hybrids suggests that chloroplast and *Lyngbya* sp. DNA have some nucleotide sequences in common. Since this is only a qualitative study, no quantitative estimation can be derived, and there-

Table 1
Buoyant densities of DNA's used in the hybridization studies.

DNA	Buoyant density (g/cm^3)			G + C content	References
	Native	Denatured	Renatured		
Broad bean chloroplast	1.696	1.712	1.698	37	6
Lyngbya sp.	1.708	1.722	1.710	46	4
* *Bacillus subtilis*	1.704	1.720	1.706	44	
* Calf thymus	1.699	1.714	1.712	39	

* Unpublished data.

fore to what extent sequence homologies exist between them is unknown. However, judging from the small amount of hybrids formed, it is quite clear that the nucleotide sequences they have in common is not large. This is not surprising in view of the distance between them on the evolutionary scale. If the DNA from two closely related organisms such as blue-green alga and red alga are hybridized, the extent of sequence homology, if any, would be expected to be much greater than that reported here.

Fig. 1e illustrates that a hybrid was also formed between bacterial (*Bacillus subtilis*) and blue-green algal (*Lyngbya* sp.) DNA. The hybrid banded at an intermediate density of 1.708 g/cm^3 between the renatured bacterial (1.706 g/cm^3) and blue-green algal DNA (1.710 g/cm^3). This again indicates that the hybrid consists of both types of DNA's. This result is in agreement with earlier reports [4, 7] that bacterial and blue-green algal DNA's are closely related.

The formation of hybrids in the above experiment is reproducible and species specific [10] since no hybrid was formed between broad bean chloroplast and calf thymus DNA's (fig. 2). As was previously reported [11], no hybrid could be detected between the DNA's of two widely separated organisms, such as broad bean chloroplast and rat liver nuclear DNA, or broad bean nuclear and rat liver mitochondrial DNA. Thus, the hybrid formed should be viewed as an indication of sequence homology as suggested by Britten and Waring [12], not simply a loose association resulting from a change occurrence. This was further supported by the evidence obtained from the control experiments. No hybrid was observed when chloroplast and *Lyngbya* sp. DNA's were annealed separately and then mixed just before CsCl density-gradient centrifugation.

4. Discussion

The application of the CsCl density gradient technique for DNA–DNA hybridization studies has proved to be very useful [10, 11, 13, 14]. The strong species specificity of the hybrid formed indicates that the DNA strands are held together by the association of complementary sequences [10, 11]. It can therefore be used as a valid test for sequence homology.

It should be mentioned that in the hybridization experiments of chloroplast and *Lyngbya* sp. DNA's (fig. 1d), the majority of chloroplast DNA renatured (1.968 g/cm^3), while a portion of the *Lyngbya* sp. DNA remained denatured (arrow). This agrees with the previous report [7] that chloroplast DNA (broad bean) renatured within 30 min, whereas *Lyngbya* sp. DNA renatured only after 24 hr. Thus, the incubation time (16 hr) used in the hybridization experiment was sufficient to allow chloroplast DNA but not the *Lyngbya* sp. DNA to renature. Based on the rate of renaturation and other observations, it is reasonable to assume that the molecular complexity (genome size) of the *Lyngbya* sp. DNA is almost certainly much greater than that of the chloroplast DNA.

Based on buoyant density measurements (table 1), the hybrid which banded at 1.706 g/cm^3 was that formed between chloroplast and *Lyngbya* sp. DNA's. The other band (1.700 g/cm^3) was probably a hybrid formed between a double-stranded (renatured) chloroplast DNA and a single-stranded (denatured) *Lyngbya* sp. DNA.

Although the density gradient technique used in this study is not quantitative, it does allow one to visualize the formation of hybrids. This report presents evidence suggesting qualitative partial homology between chloroplast (broad bean) and blue-green algal (*Lyngbya* sp.) DNA.

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