A eukaryotic genome of 660 kb: electrophoretic karyotype of nucleomorph and cell nucleus of the cryptomonad alga, *Pyrenomonas salina*

Stefan Eschbach, Claudia J.B.Hofmann, Uwe-G.Maier, Peter Sitte and Paul Hansmann
Institut für Biologie II der Universität, Zellbiologie, Schaenzlestrasse 1, D-7800 Freiburg, FRG

Received January 28, 1991; Revised and Accepted March 19, 1991

ABSTRACT

Cryptomonads are unicellular algae with chloroplasts surrounded by four membranes. Between the inner and the outer pairs of membranes is a narrow plasmatic compartment which contains a nucleus-like organelle called the nucleomorph. Using pulsed field gel electrophoresis it is shown that the nucleomorph of the cryptomonad *Pyrenomonas salina* contains three linear chromosomes of 195 kb, 225 kb and 240 kb all of which encode rRNAs. Thus, this vestigial nucleus has a haploid genome size of 660 kb, harboring the smallest eukaryotic genome known so far. From the cell nucleus of *P. salina* at least 20 chromosomes ranging from 230 kb to 3.000 kb were fractionated. Here, the rDNA was detected on a single chromosome of about 2.500 kb.

INTRODUCTION

According to the endosymbiont hypothesis, eukaryotes aquired their chloroplasts through endosymbiotic uptake of a photosynthetic prokaryote (1–3). However, a modified hypothesis has been put forward concerning the origin of chloroplasts surrounded by more than the usual two membranes, as they are found in the majority of algal taxa (e.g. Dinophyta, Chromophyta, Cryptophyta, and Euglenophyta; see ref. 4). It has been suggested that these 'complex' chloroplasts might have originated from a symbiosis between a heterotrophic host cell and a eukaryotic photosynthetic endosymbiont rather than a prokaryotic one, and that the endosymbiont has been reduced step by step during evolution, leaving finally only the chloroplast and one or two supernumerary envelope membranes in the host cell cytoplasm (5,6).

Cryptomonads like *Pyrenomonas salina* are of particular interest, as they possibly represent an intermediate stage in this evolution. These unicellular algae are composed of two eukaryotic compartments. Firstly, the cytoplasm, containing the cell nucleus and one mitochondrion, but devoid of chloroplasts. Secondly, a periplastidal compartment (7), which is separated from the cytoplasm by two membranes and harbors the chloroplast, eukaryotic ribosomes (8) and the nucleomorph.

Ultrastructurally, the nucleomorph shows typical features of a eukaryotic nucleus: a double membrane envelope with pores (9), a nucleolus-like zone (10), DNA (11,12) and RNA (13), and duplication by an amitotic process (14,15). Up to now, however, it has not been unequivocally established that the nucleomorph truly represents a vestigial eukaryotic nucleus, as no molecular data on the nucleomorph have been reported. Recently, the nucleomorph as well as the cell nucleus of *P. salina* were purified (16). In the present study we used pulsed field gel electrophoresis (PFGE; see ref. 17) to obtain the first information about the organization of DNA in the nucleomorph and the cell nucleus.

MATERIALS AND METHODS

DNA inserts

Pyrenomonas salina was cultured and harvested as described previously (13).

For preparation of total *P. salina* DNA the cells were suspended in buffer 1 (10 mM Tris/HCl pH 8.0, 100 mM Na_2EDTA pH 8.0, 100 mM NaCl) and rapidly mixed at 30°C with one volume buffer 2 (buffer 1, plus 2 mM aurintricarboxylic acid (ATA) pH 7.0, 1% low gelling temperature agarose [SeaPlaque, FMC Corporation, Rockland, USA], and 2 mg/ml proteinase K). Aliquots of 150 µl, which contained 2×10^7 cells, were then transferred into precooled sample holders. Upon solidification the agarose blocks were incubated for 10 min at 50°C in buffer 3 (10 mM Tris/HCl pH 8.0, 400 mM Na_2EDTA pH 8.0, 1% sodium N-lauroylsarkosinate, 1 mM ATA pH 7.0), plus 0.25 mg/ml pronase. Thereafter Inserts were added to buffer 3, plus 1 mg/ml pronase and incubated for 48 h at 50°C. The resulting Inserts were stored at 4°C in buffer 4 (10 mM Tris/HCl pH 8.0, 400 mM Na_2EDTA pH 8.0).

To prepare agarose blocks containing nucleomorph DNA, Pyrenoid-nucleomorph complexes were isolated using the method described previously (16). Organelles from 5×10^8 or 8×10^9 cells were suspended at 37°C in 150 µl of buffer 5 (50 mM Tris/13 mM EDTA pH 8.0, 500 mM sucrose, 1 mM ATA pH 7.0, 0.5% low gelling temperature agarose). The suspension was transferred into precooled sample holders and further processed as described above.

Lamda-ladders and chromosomes of yeast were used as size markers for PFGE and were prepared according to the protocol described elsewhere (18).

General electrophoretic conditions

Electrophoresis was carried out in a home-made apparatus constructed according to Schwartz et al. (19). The alternating field orientations were generated by the Pulsaphore Plus control unit (Pharmacia Uppsala, Sweden). Separation conditions were as follows: TBE buffer (45 mM Tris, 45 mM boric acid, 1.25 mM Na_2EDTA, pH 8.3), 1% agarose, at a temperature of 10°C. Field strength, field reorientation time, and running time are indicated in figure legends.

Southern blotting and hybridizations

Gels were blotted onto Hybond-N membranes (Amersham Buchler, Braunschweig, FRG) after UV nicking (20). The probes were prepared by the random hexamer priming method (21) using [α^{32}P]dGTP. The blots were washed at 65°C in 0.5×SSC, and 0.1×SSC (1×SSC = 0.15 M NaCl, 0.015 M Na_3citrate, pH 7.0).

RESULTS

Nucleomorph chromosomes

Fractionation of chromosome-sized DNA molecules from *P. salina* is shown in Fig. 1a. When subjected to PFGE, DNA from isolated nucleomorphs was resolved into three distinct bands. These three chromosomes with sizes of 195 kb, 225 kb, and 240 kb could also be separated from total *P. salina* cells. No additional nucleomorph specific bands could be detected by probing PFGE-blots with DNA from isolated nucleomorphs (Fig.1b), or by changing PFGE conditions (data not shown). Hence, the nucleomorph of *P. salina* contains only three linear chromosomes or multiples of them. Our hybridization data indicated that the three distinct nucleomorph DNA bands are unlikely to be generated by chromosome breakage, as no smear of DNA was observed (Fig. 1b).

In order to identify the non-nucleomorph specific bands of PFGE fractionated total DNA from *P. salina* (Fig. 1a), PFGE-blots were probed with homologous mitochondrial and plastidal DNA (Fig. 1b). The hybridization data showed that the smallest band (130 kb) represented arteficially linearized plastidal DNA. On account of the earlier finding (22) that plastidal and mitochondrial DNAs of *P. salina* are circular molecules of about 130 kb and 40 kb, respectively, the hybridization data are congruent with the results of Beverley (23) that circular DNAs remain entrapped in the agarose insert and do not migrate under PFGE conditions. Hybridization with DNA from isolated cell nuclei revealed that DNA of the smallest cell nucleus chromosome formed a band at the 230 kb position, while the other cell nucleus chromosomes migrated as a major compression band of larger molecules (Fig. 1a,b).

Cell nucleus chromosomes

With varying pulse times at least 20 individual DNA bands could be separated from total cells of *P. salina* (Fig.2), representing the cell nucleus chromosomes. Using lambda-oligomers,

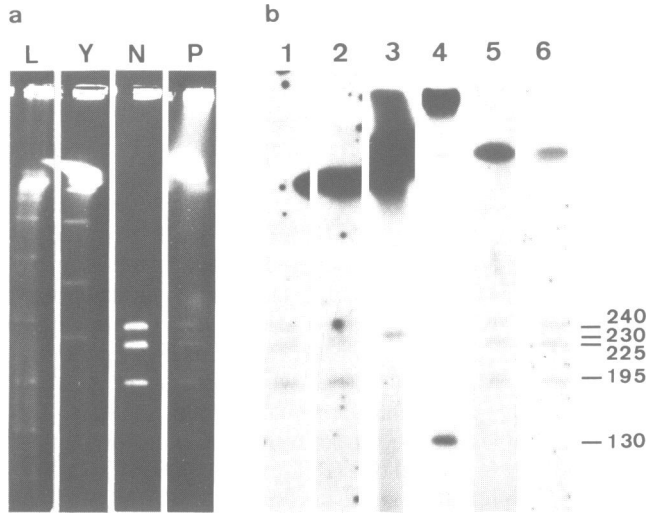

Figure 1. Nucleomorph karyotype. (a) Fractionation of nucleomorph chromosomes by pulsed field gel electrophoresis. Samples loaded are oligomers of phage lambda DNA (L), *Saccharomyces cerevisiae* chromosomes (Y), DNA from isolated nucleomorphs (N), and total DNA of *P. salina* (P). Gels were run with a field strength of 5 V/cm (totat 120 V, about 100 mA), a field reorientation time of 20 s, and a running time of 44 h. (b) PFGEs of DNA from isolated nucleomorphs (lane 1) and total cells (lanes 2–6) were blotted to nylon filters and then hybridized with DNA from isolated nucleomorphs (lanes 1 and 2), DNA from isolated cell nuclei (lane 3), plastidal DNA (lane 4), a 18S rDNA probe (lane 5) and a 25S rDNA probe (lane 6). Running conditions as in (a), with running times of 42 h (lane 1 and 2) and 44 h (lane 3–6). DNAs for the homologous probes were isolated as described previously (16,22). The rDNA probes were fragments of maize nuclear rRNA-genes (25). At the right are the estimated sizes in kilobase pairs (kb) of the three nucleomorph chromosomes (195 kb, 225 kb, and 240 kb), the smallest cell nucleus chromosome (230 kb), and the plastidal linear DNA (130 kb).

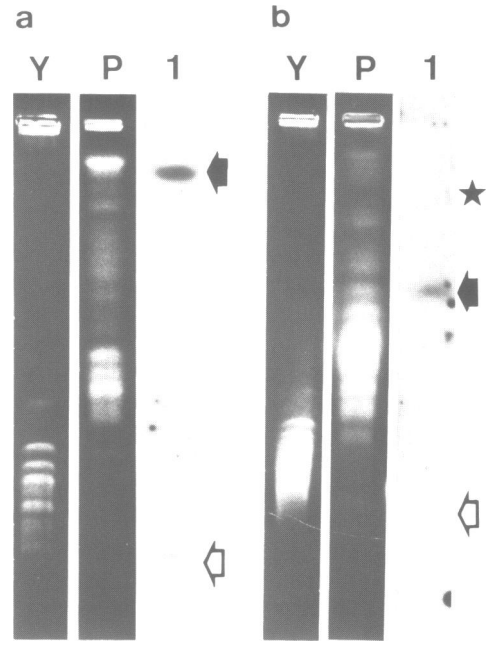

Figure 2. Separation of cell nucleus chromosomes with varying resolutions, and hybridizations with homologous 18S rDNA, respectively. Samples loaded are *S. cerevisiae* chromosomal DNA (Y) as size markers ranging from 200 kb to 2.500 kb (18, 26, 27) and total *P. salina* DNA (P). PFGE-blots of the latter (lane 1) were hybridized with the cell nuclear 18S rRNA-gene from *P. salina* (28). Running conditions as in Fig. 1, with an agarose concentration of 0.8% and 3 V/cm field strength; pulse times and running times were as follows: (a) 900 s, 168 h; (b) 1100 s, 140 h. In the blot panels, solid arrows indicate the band corresponding to the rRNA coding cell nucleus chromosome, whereas open arrows indicate the band corresponding to the three nucleomorph chromosomes. The position of the smallest *Schizosaccharomyces pombe* chromosome (3.000 kb [29]) relative to the cell nucleus chromosomes is indicated by an asterisk.

Saccharomyces cerevisiae chromosomes, and *Schizosaccharomyces pombe* chromosomes as size markers, the cell nucleus chromosomes could be estimated to be between 230 kb and 3.000 kb in size. The precise number of chromosomes in the cell nucleus of *P. salina* could not be determined, as the banding pattern varied depending on PFGE conditions.

Localization of the rDNA

In order to localize rDNA on individual cell nucleus chromosomes of *P. salina*, PFGE-blots were hybridized with a homologous probe (Fig. 2). It could be demonstrated that the rDNA of cell nuclei is located on a single chromosome with a size of about 2.500 kb. In contrast, all three chromosomes of the nucleomorph hybridized with eukaryotic 18S and 25S rDNA probes (Fig. 1b).

DISCUSSION

In this paper we demonstrate that the nucleomorph of the cryptomonad *P. salina* contains only three linear chromosomes of 195 kb, 225 kb, and 240 kb in size. Since the nucleomorph chromosomes always appeared to be present in the same copy number (Fig 1a), the haploid genome size of the nucleomorph can be calculated as the sum of sizes of the three single chromosomes, resulting in 660 kb. This is the smallest eukaryotic genome known so far. It is even smaller than the genomes of many prokaryotes (e.g., *E. coli* with 4.700 kb [24]). The DNA content of this organelle was determined earlier to be in the range of 1.300 kb and 2.800 kb (16). Hence, the nucleomorph of *P. salina* contains between two and four sets of chromosomes.

Considering the ultrastructural findings together with the presented karyotype of the nucleomorph of *P. salina*, this organelle now meets all criteria of a reduced eukaryotic nucleus. Hence, the nucleomorph turns out as a real 'King's evidence' in favor of the hypothesis that cryptomonads (and all other algae containing complex plastids) acquired their chloroplasts by permanently harboring a photosynthetic *eukaryotic* endosymbiont.

By using *P. salina* as a model system it will be possible to unravel some of the problems encountered by symbioses between two eukaryotic organisms. Since the nucleomorph of *P. salina* contains a haploid genome of only 660 kb, most of the genetic information of this endosymbiotic nucleus has been lost or has been transferred to the (host) cell nucleus. However, genetic reduction of the nucleomorph is not complete, as all three nucleomorph chromosomes code for rRNA genes. It can be postulated that these nucleomorph genes are actively transcribed, since McFadden (8) demonstrated using in situ-hybridization that the ribosomes of the periplastidal compartment contain eukaryotic 18S rRNAs. Up to now, the significance of the endosymbiont ribosomes of the cryptomonads is not known. In order to get more information on this problem we are at present looking for nucleomorph encoded proteins.

ACKNOWLEDGEMENTS

We thank Dr. Gabor Igloi for helpful discussions and critical comments on the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft (DFG).

REFERENCES

1. Margulis, L. (1981) Symbiosis in Cell Evolution. Life and its Environment on the Early Earth. Freeman, San Francisco.
2. Gray, M.W. and Doolittle, W.F. (1982) *Microbiol. Rev.*, **46**, 1–42.
3. Gray, M.W. (1989) *Trends Genet.*, **5**, 294–299.
4. Dodge, J.D. (1973) The fine structure of algal cells. Academic press, London.
5. Gibbs, S.P. (1981) *Ann. NY Acad. Sci.*, **361**, 193–208.
6. Wathley, J.M. (1981) *Ann. N.Y. Acad. Sci.*, **361**, 154–165.
7. Gibbs, S.P. (1962) *J. Cell Biol.*, **14**, 433–444.
8. McFadden (1990) *J. Cell Sci.*, **95**, 303–308.
9. Greenwood, A.D., Griffiths, H.B. and Santore, U.J. (1977) *Br. Phycol. J.*, **12**, 119.
10. Gillott, M.A. and Gibbs, S.P. (1980) *J. Phycol.*, **16**, 558–568.
11. Ludwig, M. and Gibbs, S.P. (1985) *Protoplasma*, **127**, 9–20.
12. Hansmann, P., Falk, H., Scheer, U. and Sitte, P. (1986) *Eur. J. Cell Biol.*, **42**, 152–160.
13. Hansmann, P. (1988) *Protoplasma*, **146**, 81–88.
14. McKerracher, L. and Gibbs, S.P. (1982) *Can. J. Bot.*, **60**, 2440–2452.
15. Morrall, S. and Greenwood, A.D. (1982) *J. Cell Sci.*, **54**, 311–328.
16. Hansmann, P. and Eschbach S. (1990) *Eur. J. Cell Biol.*, **52**, 373–378.
17. Schwartz, D.C. and Cantor, C.R. (1984) *Cell*, **37**, 67–75.
18. Vollrath, D. and Davis, R.W. (1987) *Nucleic Acids Res.*, **15**, 7865–7876.
19. Schwartz, D.C., Smith, L.C., Baker, M. and Hsu M. (1989) *Nature*, **342**, 575–576.
20. Orbach, M.J., Vollrath, D., Davis, R.W. and Yanofsky, C. (1988) *Mol. Cell. Biol.*, **8**, 1469–1473.
21. Feinberg, A. and Vogelstein. B. (1983) *Anal. Biochem.*, **132**, 6–13.
22. Hansmann, P., Maerz, M. and Sitte, P. (1987) *Endocyt. C. Res.*, **4**, 289–295.
23. Beverley, S.M. (1988). *Nucleic Acids Res.* **16**, 925–939.
24. Kohara, Y.K., Akiyama, K. and Isono, K. (1987) *Cell*, **50**, 495–508.
25. Toloczyki, C. and Feix, G. (1986) *Nucleic Acids Res.*, **14**, 4969–4986.
26. Carle, G.F. and Olson, M.V. (1985) *Proc. Natl. Acad. Sci.*, **82**, 3756–3760.
27. Mortimer, R.K. and Schild, D. (1985) *Micro. Rev.*, **49**, 181–212.
28. Eschbach, S., Wolters, J. and Sitte, P. (1991) *J. Mol. Evol.*, in press.
29. Smith, C.L., Matsumto, T., Niwa, O., Klco, S., Fan, J.-B., Yanagida, M. and Cantor, C.R. (1987) *Nucleic Acids Res.*, **15**, 4481–4489.