Biochimica et Biophysica Acta, 653 (1981) 412—415
Elsevier/North-Holland Biomedical Press

BBA Report

BBA 91517

THE CHLOROPLAST GENOME OF BLEACHED MUTANTS OF *EUGLENA GRACILIS*

P. HEIZMANN, J. DOLY, Y. HUSSEIN, P. NICOLAS, V. NIGON and G. BERNARDI

Laboratoire de Biologie Cellulaire, Université de Lyon I, Lyon and Laboratoire de Génétique Moléculaire, Institut de Recherche en Biologie Moléculaire, Paris (France)

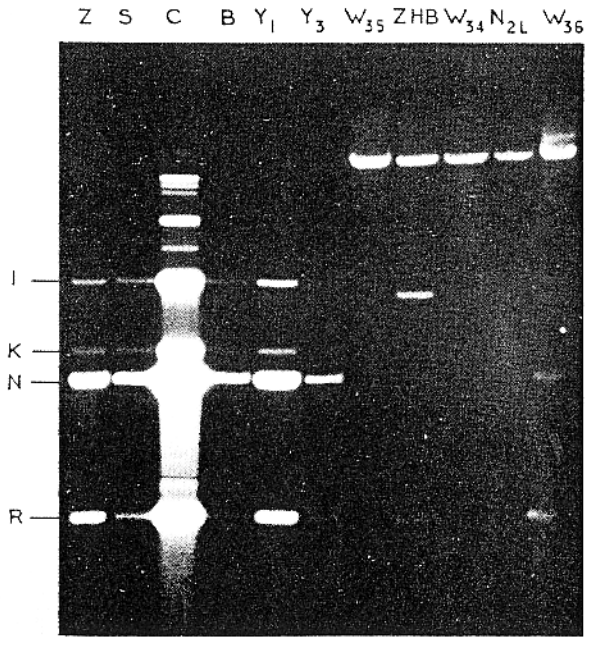
(Received January 29th, 1981)

Key words: Chloroplast DNA; Defective genome; rRNA gene; Bleached mutant; (E. gracilis)

Summary

Bleached mutants of *Euglena gracilis*, traditionally thought to be completely deprived of chloroplast DNA, have been shown to contain a defective chloroplast genome, present at a very low copy number, and preferentially retaining ribosomal RNA genes. We propose to call these mutants ϕ^-, because of their resemblance with the ρ^- mutants of yeast.

The unicellar flagellate *Euglena gracilis* can undergo mutations causing the irreversible loss of chloroplast function [1—3]. Some of these mutants seem to contain normal amounts of non-functional chloroplast DNA; other mutants show no evidence of chloroplast DNA and have been considered as deprived of it [1—3]. The first indication that mutants of this second class might contain remnants of the chloroplast genome came from the demonstration of small amounts of chloroplast ribosomal RNAs accumulating as 23 S, 16 S and 16 S precursor in bleached mutants treated with cycloheximide plus lincomycin [4,5]. Here we provide direct evidence for the existence in these mutants of small amounts of chloroplast DNA exhibiting massive deletions. The experimental approach used has been to detect the chloroplast DNA of the mutants by hybridizing cloned restriction fragments from chloroplast DNA of wild-type cells on restriction fragments from total DNA of the mutants. The use of this strategy is imposed by the requirement of complete purity of the chloroplast DNA probes from wild-type cells, contamination by nuclear DNA being a source of ambiguity.



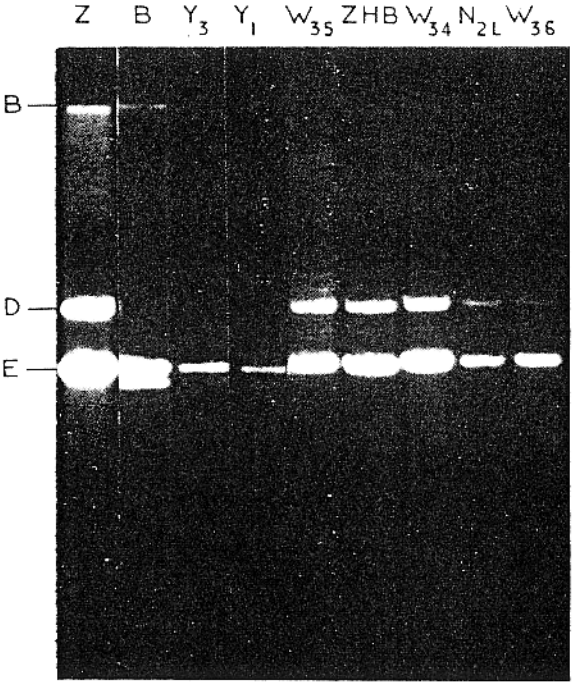


Fig. 1. Hybridization of ^{32}P-labelled $2 \cdot 10^7$ to $5 \cdot 10^7$ cpm/μg DNA) pSF2124 hybrid plasmids containing EcoRI fragments I, K, N and R from chloroplast DNA of *E. gracilis* var. Z on EcoRI hydrolysates of (a) 1 μg of chloroplast DNA from *E. gracilis* var. Z (C); (b) 3 μg of total DNAs from *E. gracilis* var. Z (Z) and var. B (B), and from mutants S^r_m 11.16 (S), Y_1BXD (Y_1), Y_3BUD (Y_3); (c) 12 μg of total DNAs from mutants W_{35}, ZHB, W_{34}, N_2L and W_{36}. Autoradiograms were obtained using XOmat-R films (Kodak) with Fast tungstate screens (Ilford) at —70°C after 24—72 h for series a and b, 15 days for series c. Hybridization bands above I in the case of sample C correspond to partially digested fragments. The common strong hybridization band exhibited by samples of series c is due to a spurious hybridization of the vector plasmid.

Fig. 2. Hybridization of ^{32}P-labelled ($6 \cdot 10^7$ cpm/μg DNA) pSF2124 hybrid plasmids containing EcoRI fragments P and J from chloroplast DNA of *E. gracilis* var. Z on BamHI hydrolysates of (a) 1 μg of chloroplast DNA from *E. gracilis* var. Z (C); (b) 3 μg of total DNAs from *E. gracilis* var. B (B), and mutants Y_1BXD (Y_1), Y_3BUD (Y_3); (c) 12 μg of total DNAs from mutants W_{35}, ZHB, W_{34}, N_2L and W_{36}. Autoradiograms were obtained as described in the legend of Fig. 1.

EcoRi fragments from DNA extracted from chloroplasts isolated from *E. gracilis*, var. Z, and purified by centrifugation in a CsCl density gradient, were cloned using plasmid pSF 2124 [6] as the vector and *Escherichia coli* WS445 as the host cells. Hybrid plasmids were isolated, labelled by nick-translation [7] to $2 \cdot 10^7$ to $5 \cdot 10^7$ cpm/μg, and used as chloroplast DNA probes. These probes were hybridized [7] on EcoRI or BamHI fragments obtained from: (a) chloroplast DNA from *E. gracilis* var. Z, in order to obtain a self-annealing control; (b) total DNAs from *E. gracilis* var Z and var. B (bacillaris), chlorophyll-less strains Y_1BXD and Y_3BUD and streptomycin-resistant strain S^r_m 11.16, all known to contain chloroplast DNA [2,3]; (c) total DNAs from bleached strains W_{34}ZUD, W_{35}ZEmsD. W_{36}ZHD, ZHB and N_2L, all supposed to contain no chloroplast DNA [8,9].

Fig. 1 shows the expected detection of EcoRI fragments I, K, N and R on the DNAs from series a and b. The DNA from mutant W_{36} showed the presence of fragments N and R and of a very large fragment not corresponding to any control DNA fragment; the DNA from mutant ZHB fragment R and another fragment different in size from those present in the control DNA. Preliminary experiments (not shown) with EcoRI fragments J, M and Q, were again positive for series a and b, but only showed the presence of fragment Q in W_{35}, W_{34}, N_2L and, possibly, in W_{36}.

Fig. 2 presents the hybridization of EcoRI fragments P + J on BamHI fragments. Fragment P is a ribosomal DNA probe (see Fig. 3 and Refs. 10 and 11). Again this revealed the expected positive results for the DNAs of series a and b; the differences found for DNAs from strains Z and B are also expected since the ribosomal DNA region has a different BamHI restriction map in chloroplast DNAs from strains Z and B [12]. In contrast with the results of Fig. 1, however, this ribosomal probe gave definitely positive results with all DNAs of series c.

The main conclusion of the present work is that bleached Euglena mutants supposed for the past 15 years to contain no chloroplast DNA, do contain it. The amount of chloroplast DNA in these mutants appears to be very low, compared with wild-type strains and other bleached mutants known to contain chloroplast DNA. Furthermore, a number of restriction fragments appear to be absent from these DNAs and fragments having sizes different from those of chloroplast DNA from wild-type cells have been detected. Among the residual fragments, those corresponding to ribosomal cistrons appear to be regularly present. The situation found in these bleached mutants is reminiscent of that of

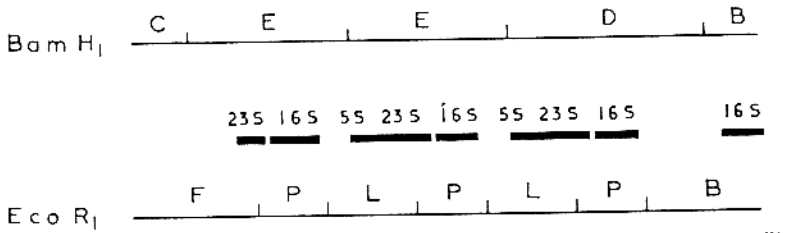


Fig. 3. BamHI and EcoRI restriction map of the chloroplast genome of *E. gracilis* var. Z in the region of ribosomal genes. The three pairs of ribosomal gene are indicated by solid lines (adapted from Refs. 8 and 9).

ρ^- mutants of yeast [13], except that the defective chloroplast genomes show no sign of amplification. We propose to all these bleached mutants ϕ^-. The strongly reduced amount of chloroplast DNA and the predominance in these defective genomes of the genome chloroplast-rich ribosomal cistrons explain why these mutants were not found to contain any chloroplast DNA. It is of interest that ribosomal genes appear to be preferentially retained in ϕ^- mutants; one explanation for this observation may be that the ribosomal genes are located near the origin of replication of the chloroplast genome and that this origin is preserved in the defective genomes, as is the case in spontaneous ρ^- mutants of yeast [13]. In connection with this point, it should be recalled that 'stopper' mutants of *Neurospora crassa* also present defective mitochondrial genomes preferentially retaining ribosomal genes and that the same explanation has been proposed [14].

Another conclusion of this work concerns the other class of bleached mutants which exhibit all the restriction fragments of the genomes from the parental wild-type cells, a normal amount of chloroplast DNA, and yet have irreversibly lost any chloroplast function. These mutants resemble the mit^- mutants of yeast, which are characterized by small deletions in mitochondrial genes. We propose to call these bleached mutants cp^-. Finally, it is not impossible that there is yet another class of mutants which is completely deprived of chloroplast DNA; if it exists, this class could be called ϕ°, because of its resemblance with the ρ° mutants of yeast which do not contain any mitochondrial DNA.

Mutants Y_1BXD and Y_3BUD have been provided by J.A. Schiff, mutant ZHB by J.R.Y. Rawson, mutant N_2L by R. Calvayrac.

References

1 Schiff, J.A. and Epstein, H.T. (1965) in Reproduction: Molecular, Subcellular, Cellular (Locke, M., ed.), pp. 131—189, Academic Press, New York
2 Schiff, J.A., Lyman, H. and Russell, G.K. (1971) Methods Enzymol. 23, 143—162
3 Schiff, J.A., Lyman, H. and Russell, G.K. (1980) Methods Enzymol. 69, 23—29
4 Heizmann, P., Salvador, G.F. and Nigon, V. (1976) Exp. Cell Res. 99, 253—260
5 Heizmann, P., Verdier, G. and Younis, H. (1978) in Chloroplast Development (Akoyunoglou, G. and Argyroudi-Akoyunoglou, J.H., eds.), pp. 623—628, Elsevier/North-Holland, Amsterdam
6 So, M., Gill, R. and Falkow, S. (1975) Mol. Gen. Genet. 142, 239—246
7 Jeffreys, A.J. and Flavell, R.A. (1977) Cell 12, 429—439
8 Gruol, D., Rawson, J.R.Y. and Haselkorn, R. (1975) Biochim. Biophys. Acta 414, 20—29
9 Rawson, J.R.Y. and Boerma, C. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 2401—2404
10 Jenni, B. and Stutz, E. (1978) Eur. J. Biochem. 88, 127—134
11 Jenni, B. and Stutz, E. (1979) FEBS Lett. 102, 95—99
12 Helling, R.B., Raafat El-Gewely, M., Lomax, M.I., Baumgartner, J.E., Schwartzbach, S.D. and Barnett, W.E. (1979) Mol. Gen. Genet. 174, 1—10
13 Bernardi, G., Baldacci, G., Bernardi, G., Faugeron-Fonty, G., Gaillard, C., Goursot, R., Huyard, A., Mangin, M., Marotta, R. and de Zamaroczy, M. (1980) in The Organization and Expression of the Mitochondrial Genome (Kroon, A.M. and Saccone, C., eds.), pp. 21—31, Elsevier/North-Holland, Amsterdam
14 Bertrand, H., Collins, R.A., Stohl, L.L., Goewert, R.R. and Lambowitz, A.M. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 6032—6036