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The Chloroplast genome of Algae

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The genetic information required for synthesis and function of chloroplasts is located partially on chloroplast DNA and partially on nuclear DNA. The advances in molecular biology allow to study in detail the anatomy and function of chloroplast DNA and to precisely define the sites of DNA segments coding for rRNA, tRNA and protein coding genes and to study its transcription and transcription regulation. We give a survey of the status quo in algal chloroplast genome research on a molecular level considering in particular the results obtained with *Chlamydomonas reinhardii* and *Euglena gracilis*.

A. General observations

Plastids from lower and higher plants are of dual genetic origin, the majority of relevant genes being accomodated on nuclear DNA (nu DNA) while a minor number of essential genes is located on chloroplst DNA (cp DNA). During the past few years a rapidly increasing amount of information about the anatomy and function of chloroplast DNA has appeared this mainly due to the introduction of restriction endonucleases as analytical tool. As a consequence many complete restriction site maps of several chloroplast genomes were constructed allowing thereby to precisely define the site of DNA segments and to sequence structural genes, e.g., for tRNAs, rRNAs and more recently also the genes coding for proteins. It seems to be just a matter of time until an entire circular chloroplast genome will be sequenced, as is already achieved, e.g., for the human mitochondrial genome (Anderson et al. 1981).

Reviewing the literature on the structural analysis of algal chloroplast genomes reveals that only two kinds of algae have been studied on a molecular level in detail, namely *Chlamydomonas reinhardii* and *Euglena gracilis*. In all other cases the chloroplast DNA is yet poorly characterized. A more detailed study of other algal cpDNA, however, would be of great interest in view of the very wide spectrum of anatomical and functional properties of algal chloroplasts what should have its parallel in a wide spectrum of different kinds of chloroplast genomes.

In table I we list physico-chemical data of chloroplast DNA from several algae. The list is not exhaustive at all but this small collection already indicates that algal cpDNAs

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Travail dédié au professeur Cl. Favarger, à l'occasion de son 70e anniversaire.

can be very different in terms of overall genetic complexity and G + C content. The most complex algal cpDNA so far reported is that from *Acetabularia cliftonii* (150×10^8), the smallest is that of *Codium fragile* (0.56×10^8). Unfortunately, we do not yet know whether a correspondingly large difference in genetic information exists, between the two plastidial genomes, this certainly being a question to be persued. Similarly large differences exist in the average G + C content of cpDNAs, e.g., 25% in *Euglena gracilis* and 53% in *Scenedesmus obliquus* indicating important structural differences in the organization of the two genomes. All chloroplast genomes, probably with the exception of *Acetabularia,* occur in circular form. Furthermore it is accepted that chloroplasts, retaining the capacity to proliferate, must contain at least one copy of the genome. However, functional chloroplasts seem to exist in *Acetabularia mediterranea* (Woodcock & Bogorad 1970) which lack DNA, probably as a result of asymmetric DNA partitioning during chloroplast division (Lüttke & Bonotto 1981).

We include in this list *Polytoma obtusum* which is a colorless algae and a relative of *Chlamydomonas,* containing leucoplasts. It has an extremely low G + C content probably due to extensive deletions, but most remarkably, this genome has retained the genes for rRNA (Siu et al. 1975).

Cyanophora paradoxa, a flagellate of uncertain taxonomic position is capable of growing photoautotrophically and its cyanelles have many features in common with chloroplasts. The cyanelle DNA is about 115 megadaltons, contains two inverted repeats with rDNA sequences, i.e., its a chloroplast type genome comparable to that found in higher plants (Mucke et al. 1980).

Tab. 1. Properties of algal plastid DNA

	% [G + C]	Size (μm); form (EM)	Complexity (dalton) $\times 10^{-8}$
Chlorophytes			
Acetabularia (Polyphysa) cliftonii	37 (D)[a]	up to 400; linear[a]	150 (K)[a]
Chlamydomonas reinhardii	36 (D, M)[b]	62; circular[c]	1.9 (K)[d]; 1.3 (R)[e]
Chorella pyrenoidosa	28 (D, M)[f]	–	1.3 (K)[f]
Codium fragile	37 (M)[g]	27; circular[g]	0.56 (R)[g]
Scenedesmus obliquus	53 (D)[h]	–	–
Polytoma obtusum (leucoplast)	17.4 (D)[i]	–	–
Euglenophytes			
Euglena gracilis	25 (D, M)[j]	44; circular[k]	0.9 (K)[l]; 0.9 (R)[m]
Chrysophyta			
Ochromonas danica	30 (D)[h]	–	–
Olisthodiscus luteus	31 (D)[n]	46; circular[n]	–
Vaucheria sessilis	–	37; circular[o]	–
Rhodophyta			
Poryphyridium cruentum	35 (D)[h]	–	–

[a] Padmanabhan & Green 1978; [b] Sager & Ishida 1963; [c] Behn & Herrmann 1977; [d] Wells & Sager 1971; [e] Rochaix 1978; [f] Bayen & Rode 1973; [g] Hedberg et al. 1981; [h] Charles 1977; [i] Siu et al. 1975; [j] Brawerman & Eisenstadt 1964; Edelman et al. 1964; [k] Manning & Richards 1972; [l] Stutz 1970; [m] Gray & Hallick 1978; [n] Aldrich & Cattolico 1981; [o] cit. in: Herrmann et al. 1980.
EM: determined by electron microscopy; D: calculated from buoyant density results; M: calculated from thermal melting data; K: calculated from reannealing kinetics data; R: calculated from restriction endonuclease analyses.

B. The chloroplast genomes of *Euglena gracilis* and *Chlamydomonas reinhardii*

1. The general anatomy

The chloroplast genomes from *E. gracilis* and *C. reinhardii* are extensively studied both with respect to structure and function (for recent reviews see, respectively, Hallick, 1982; Rochais, 1981). A comparative analysis of the two genomes is interesting for several reasons and especially in view of the large difference in taxonomic position of the two unicellular algae: *C. reinhardii* belongs to the division of chlorophytes, order *volvocales*, while *E. gracilis* belongs to the division of euglenophytes, order *euglenales* (Round 1966). Euglenophytes are not in a direct line with higher plants rather they represent an evolutionary dead end.

In Fig. 1 we show the EcoRI restriction sites map of both genomes in a linearized form. The two maps are approximately aligned with respect to the locus coding for the large subunit protein of the 1,5-bisphosphate-carboxylase (LS). The results of a comparative structural analysis may be summarized as follows: a) The two EcoRI restriction sites maps have no common fragment pattern in any region of the genome, as expected for two only distantly related genomes. b) The *C. reinhardii* genome is about 46 kbp longer than that of *E. gracilis* and carries two inverted repeats of about 19 kbp in size which are 48 kbp apart (small single copy region). By the way, this structural feature is typical for many higher plant chloroplast genomes. c) In *C. reinhardii* the rDNA region is part of two distant inverted repeats but in *E. gracilis* the rDNA is part of three direct contiguous repeats of about 6.2 kbp. d) The tRNA genes are scattered in both cases throughout the genome. Clusters of tRNA genes have been mapped in case of *E. gracilis*. e) The relative position of the few protein genes mapped so far with certainty on both genomes is similar, i.e., starting at the 5′ end of the rRNA operon of *E. gracilis* and *C. reinhardii* (left side of the map) and moving towards the right we find for *E. gracilis* and *C. reinhardii* cpDNA in a distance of about 6 kbp and 4 kbp, respectively, the structural gene for the β-subunit of the coupling factor (β), close to it the structural gene for the large subunit of the ribulose 1,5-bisphosphate carboxylase (LS) and 12 kbp and 15 kbp, respectively, away, the structural gene of the 32 kd thylakoid membrane protein of the photosystem II reaction center (P).

Both genomes seem to contain small repetitive sequences which are interspersed throughout the genome in case of *C. reinhardii* (Rochaix 1972). For *E. gracilis* cpDNA short inverted repeats were physically mapped in the vicinity of the rDNA region (Koller & Delius 1982a).

A detailed cross hybridization study between the two cpDNAs was not yet made, therefore a more detailed comparison of the anatomy of the two algal chloroplast genomes as far as gene arrangement and rearrangement is concerned, is not yet possible as was done with higher plant chloroplast genomes (e.g. Palmer & Thompson 1982).

The EcoRI map shown in Fig. 1 is that of *E. gracilis*, strain Z. A similar structural analysis was made with *E. gracilis*, strain bacillaris (Helling et al. 1979) which gave essentially the same fragment pattern, except for differences in the rDNA regions.

2. The ribosomal DNA region

In Fig. 2 we show structural details of the chloroplast rDNA region of *E. gracilis* and *C. reinhardii*. It is known that in both cases the rRNA genes are arranged on the same strand and in the order 5′ 16S RNA-spacer large ribosomal subunit RNAs.

The rRNA operons of *E. gracilis* are part of three contiguous repeats (Gray & Hallick 1978; Jenni & Stutz 1978; Rawson et al. 1978) and the genes for stable rRNAs

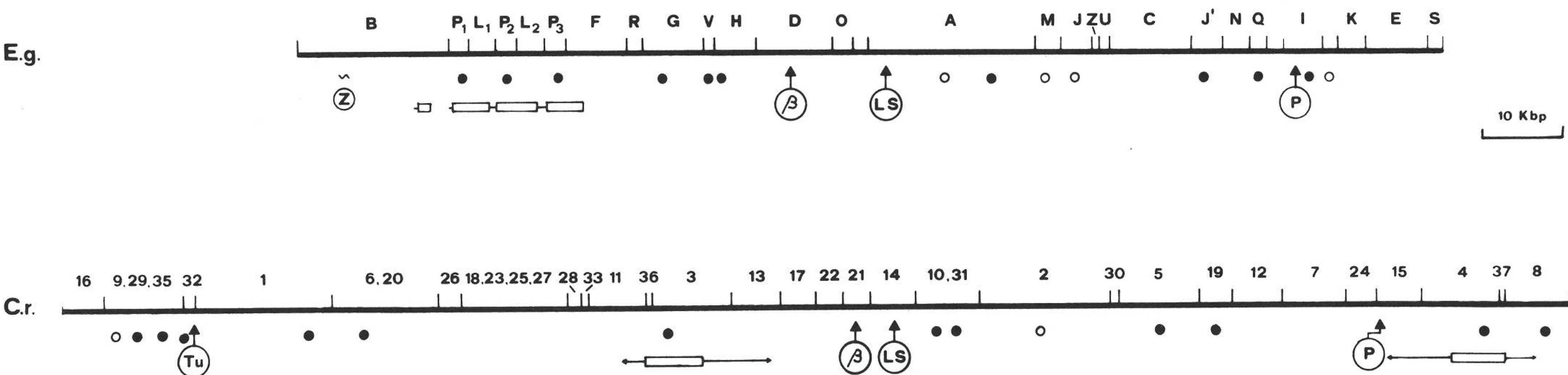

Fig. 1. Linearized EcoRI·restriction sites map of the chloroplast genomes of *Euglena gracilis* (E.g.) and *Chlamydomonas reinhardii* (C.r.) The *E. gracilis* map and fragment nomenclature is according to Hallick, 1982. The *C. reinhardii* map is according to Rochaix (1978) and the fragment numerotation according to Myers et al., 1982. The *E. gracilis* map is oriented so as to read the tandem rRNA genes (5'→3') from left to right. The *C. reinhardii* map is aligned with the *E. gracilis* map with respect to the LS gene carrying EcoRI fragment and the rRNA gene on the left side inverted repeat is oriented (5'→3') from left to right. ⊣□⊢ tandem repeats with rDNA; ←□→ inverted repeats with rDNA; ● fragments strongly hybridizing with tRNA; ○ fragments weakly hybridizing with tRNA; Ⓛⓢ gene for the large subunit protein of the ribulose 1,5-bisphosphate carboxylase; Ⓟ gene for the 32 kd thylakoid protein of the photosystem II reaction center; Ⓣⓤ gene for elongation factor EFTu; Ⓑ gene for the β-subunit of the coupling factor CF1; Ⓩ site of variable length.

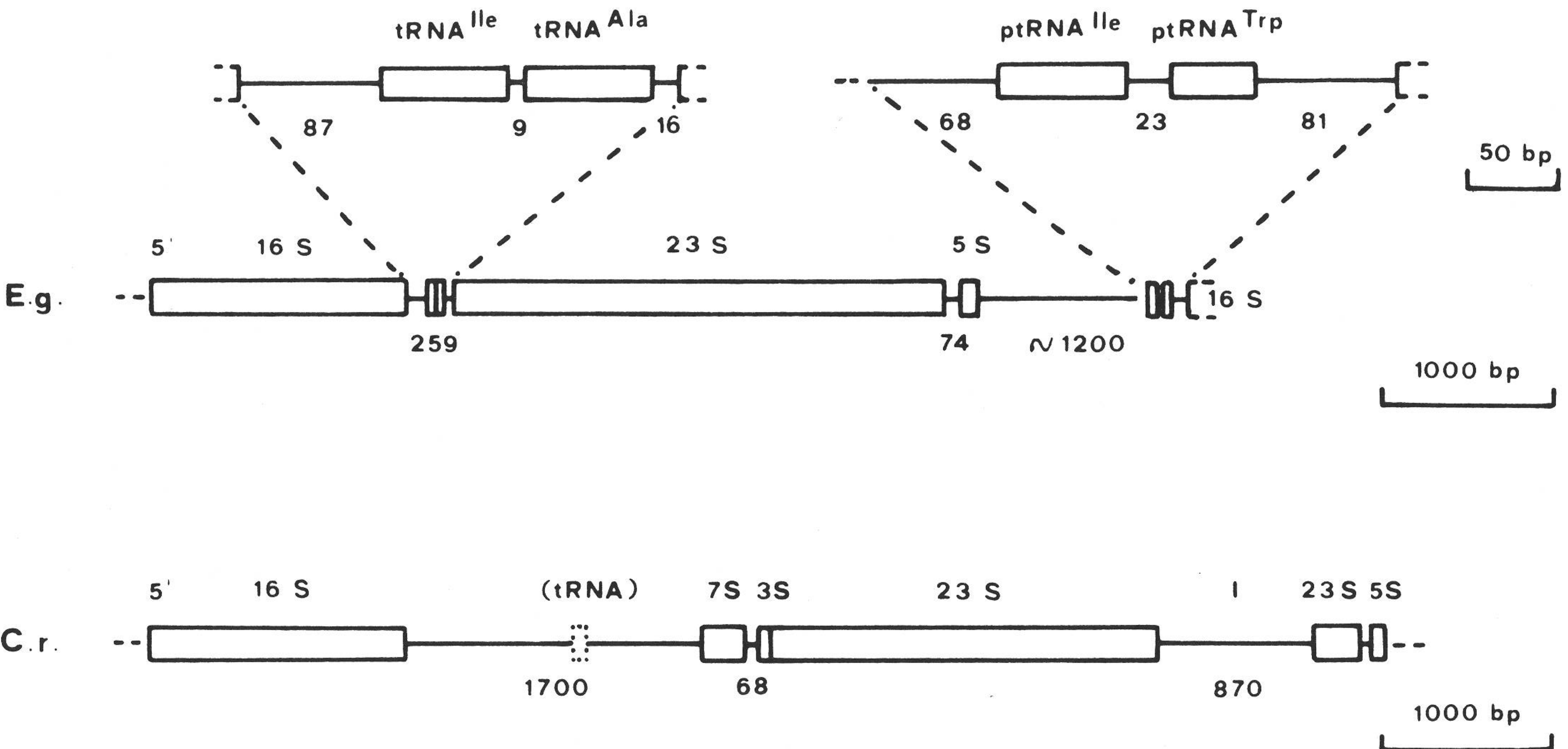

Fig. 2. Detailed structural features of the chloroplast rDNA region of *Euglena gracilis* (E.g.) and *Chlamydomonas reinhardii* (C.r.). For references see text. The rRNA genes are oriented (5'→3') from left to right. For *E. gracilis* rDNA the 16S–23S intergenic spacer is amplified showing the arrangement of the tRNA genes for isoleucine and alanine. The −100 to −400 region 5' to the next 16S RNA gene is also amplified showing the approximate position of the pseudo-tRNA genes for isoleucine and tryptophane. In the 16S–7S intergenic spacer of the *C. reinhardii* rDNA we placed a tRNA gene to indicate that this region hybridized with 4S RNA, however no specific results are yet available to the best of our knowledge. Numbers below the bars indicate the number of nucleotides in spacers and intron (I).

are encoded within a region of about 5 kbp leaving a stretch of about 1.2 kbp between the 3′ end of the 5S gene of one operon and the 5′ end of the 16S gene of the next repeat (Orozco et al. 1980a). From hybridization (Keller et al. 1980) and nucleotide sequencing experiments (Graf et al. 1980; Orozco et al. 1980b) it is established that the 16S–23S spacer contains the tRNA genes for isoleucine and alanine. It is noteworthy that three out of seven rRNA operons of *E. coli* contain the same kind of tRNA genes (Nomura & Morgan 1977) what gives further support for the hypothesis that chloroplasts are descendants of prokaryotic cells.

```
        10         20         30         40         50         60
TGGAAATGAC GAGTTTGATC CTTGCTCAGG GTGAACGCTG GCGGTATGCT TAACACATGC
        70         80         90        100        110        120
AAGTTGAACG AAATTACTAG CAATAGTAAT TTAGTGGCGG ACGGGTGAGT AATATGTAAG
       130        140        150        160        170        180
AATCTGCGCT TGGGCGAGGA ATAACAGATG GAAACGTTTG CTAATGCCTC ATAATTTACT
       190        200        210        220        230        240
AGATCTATGT GAGTAGCTAG TTAAAGAGAA TTTCGCCTAG GCATGAGCTT GCATCTGATT
       250        260        270        280        290        300
AGCTTGTTGG TGAGGTAAAG GCTTACCAAG GCGACGATCA GTAGCTGATT TGAGAGGATG
       310        320        330        340        350        360
ATCAGCCACA CTGGGATTGA GAACGGAACA GACTTCTACG GAAGGCAGCA GTGAGGAATT
       370        380        390        400        410        420
TTCCGCAATG GGCGCAAGCC TGACGGAGCA ATACCGCGTG AAGGAAGACG GCCTTTGGGT
       430        440        450        460        470        480
TGAAAACCTC TTTTCTCAAA GAAGAAGAAA TGACGGTATT TGAGGAATAA GCATCGGCTA
       490        500        510        520        530        540
ATTCCGTGCC AGCAGCCGCG GTAATACGGG AGATGCGAGC GTTATCCGGA ATTATTGGGC
       550        560        570        580        590        600
GTAAAGAGTT TGTAGGCGGT CAAGTGTGTT TAATGTTAAA AGTCAAAGCT TAACTTTGGA
       610        620        630        640        650        660
AGGGCATTAA AAACTGCTAG ACTTGAGTAT GGTAGGGGTG AAGGGAATTT CCAGTGTAGC
       670        680        690        700        710        720
GGTGAAATGC GTAGAGATTG GAAAGAACAC CAATGGCGAA GGCACTTTTC TAGGCCAATA
       730        740        750        760        770        780
CTGACGCTGA GAAACGAAAG CTGAGGGAGC AAACAGGATT AGATACCCTG TAGTCTTGGC
       790        800        810        820        830        840
CGTAAACTAT GGATACTAAG TGGTGCTGAA AGTGCACTGC TGTAGTTAAC ACGTTAAGTA
       850        860        870        880        890        900
TCCCGCCTGG GGAGTACGCT TGCACAAGTG AAACTCAAAG GAATTGACGG GGGCCCGCAC
       910        920        930        940        950        960
AAGCGGTGGA GCATGTGGTT TAATTCGATG CAACACGAAG AACCTTACCA GGATTTGACA
       970        980        990       1000       1010       1020
GGATCTAGGA AGTTTGAAAG AACGCAGTAC CTTCGGGTAT CTAGACACAG GTGGTGCATG
      1030       1040       1050       1060       1070       1080
GCTGTCGTCA GCTCGTGTCG TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTTT
      1090       1100       1110       1120       1130       1140
TTTTTAATTA ACGCTTGTCA TTTAGAAATA CTGCTGGTTA TTACCGGAGG AAGGTGAGGA
      1150       1160       1170       1180       1190       1200
CGACGTCAAG TCATCATGCC CCTTATATCC TGGGCTACAC ACGTGCTACA ATGGTTAAGA
      1210       1220       1230       1240       1250       1260
CAATAAGTTG CAATTTTTGTG AAAATGAGCT AATCTTAAAA CTTAGCCTAA GTTCGGATTG
      1270       1280       1290       1300       1310       1320
TAGGCTGAAA CTCGCCTACA TGAAGCCGGA ATCGCTAGTA ATCGCCGGTC AGCTATACGG
      1330       1340       1350       1360       1370       1380
CGGTGAATAC GTTCTCGGGC CTTGTACACA CCGCCCGTCA CACCATGGAA GTCGGCTGTG
      1390       1400       1410       1420       1430       1440
CCCGAAGTTA TTATCTTGCC TGAAAAGAGG GAAATACCTA AGGCCTGGCT GGTGACTGGG
      1450       1460       1470       1480       1490
GTGAAGTCGT AACAAGGTAG CCGTACTGGA AGGTGTGGCT GGAACAACTC C
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Fig. 3. Nucleotide sequence of the *E. gracilis* chloroplast 16S rRNA gene.

The entire 16S rDNA was sequenced including flanking regions (Graf et al. 1982). The 5′ and 3′ termini of the 16S gene were determined by comparison with the results from T1-ribonuclease analysis of the 16S rRNA (Zablen et al. 1975). The structural gene comprise 1491 nucleotides (see Fig. 3) and its sequence is 72% homologous to that of *E. coli* (Brosius et al. 1978) and 80% to that of maize chloroplasts (Schwarz & Kössel 1980). Two deletions of 9 and 23 nucleotides are identical in size and position with deletions found in 16S rDNA of maize and tobacco (Tohdoh & Sugiura 1982) chloroplasts, and what seems to be a characteristic structural feature of chloroplast 16S rRNA. A crucial difference in the sequence occurs at position 1487 where an A is found instead of a C what changes the Shine-Dalgarno sequence, required for mRNA and rRNA interaction (Shine & Dalgarno 1974).

The 1.2 kbp region of the repeat was partially sequenced (Orozco et al. 1980b). It was shown that a DNA segment between position −100 to −400 5′ to the 16S rRNA gene in the leader part shows great homology with the intergenic 16S–23S spacer and contains a cluster of pseudo-tRNA genes. These authors and others (Miyata et al. 1982) conclude that the leader region with its pseudogene cluster is the result of a (imperfect?) gene duplication event, and subsequent rapid mutations during evolution.

In *E. gracilis,* strain Z, the three repeats are probably identical in length and sequence. Minor differences may exist e.g., in the spacer regions and the 5S DNA region (Hallick et al. 1981). A somewhat different situation is found in *E. gracilis,* strain bacillaris, where the rDNA repeats are not of identical length due to small deletions in the leader part (El-Geweley et al. 1981). In another strain, *E. gracilis,* strain Z.S., there exists only one operon (Wurtz & Buetow 1981) while still in another bacillaris strain five rDNA repeats seem to exist (B. Koller, personal communications). The conclusion, so far, would be that within the species *E. gracilis* different strains exist with variable numbers of rDNA repeats.

Another pecularity concerning the rDNA region was observed in *E. gracilis,* Z, where in addition to the three rDNA repeats with a complete set of RNA genes, an extra 16S rRNA gene was mapped (Jenni & Stutz 1979). This extra 16S gene (or pseudogene?) and adjacent regions were sequenced (Roux et al. 1983). The structural part of this extra 16S gene is 98% homologous to the "normal" 16S gene. Sequence homology stops abruptly 16 positions after the 3′ end of the structural part and no genes nor pseudogenes for tRNAs and/or 23S RNA are found within the next 573 positions. 5′ to the structural part of the extra 16S gene homology to the leader sequences in the regular operons continues for at least 85 positions (sequencing data) and probably more as shown by reannealing studies (Koller & Delius 1982b). It seems that this extra 16S gene is not actively transcribed (Roux et al. 1983).

The genes within the rDNA segment are most likely organized into a single transcription unit analogous to bacterial operons. Correspondingly large precursor RNAs were described (Scott 1976). This means that a series of processing sites for specific endonucleases must exist in the leader and spacer parts. In view of the detailed sequencing data available now, the synthesis of precursor RNAs and their maturation should be reexamined.

The organization of the rDNA segment in *C. reinhardii* is depicted in Fig. 2. Two unique features can be recognized which are not seen in *E. gracilis,* nor in chloroplast rDNA of other plants (Rochaix & Malnoe 1978). a) The 23S rRNA gene is intercepted by a 870 bp intron (Allet & Rochaix 1979). b) The 23S rRNA gene is preceded by a 7S and a 3S rRNA gene, which are both associated with the large ribosomal subunit. The sequence of the 7S gene shows considerable sequence homology with the 5′ terminus of

Table 2. Location of specific tRNA genes on EcoRI fragments of the *E. gracilis,* Z, chloroplast genome

EcoRI fragment	tRNA	Reference
A [S]	Phe	Nickoloff & Hallick 1982
A [H]	Phe, Gly	Kuntz et al. 1982
B [H]	Leu_1	Kuntz et al. 1982
G [S]	Val, Asn, Arg, Leu	Orozco & Hallick 1982b
G [H]	Asn	Kuntz et al. 1982
V–H [S]	Tyr, His, Met, Trp, Glu, Gly	Hollingsworth & Hallick 1982
I [H]	Leu_2	Kuntz et al. 1982
J or J′ [H]	Lys	Kuntz et al. 1982
P_1, P_2, P_3 [H] [S]	Ile, Ala	Keller et al. 1980 Orozco et al. 1980b Graf et al. 1980
Q [H]	Thr	Kuntz et al. 1982
T, U or V	Tyr	Kuntz et al. 1982

[H]: identified by hybridization with specific tRNAs
[S]: identified by DNA sequencing

the *E. coli* 23S gene, the conclusion being that the 5′ end of the large subunit rRNA gene of *C. reinhardii* has been split into two small genes (Rochaix & Darlix 1981). *C. reinhardii* like *E. gracilis* cpDNA lacks a 4.5S rRNA gene, seen in flowering plants and e.g. in the fern *Dryopteris acuminata* (Takaiwa et al. 1982).

The 5′ terminus of the 23S rRNA chloroplast gene of *E. gracilis* was sequenced (Graf et al. 1980; Orozco et al. 1980b) and considerable sequence homology was found with the corresponding parts of the *E. coli* (Brosius et al. 1980) and *Zea mays* (Edwards & Kössel 1981) 23S RNA genes. Furthermore Hallick et al. (1981) sequenced the 3′ end of the *E. gracilis* chloroplast 23S rRNA gene and adjacent regions and they found that it is about 60% homologous with the bacterial 23S rRNA gene and the 4.5S chloroplast RNA gene of several higher plants, e.g. tobacco (Takaiwa & Sugiura 1980). The tentative conclusion would be that both the algal and bacterial 3′ end of the 23S RNA have the same position and function in the large ribosomal subunit as the 4.5S RNA this especially since these RNA regions can be folded into a common secondary structure.

3. The transfer DNA region

In Fig. 1 we have marked for both algal genomes those areas which hybridize with tRNAs. Malnoe & Rochaix (1978) mapped 12 to 14 regions on the circular chloroplast genome of *C. reinhardii* which hybridized with 4S RNA. Further details are lacking.

From hybridization experiments it was known that *E. gracilis* cpDNA contains tRNA genes (Schwartzbach et al. 1976; McCrea & Hershberger 1976; Gruol & Haselkorn 1976). According to the newest hybridization data (Kuntz et al. 1982) a minimum of 10 EcoRI fragments, namely EcoRI·A,B,G,H,I,J or J′ (these two co-migrate), K or L (these two co-migrate), P,Q,TU or V (these three co-migrate) contain genes for tRNA. More specific data are listed in Table II. In addition to the two tRNA genes located in the rDNA region Hallick & collaborators have sequenced two tRNA gene clusters. The EcoRI·G fragment contains four tRNA genes which are arranged 5′-$tRNA^{Val}$ – 16 bp spacer – $tRNA^{Asn}$ – 3 bp spacer – $tRNA^{Arg}$ – 45 bp spacer – $tRNA^{Leu}$.

The $tRNA^{Leu}$ gene is of opposite polarity as the three other ones (Orozco & Hallick 1982b). The EcoRI fragments V–H have a cluster of six tRNAs arranged $tRNA^{Tyr}$ – 64 bp spacer – $tRNA^{His}$ – 14 bp spacer – $tRNA^{Met}$ – 4 bp spacer – $tRNA^{Trp}$ – 27 bp spacer – $tRNA^{Glu}$ – 6 bp spacer – $tRNA^{Gly}$. The $tRNA^{Met}$ is believed to be an elongator tRNA (Hollingsworth & Hallick 1982). According to these authors and in line with the results of Kuntz et al. (1982) there are at least five additional tRNA coding regions in EcoRI·A, EcoRI·I, EcoRI·J′ and EcoRI·Q. If each of these strongly hybridizing regions contains an average of 4 to 6 genes, there would be about 35 tRNA genes, what would be sufficient to translate the 61 codons of the genetic code considering normal wobble pairing. The chloroplast genome of the bacillaris strain shows a similar distribution of the tRNA genes, but detailed sequencing data are lacking (El-Geweley et al. 1982).

Orozco and Hallick (1982b) and Hollingsworth and Hallick (1982) have compared the sequence of the *E. gracilis* tRNA genes with the corresponding tRNA species from *E. coli,* and chloroplasts, mitochondria and cytoplasm of various eukaryotic organisms. It certainly does not come as a surprise that the *E. gracilis* chloroplast tRNAs generally are more prokaryotic than eukaryotic in nature, although exceptions to that rule may not be excluded in the future as more chloroplast tRNA species will be sequenced.

A novel approach to retrieve tRNA genes was published by Nickoloff and Hallick, 1982: the oligonucleotide 5′ CTACCAACTGAGCT, e.g., is complementary to conserved regions of chloroplast $tRNAs^{Ala}$, $tRNA^{Gly}$, $tRNA^{Phe}$, $tRNA^{Val}$ of *E. gracilis,* and some chloroplast tRNAs from higher plants (not listed here). Using such synthetic probes it was possible to show that e. g., EcoRI·A carries a tRNA gene for Phe. El-Geweley et al. (1982) mapped the $tRNA^{Phe}$ gene at a corresponding locus of the chloroplast genome of *E. gracilis,* bacillaris.

4. DNA regions coding for protein genes

Detailed mapping data concerning specific protein genes are scant, so far. In fact only the gene for the large subunit protein of the ribulose 1,5-bisphosphate caboxylase (LS) was precisely mapped in case of *E. gracilis* (Stiegler et al. 1982a), and mapped and sequenced in *C. reinhardii* (Dron et al. 1982) (see also Fig. 1). The LS gene of *C. reinhardii* is 1425 bp in length and highly homologous to those of spinach (Zurawski et al. 1981) and maize (McIntosh et al. 1980). The Chlamydomonas LS gene does not contain any introns. On the other hand Stiegler et al. (1982) give good experimental evidence that the *E. gracilis* LS gene contains an intervening sequence with a minimum size of 0.6 to 0.7 kb. This would represent the first evidence for an intron within a chloroplast protein gene and it would indicate that the *E. gracilis* chloroplast genome displays both prokaryotic and eukaryotic features, if we accept that introns are essentially an eukaryotic property.

According to preliminary reports (Stiegler et al. 1982b) and recent observations in our own laboratory the genes for the β-subunit of coupling factor CF_1 and for a 32 kd thylakoid membrane protein of the photosystem II reaction center are on EcoRI·D and I, respectively, this according to DNA:DNA hybridization data using internal parts of the respective genes of spinach chloroplasts as probes (Fig. 1). These experiments suggest that higher plant and *E. gracilis* chloroplast genes are sufficiently homologous to allow cross-hybridization if proper and not too stringent hybridization conditions are chosen. Using as probes transducing phages carrying bacterial genes for the α, β, β' subunits of RNA polymerase and for the elongation factors EFTu, EF·G and for 31 ribosomal proteins Watson and Surzycki (1982) were trying to locate the respective

genes on the chloroplast genome of *C. reinhardii*. So far they mapped the gene for EFTu on a DNA segment with an EcoRI site separating fragment 1 and 32 (Fig. 1). According to indirect evidence (protein synthesis in the presence of selective inhibitors) *E. gracilis* cpDNA contains the gene for EFTu (Spremulli 1982), however, it seems not to contain the genes for EFTs and EFG (Breitenberger et al. 1979; Fox et al. 1980). If correct, this is another example where a functional unit (EFTu–EFTs) is of dual genetic origin supporting the multisubunit completion principle formulated by Ellis (1977). It is noteworthy that the chloroplast elongation factors EFTs and EFG in spinach are coded on cpDNA (Ciferri et al. 1979). Obviously the partitioning of genetic information between nu DNA and cpDNA is not uniformly handled by nature, a point of considerable interest.

Rutti et al. (1981) have shown that the fragment EcoRI·N interacts with the synthesis of a 53 kd chloroplast protein using the techniques of hybrid arrested and hybrid selected *in vitro* protein synthesis in a rabbit reticulocyte lysate. A stromal polypeptide of identical size and proteolytic digest pattern (papain and chymotrypsin) is synthesized in isolated *E. gracilis* chloroplasts under light dependent conditions*. Using the same protocol they also found that EcoRI·I interacts with a 33 kd and a 46 kd protein. Both the 53 kd and the 46 kd are of yet unknown function, while the 33 kd protein most likely is a thylakoid protein of the photosystem II reaction center (Keller et al. 1982).

Orozco et al. (1982b) have sequenced within EcoRI·G a DNA segment which may be a putative gene or pseudogene for a chloroplast protein. The respective open reading frame of 91 codons, however, has the trinucleotide UGA as codon 31 in a possible mRNA. The following features are noteworthy: a) 15 bp 5′ to the ATG trinucleotide is the sequence 5′GGAGT, which is complementary to the sequence 3′-CCUCA which occurs at the 3′ terminus of the *E. gracilis* chloroplast 16S rRNA and represent a one base variant of the *E. coli* 16S rRNA Shine and Dalgarno sequence (ribosome binding site). b) Sequences similar to the Pribnow box (Pribnow 1975) and the "−35" region are present at the appropriate positions 5′ to the initiation codon. c) AT-rich sequences are present both at the "−35" region and 3′ to the TAA stop codon. Other structural features strongly indicate that this DNA sequence is either a chloroplast gene provided the UGA (31st codon) is translated as tryptophane, like, e.g. in yeast mitochondria (Bonitz et al. 1980) or it is derived from a gene being now a pseudogene. (For a discussion of structural features of regulatory sites in prokaryotic genomes, see Siebenlist et al., 1980.)

5. *Selective transcription and regulatory sites in E. gracilis chloroplast DNA*

At al developmental stages examined sofar, chloroplast rRNAs are the most abundant chloroplast transcripts (Chelm et al. 1979; Rawson et al. 1981). This is also true for *in vitro* transcription experiments using a transcriptionally active chromosome (Rushlow et al. 1980). This means that the chloroplast specific DNA dependent RNA polymerase preferentially recognizes specific sequences in the rDNA region.

Note added to proof: Montandon and Stutz (Nucleic Acids Res. 11: 5877–5892, 1983) showed that EcoRI·N codes for the elongation factor Tu which is composed of 408 aminoacids as deduced from the nucleotide sequence data. The chloroplast *tuf* gene is split containing two introns of 103 and 110 nucleotides, respectively.

The structural requirements (sequences) for this preferential rRNA synthesis are presently unknown. As already mentioned 5′ to the 16S RNA gene pseudo tRNA genes have been identified in the strain Z (Orozco et al. 1980b) as well as in the bacillaris strain (Helling 1982, personal communication). Their presence may be crucial for the control of transcription and the secondary/or tertiary structure of the co-transcript may be important for post-transcriptional processing signals as found in bacterial systems (Young & Steitz 1978). Regulatory sequences (5′TATRATR) were also identified 5′ to several of the sequenced tRNA gene clusters (Orozco et al. 1982b). Furthermore, it could be that the tRNA genes in the proximity of protein genes scattered throughout the genome have dual function, i.e., in addition to act as structural genes they may assure regulatory functions controllig the transcription of the correlated protein gene (Hudson et al. 1981).

6. Length heterogeneity of chloroplast genomes

As a first approximation, we may consider the multiple circular DNA molecules in a chloroplast to be identical in length and nucleotide sequence. Restriction enzyme analyses of all chloroplast genomes sofar tested support this assumption. For the *E. gracilis* chloroplast genome, however, a site specific size variation was located in EcoRI·B (Jenni et al. 1981). This region (Z-region, Fig. 1) can vary in length, due to deletions or insertions, by about 600 to 800 bp. It was shown by further mapping experiments and by electron microscopic studies (Schlunegger et al. 1983) that this variable zone is framed by two small inverted repeats and must be very rich in A–T. Furthermore, it could be shown by EM analysis that the origin of DNA replication is close to that Z-region, suggesting that the observed size variation is functionally related to the replication event (Koller & Delius 1982c).

Heizmann et al. (1981) have analyzed the plastid DNA of bleached mutants of *E. gracilis*. Some of these plastidial DNAs contain a defective chloroplast genome preferentially retaining the rDNA region (Φ^-mutants). They argue that the origin of replication may be near the rDNA region and that this site is preserved in the defective DNA as is the case in spontaneous ρ^-mutants of yeast.

Rather extensive research was done on mutants of *C. reinhardii* with physical alterations in the chloroplast genome. It is beyond the scope of this paper to deal with this very interesting genetic aspect. However, just two very recent results may be quoted. Myers et al. (1982) have isolated the chloroplast DNA of numerous non photosynthetic acetate requiring mutants of *C. reinhardii* and searched for deletions and gene rearrangements including inversions or duplications. They describe a series of mutants with altered DNA structures and observe that in some cases the alterations are symmetrical, i.e., affect both inverted repeats. This could be explained by spreading a mutation at one inverted repeat via copy correction. Also Spreitzer and Mets (1980) have described a chloroplast mutant that alters the catalytic activity of the LS gene in *C. reinhardii*. In both kinds of studies, it was possible to correlate genetically defined loci with physically mapped restriction fragments. It is obvious that in this respect the "Chlamydomonas system" out-does the "Euglena system" which lacks a sexual life cycle.

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