Sequence organization of the chloroplast ribosomal spacer of *Chlamydomonas reinhardii*: uninterrupted tRNAile and tRNAala genes and extensive secondary structure*

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Summary
The 1805 bp spacer between the chloroplast ribosomal 16S and 7S RNA genes of *Chlamydomonas reinhardii* has been sequenced. It contains the genes of tRNA ala and tRNA ile which are both uninterrupted. The spacer includes several short direct and inverted repeats and a large palindromic structure which maps in the region where DNA rearrangements have occurred in other *Chlamydomonas* species.

Introduction
In several higher plants and algae the chloroplast ribosomal units are located within a large inverted repeat of the chloroplast genome (26). In *C. reinhardii* the two 19 kb segments of the inverted repeat are separated by two large single copy regions of nearly equal size (20). The ribosomal unit of this green alga consists, in the order of transcription, of the 16S, 7S, 3S, 23S and 5S rRNA genes (22). Unique features of this unit include the presence of an 888 bp intron in the 23S rRNA gene and of the two small 3S and 7S rRNA genes which precede the 23S rRNA gene (22). While higher plant chloroplasts contain a 4.5S rRNA gene between the 23S and 5S rRNA genes (26), this is not observed in *C. reinhardii* (22).

The arrangement and the sequences of the chloroplast rRNA genes are remarkably related to those of prokaryotes (3, 23, 25). In *E. coli* the spacer between the 16S and 23S rRNA genes in the seven ribosomal operons contains either a tRNA glu gene or both tRNA ala and tRNA ile genes (11, 28). These two tRNA genes have also been found in the ribosomal spacer in *Bacillus subtilis* (10), in the cyanelles of *Cyanophora paradoxa* (9), in the chloroplasts of *Euglena gracilis* (6, 18), tobacco (24), maize (8), bean (14), broad bean (15) and wheat (16). The spacers of *Euglena gracilis* (6, 18), tobacco (24) and maize (8) have been entirely sequenced. In higher plant chloroplasts the ribosomal spacer ranges between 1700 and 2400 bp, considerably longer than those of *Euglena* chloroplasts (259 bp) and of *E. coli* (437 bp). The large size of the spacers from higher plants is due mainly to the introns in the tRNA genes which range between 707 and 949 bp (8, 24).

Since the *C. reinhardii* spacer has a size comparable to that of higher plants and since it is known to hybridize with 4S RNA (12), it was of interest to examine the structure of this spacer in more detail. Here we present the complete sequence of this spacer. It consists of 1805 bp and it contains the genes for tRNA ala and tRNA ile both of which lack introns.

Materials and methods
The recombinant plasmid HR1.14 containing most of the chloroplast ribosomal spacer of *C. reinhardii* has been described (22). Plasmid DNA

was prepared by the method of Katz et al. (7). DNA fragments were 3' or 5'-endlabelled and sequenced by the chemical cleavage method as described (13). The DNA sequence analysis was performed on a Hewlett Packard computer, model 9845.

Results and Discussion

The plasmid HRI.14 contains a chloroplast EcoRI-HindIII fragment that covers most of the spacer between the 16S and 7S rRNA genes. The first 67 bp of this spacer that flank the 3' end of the 16S rRNA gene were determined previously (4). Figure 1 displays the physical map of the spacer.

![Fig. 1. Restriction map and sequencing strategy of the chloroplast ribosomal spacer of C. reinhardii. The genes of tRNAIle and tRNAala, and the 3' and 5' ends of the 16S and 7S rRNA genes, respectively, are indicated. Restriction sites are indicated by • EcoRI, ○ AluI, ▲ DdeI, ◊ Hinfl, □ KpnI, ○ TaqI.](image)

Fig. 2. Nucleotide sequence of the non-coding strand of the entire 16S–7S ribosomal spacer. The 3' end of the 16S rRNA gene immediately precedes base 1 and the 7S rRNA gene starts at base 1806. The genes of tRNAIle and tRNAala are framed. IR7 and IR2 are two nearly perfect inverted repeats (cf. Figs. 4, 5).