The genes for the ribosomal proteins S12 and S7 are clustered with the gene for the EF-Tu protein on the chloroplast genome of *Euglena gracilis*

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**ABSTRACT**

We characterize a DNA segment of the *Euglena gracilis* chloroplast DNA fragment Eco-N by nucleotide sequencing and S1 nuclease analysis. We show that this region, which is upstream of the previously sequenced *tuf A* gene, contains the genes for the ribosomal proteins S12 and S7. The gene arrangement is 5'—rps 12—80 bp spacer—rps 7—174 bp spacer—tuf A, somewhat similar to the *str* operon of *E. coli*. The chloroplast S12 and S7 proteins contain 124 and 155 aminoacids, respectively, and are to 68% and 38% homologous with the corresponding *E. coli* proteins. The region is transcribed into a distronic mRNA of about 1.1 to 1.2 kb. The *rps* 12 and *rps* 7 genes, contrary to the *tuf A* gene, are not split.

**INTRODUCTION**

We have recently sequenced the *Euglena gracilis* chloroplast gene for the elongation factor EF-Tu (*tuf A*) (1) which was previously mapped on the DNA fragment Eco-N (2). The chloroplast *tuf A* gene is expressed as a 1.95 kb mRNA which is the product of three splicing events (1). The coding part of this chloroplast gene is to about 55% homologous with the *E. coli* *tuf A* gene which is known to be part of a large operon containing in the 5' to 3' direction the genes for the small subunit ribosomal proteins S12 and S7 (*rps* 12, *rps* 7), the elongation factor EF-G (*fus*) and the elongation factor EF-Tu (*tuf A*) (3). The bacterial *tuf A* (4), *rps* 12 and parts of *rps* 7 have been sequenced (5) as well as the S12 (6) and S7 proteins (7). The S12 protein is of particular interest since it is involved in the translation initiation step and controls the fidelity of the reading process (8). Streptomycin interacts with S12 abolishing normal protein synthesis (9 and references there). Streptomycin also interacts with chloroplast ribosomes, preventing normal chloroplast growth what can lead to irreversibly bleached cells. Streptomycin resistant Euglena strains have been described (10,11), i.e.,
streptomycin resistance is most likely a convenient chloroplast bound genetic marker.

Considering these facts it became of interest to sequence the 5' flanking region of the tuf A gene and to size its stable transcript. We show in this report that adjacent and upstream of the tuf A gene there are in the 5' to 3' direction the rps 12 and rps 7 genes. The two genes are transcribed into a dicistronic mRNA. A preliminary account of this work was given at the eleventh A. Katzir-Katchalsky conference, Jerusalem, 1984.

MATERIALS
Enzymes were purchased from Boehringer-Mannheim and used following instructions of the supplier. [γ-32P]-ATP (400 Ci/mmol) was from Radiochemical Center, Amersham.

METHODS
Cell culture and preparation of chloroplast RNA
E. gracilis (Z-strain) was grown as published (1). Chloroplast RNA was extracted (12) from purified chloroplasts (13).

DNA cloning
The chloroplast DNA fragment Eco·N was previously cloned into pBR322 (14). Selected subfragments of Eco·N were isolated according to published procedures (15).

S1 nuclease mapping and DNA sequencing
S1 nuclease protection analysis was according to (16,17). Protected DNA fragments were detected as described (18,1). For DNA sequencing subfragments of Eco·N were blunt ended with DNA polymerase (Klenow), cloned into the HincII site of M13 mp 9 (19,20) and sequenced according to (21). DNA fragments with HhaI ends were blunt ended with S1 nuclease (22).

RESULTS AND DISCUSSION
1. Mapping and sequencing of the rps 12 and rps 7 genes
We have totally sequenced the chloroplast DNA fragment Eco·N (3067 positions) and we found upstream and in close vicinity to the previously analyzed tuf A gene (1) the chloroplast rps 12 and rps 7 genes. As shown in Fig. 1 the overall gene arrangement is 5'-rps 12-rps 7-tuf A what is very similar to the situation found in the str operon of E. coli (5), the crucial difference being that the chloroplast genome lacks the fus gene. The gene for
Fig. 1. Arrangement of the genes for the chloroplast ribosomal proteins S12 (rps 12), S7 (rps 7) and the EF-Tu protein (tuf A) on the DNA fragment Eco·N. The three genes have the same polarity starting 5' with the gene for S12. The coding regions are boxed. In order to correlate the sequence data given in Fig. 2 with the results previously reported (1) we show also the split tuf A gene (exon 1, exon 2, part of exon 3). [***] indicates continuation of an ORF on Eco·Q (1). Some restriction sites are given as position markers.

the Euglena chloroplast EF-G protein seems to be located in the nuclear genome (23); our data support this assumption indirectly. It is noteworthy that the spinach chloroplast genome seems to contain the genetic information for the EF-G protein (24).

In Fig. 2 we give the nucleotide sequence of the relevant DNA region. The sequence starts with the 5' end of the EcoRI site (N/J') and continues towards the tuf A gene, including 28 codons of the first exon of tuf A. The aminoacid sequence is given for the coding parts and aligned with it is the aminoacid sequence of the corresponding E. coli proteins.

The chloroplast rps 12 gene has an open reading frame for 124 aminoacids (not counting the initiation codon). The deduced chloroplast protein is one position longer than the bacterial S12 protein. The chloroplast S12 protein has a calculated Mr of 13'828 and is to 68% homologous with the bacterial protein. We find several important conserved regions, especially the domains around position 42 (Lys) and 87 (Lys) which are involved in streptomycin binding (9). Streptomycin resistant strains of E. gracilis were analyzed and compared with the wild type strain on a ribosomal protein level (11). The strain Smf2 BNgL seems to contain a modified acidic protein in the small ribosomal subunit (two-dimensional gel-electrophoresis). Since the Euglena chloroplast S12 protein (Fig. 2) like the E. coli S12 protein belongs to the basic ribosomal proteins the observed resistance of strain Smf2 BNgL seems not to be due to a mutated S12 protein. The analytical data need, however, to be verified. Future tests will show whether the same type of mutations as
Fig. 2. Nucleotide sequence of the region containing the rps 12, rps 7 and parts of the tuf A gene.

A DNA stretch of 1358 positions, starting with the right hand EcoRI restriction site is given (arrow). We only show the RNA like strand. Within the coding parts the deduced aminoacid sequences are given for the S12 and S7 proteins and aligned with them are the aminoacid sequences of the corresponding E. coli proteins (6,7). For convenience we add 28 codons of the first exon of the tuf A gene. Asterisks indicate aminoacid identity. -Δ- (base position 945-947) indicates a deletion. The deletion may be placed alternatively at nucleotide positions 912-914 increasing somewhat the degree of aminoacid sequence homology. Aminoacids positions 42 and 87 of the E. coli S12 protein which are involved in streptomycin binding are marked.

observed in bacteria are also found in certain streptomycin resistant Euglena strains.

The rps 7 gene codes for a protein of 155 aminoacids. This gives a calculated molecular weight of 17'831. The S7 protein occurs in E. coli in two sizes depending on the strain (7). The Euglena chloroplast S7 protein is two positions longer than the E. coli B S7 (note the insertion at nucleotide position 945), and 22 aminoacids shorter than the E. coli K S7 protein. The aminoacid sequence homology between the chloroplast and bacterial S7 protein is very low, only 38%. As a consequence there exist only a few short conserved regions, e.g., aminoacid positions 85 to 91 and 145 to 151. We may add that the same domains are also conserved in the S7 protein of soybean chloroplasts (J.M. von Allmen, personal communication) what strongly suggests that these regions are functionally important.

Table I shows codon usages for translating the chloroplast and bacterial S12 proteins. Euglena chloroplasts prefer codons rich in A and/or U while
### Table 1. A comparison of the codon usage in the *Euglena gracilis* chloroplast and *E. coli* rps 12 gene

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*Initiation codon not included; reference, see text.*
Fig. 3. S1 nuclease protection analysis of the DNA region containing the rps 12, rps 7 genes and parts of the tuf A gene. Chloroplast RNA was hybridized to the 1.7 kb DNA fragment HindIII-EcoRI (right side of Eco-N) and the protected DNA fragments electrophoresed under alkaline conditions in a 2% agarose gel. The blotted DNA fragments (nitrocellulose paper) were detected by hybridization with 32p-labelled Eco-N DNA.

E. coli prefers codons rich in G and/or C. As a result the sequence divergence already seen on the aminoacid level is accentuated on the nucleotide level, being only 52% for the S12 genes. For the chloroplast and bacterial rps 7 genes sequence homologies on the nucleotide and aminoacid level are about equal (38%) at least for the first 81 codons, as far as data are available (5). Also in this case a difference in codon usage is observed (data not shown) similar to that for the rps 12 genes. But for statistical reasons the difference in codon usage is not apparent.

2. Transcription of the chloroplast rps 12 and rps 7 genes

We have previously shown that the chloroplast tuf A gene is split (1). It was important to test whether the adjacent rps 12 and rps 7 genes are also split or whether the stable transcripts are colinear with the genes like in E. coli. We used as hybridization probe the 1.7 kb HindIII-EcoRI fragment in S1 nuclease protection analysis (see Fig. 1). The experiments were performed under neutral and alkaline conditions. In Fig. 3 we only show the result obtained under alkaline conditions since the result obtained under neutral conditions was identical. We see three bands on the radiograph, the 0.45 kb fragment corresponds to a DNA segment containing the first exon of the tuf A gene (consult Fig. 1 and ref. 1). The weak double band of 1.1 to 1.2 kb can only stem from a contiguous DNA fragment protected by a mRNA coding for the S12 + 7 protein including the intergenic spacer (375 + 80 + 468 bases). The 1.7 kb band represents some renatured DNA probe. No smaller protected DNA
fragments can be detected, what strongly suggests that the two genes are co-transcribed into a dicistronic mRNA. Furthermore, since the "neutral" and "alkaline" data coincided, the two ribosomal protein genes are not split. For the very moment we cannot unequivocally explain the appearance of a 1.1 to 1.2 kb double band in Fig. 3. Some preliminary data (not shown) obtained with other DNA probes suggest that the size difference is at the 5' end of the transcripts.

Koller and Delius (25) showed by FM analysis very recently that the entire Eco-N is transcribed in one direction only, but transcription switches to the other strand on either side of Eco-N. Karabin and Hallick (26) showed that indeed on Eco-Q and in close vicinity to Eco-N (see Fig. 1, ref. 1) two tRNA gene clusters of opposite polarity exist. The transcripts on the other side of Eco-N in Eco-J' are not yet characterized.

In E. coli the rps 12 and rps 7 genes are part of the str operon and co-transcribed with the genes for EF-G and EF-Tu proteins (3). In E. gracilis chloroplasts this gene cluster is transcribed into a stable dicistronic 1.1-1.2 kb mRNA (rps 12 + 7) and a 1.95 kb spliced mRNA (tuf A plus ORF for unknown proteins). Under the prevailing growth and analytical conditions, the 1.95 kb transcript is much more abundant than the 1.1 - 1.2 kb mRNAs as estimated from the relative intensities of the signals in the radiograph (Fig. 3). This suggest that transcription of the two regions is under separate control (two promoters), but it is also possible that the entire region is co-transcribed (one promoter) and the steady state concentration of stable transcripts depends primarily on subsequent transcript processing. Nothing specific is yet known about promoter sequences in E. gracilis chloroplast genomes. Therefore, we do not mark in Fig. 2 any possible promoter like "boxes" upstream of the coding parts of the genes.

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REFERENCES