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Euglena gracilis chloroplast DNA: the untranslated leader of *tuf*A-ORF206 gene contains an intron

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ABSTRACT

Structural features of a dicistronic 1.95 kb mRNA coding for the chloroplast specific elongation factor Tu and ORF206 are described. The unspliced pre-mRNA is composed of 2562 nucleotides and undergoes four splicing events which remove a total of 606 nucleotides. The first intron splits the untranslated leader, two introns dissect the tufA coding region and the forth intron is within ORF206, which codes for a putative protein that is to 34% homologous with the putative protein of chloroplast ORF184 of tobacco. Introns neither belong to group I nor II, and 5' and 3' intron boundaries do not follow consensus sequences. Potential ribosome binding sites are located 58 and 32 positions upstream of the tufA and ORF206 start codon, respectively.

INTRODUCTION

Major parts of the Euglena gracilis chloroplast DNA fragment Eco·N and about 800 bp of the adjacent fragment Eco·Q interact upon hybridization with a stable mRNA of about 1.95 kb, which codes for the chloroplast specific elongation factor Tu (1). From our previous studies it became evident that the 1.95 kb mRNA was the product of three, possibly four splicing events. The tufA coding part is interrupted twice and we had tentatively assigned a third splicing site within an ORF downstream of the tufA gene. A forth intron was suspected to interrupt the untranslated leader.

We continued the analysis of the 1.95 kb mRNA by precisely establishing all four splice junctions by primer extension sequencing, and we determined the 5' and 3' ends of the stable transcript. It turns out that a) the leader is indeed spliced what is unique so far for a chloroplast gene; b) the 5' and 3'-intron boundaries only marginally follow established consensus sequences (2) and c) the spliced ORF region codes for a putative protein (206 aminoacids) related to an ORF of tobacco chloroplasts (3).

Untranslated leader sequences of mRNAs are somehow involved in ribosome binding steps. Procaryotic mRNAs usually contain, in close proximity of the start codon, short purine rich sequences complementary to the 3' terminal region of 16S rRNA (4). This signal together with initiation factors controls cistron specificity. A similar concept seems to hold for chloroplasts, i.e., Shine-Dalgarno type motifs were found in higher plant chloroplast mRNAs in numerous cases (e.g. 5,6). Euglena chloroplast mRNAs, however, do not have such oligobase elements near the start codon (e.g. 7,8). Having a well defined mRNA leader sequence available we searched for short sequences complementary to the 3' end of the 16S rRNA of Euglena gracilis (9) including other defined leader sequences of Euglena chloroplast mRNAs. According to this limited comparative study oligobase sequences complementary to the 3' end of 16S rRNA occur in each leader at some distance away from the start codon, but never in close proximity. The DNA segment between the short DNA element and the start codon is extremely rich in AT and never contains start codons. Interestingly, the unspliced tufA leader contains an AUG which, however, is located in the intron and therefore eliminated.

MATERIALS

Enzymes, if not stated otherwise, were purchased from Boehringer-Mannheim and used following instructions of the supplier. [α-^{32}P]dATP (400 Ci/mmole) and [γ-^{32}P]ATP (3000 Ci/mmole) were from Radiochemical Center, Amersham.

METHODS

Cell culture and preparation of chloroplast RNA

Euglena gracilis (Z-strain) was grown heterotrophically and total RNA from purified chloroplasts was obtained as reported (10,11). To prevent RNA degradation all buffers used for chloroplast preparation contained 1 mM aurintricarboxylic acid [ATA] (12). ATA was removed by chromatography (Sephadex G-100; equilibrium buffer, 0.3 M NaCl, 20 mM Tris-HCl, pH 7.5). DNA was removed by digestion (DNAase I, ribonuclease free, Worthington) and subsequent chromatography of the RNA preparation through Sephadex G-100.

Northern blots and RNA:DNA hybridisation

Chloroplast RNA was denatured according to (13). RNA was electrophoresed in 1.5% agarose gels and transferred electrophoretically to nylon membranes

following instructions from the supplier (Gene Screen Plus, NEN). Hybridisation with ^{32}P-labelled DNA probes was as specified in the legends to Figs 4 and 5. In all cases membranes were washed once in 2x SSC (1x SSC is 0.15 M NaCl, 0.015 M Na-citrate), at room temperature, 5 min, twice in the same buffer with 1% SDS (sodium dodecylsulfate) at 55°, 30 min and once in 0.1x SSC at room temperature.

DNA-primers and sequencing by primer extension

Two DNA-primers were synthesized at the Swiss Institute of Experimental Cancer Research, Epalinges : 5'-TGTTTTACATTAAAGTACTC (pos. 3506-3487) was used to sequence the exon IV-exon III junction; 5'-CACCTTCTCAATTGC (pos. 2672-2657) to sequence the exon III - exon II junction. The synthetic oligodeoxynucleotides were electrophoresed on denaturing acrylamide gel, eluted by diffusion and 5'-end labelled with [γ-^{32}P]ATP (14). Two DNA primers were synthesized using as template single strand DNA inserted in M13. For sequencing the exon II - exon I junction we prepared a 55 b fragment obtained by cutting the copied 571 HinfI fragment (pos. 1995-1425) with Sau 3A1 (pos. 1893) and TaqI (pos. 1839). For sequencing the exon I - leader junction we prepared a 37 b fragment obtained by cutting the 907 bp HinfI-BglII (pos. 1426-518) fragment with HincII (pos. 1336) and DdeI (pos. 1300). The DNA fragments were electrophoretically purified and eluted by diffusion.

Primer extension dideoxy sequencing was essentially as described (15). Oligonucleotides (20-mer and 16-mer) were hybridized with 10 ug chloroplast RNA for 2 h at 42°. DNA fragments were hybridized for 1 hour at 55°.

DNA-sequencing

As starting material for the sequencing experiments (dideoxy method) we used Eco·N+Q cloned into pBR322 (16) and the HindIII fragment which extends from the single HindIII site in Eco·N to the HindIII site near the Eco·Y·Q junction (17). A subfragment containing the Eco·Q·N junction was also sequenced. The results (not shown) confirm that the fragments Eco·N and Eco·Q are indeed adjacent.

RESULTS

1. Mapping of DNA regions interacting with the 1.95 kb mRNA

The entire fragment Eco·N had been sequenced including adjacent parts of Eco·Q (1,18). In Fig. 1 we give an overview of the relative positions of leader and exons I to IV as we understand it now based on the previous and

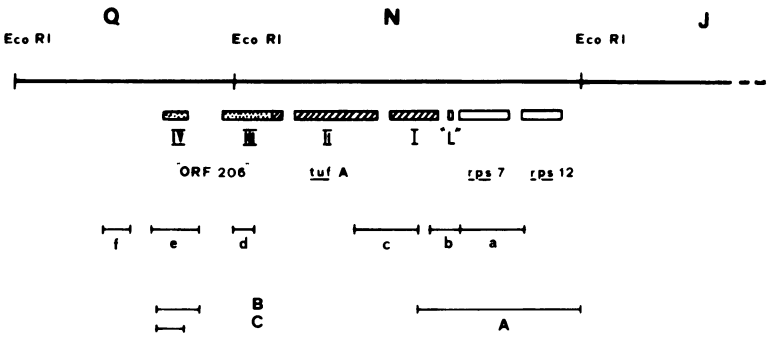

Fig. 1. Relative positions of genes rps12, rps7, tufA and ORF206 on the DNA fragment Eco·N·Q.
L, leader; I to IV, exons of tufA - ORF206 transcript; hatched and stippled boxes correspond to tuf and ORF206 region, respectively; a to f, position of DNA probes used in experiments of Fig. 3; A and B,C, DNA probes used in experiments of Fig. 4 and 5, respectively.

the new data presented in the following. In Fig. 2 we show for convenience the entire nucleotide sequence (non-coding strand) of Eco·N and adjacent parts of Eco·Q as far as relevant for the structural analysis of the 1.95 kb mRNA. Based on these sequence data we were able to obtain several DNA probes which were used for Northern hybridisation experiments (probes a to f) and S1 nuclease protection analysis (probes A,B,C). In Fig. 2 we also show the decoded ORF 206 region with its intron as will be discussed below.

In a first series of experiments we tried to delimit the DNA segment interacting with the 1.95 kb mRNA. In Fig. 3 we display the autoradiograms of Northern hybridization experiments using the DNA probes as specified in the legend. Probes b to e strongly interact with the 1.95 kb mRNA. Probe f yields no signal in this region and probe a which maps in the rps7 region does not interact with the 1.95 kb mRNA. In conclusion we can place the 1.95 kb mRNA between positions 1066 and 4152 (see Fig. 2).

2. The 5' terminal part : evidence for a spliced untranslated leader

Previous results (1) let us postulate that a spliced untranslated leader might exist. We have now further characterized the 5' terminal part by S1 protection analysis and primer extension sequencing (Fig. 4). Using probe A, as defined in the legend, we obtain protected DNA fragments of about 165 and 48 bases. A reasonable interpretation is that the 165 bases fragment represents the 5' terminal part of exon I and the 48 bases fragment

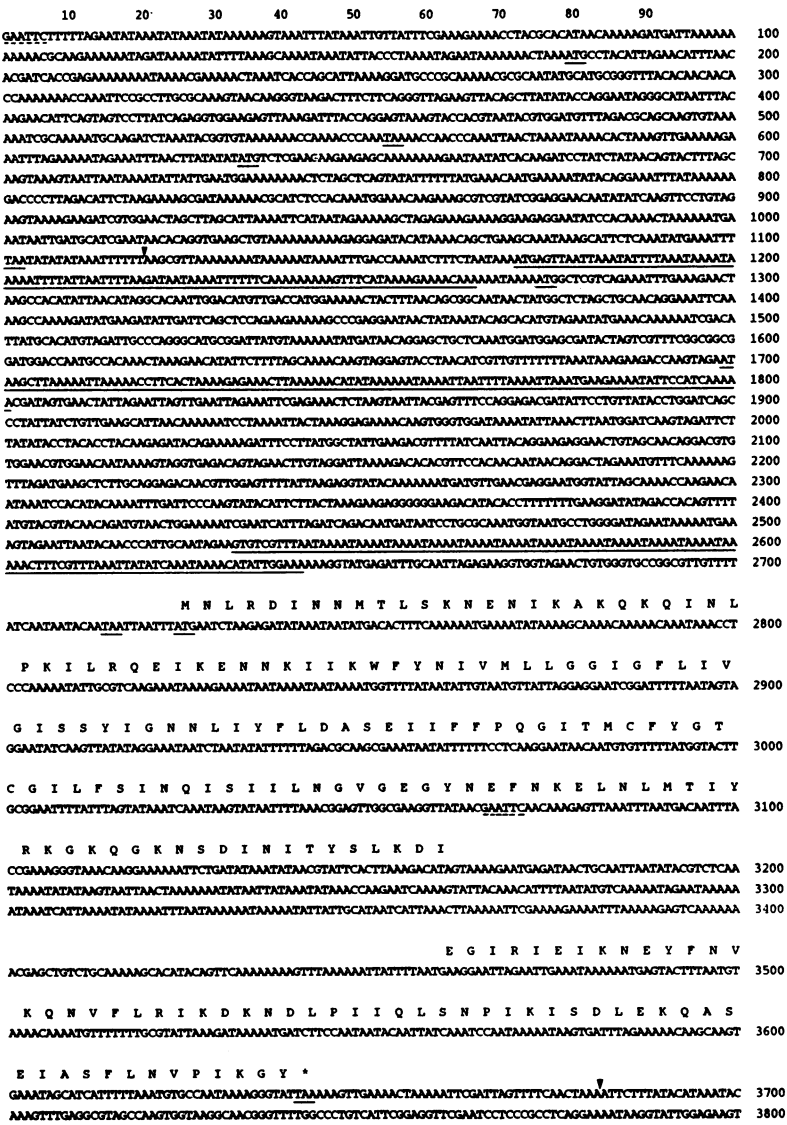

Fig. 2. Nucleotide sequence (RNA-like DNA strand) of the entire Eco·N (3066 bp) and adjacent parts of Eco·Q.
Numbering starts with junction Eco·J'·N (17) short solid lines mark start and stop codons of rps12, rps7, tufA and ORF206. Long solid lines mark introns I to III. Dashed lines mark EcoRI sites. For the split (intron 4) ORF206 region the amino acid sequence is given. Arrows mark the 5'- and 3'-end of the 1.95 kb transcript. Pos. 3681 (3'-end) precedes the trnQ gene with the same polarity (19) by 25 nucleotides.

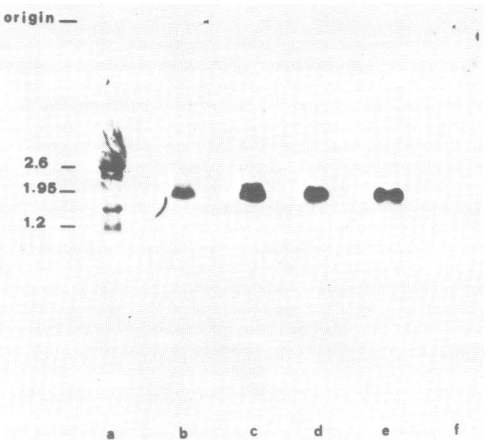

Fig. 3. Sizing the tufA-ORF206 transcript by Northern hybridisation. DNA probes a to f were used to delimit the length of the transcript. Probes a (pos. 1065-518); b (pos. 1336-1066); c (pos. 1995-1425); d (pos. 3066-2882) were ^{32}P-labelled by copying the respective inserts in M13 DNA (20). Probes e (pos. 3391-3762), f (pos. 3899-4152) were labelled by nick-translation (21). For positions consult Fig. 2. Note that the base numbers of probe f are not given on Fig. 2 but the sequences were published (19). Film exposures (Kodak XRA5) : a) 5 days; b+c) 1 day; d) 15 hours; e+f) 4 days; in case of d, e and f an intensifying screen (Cronex Lightning, Dupont) was used. Note, a,b,c represent one set, d,e,f represent another set of experiments. Numbers on the margin are in kilobases.

corresponds to the leader. If so, analysis of the RNA:DNA hybrids under non-denaturing (neutral) conditions should yield a major band of 220 bases on an agarose gel. Indeed such a band (not shown) was observed.

Unequivocal evidence for a spliced leader was obtained by sequencing the anticipated exon I - leader junction site by the primer extension method (Fig. 4B). Comparing this sequence with the sequence given in Fig. 2 allows to locate the splice junction and as a consequence to size the intron. Since both the donor and acceptor side contain short runs of As the splice site cannot be exactly assigned within the A cluster. But in analogy with other splice sites and intron termini in the tufA gene we opt for boundaries as given in Table 1.

The enlarged Fig. 4C gives the last positions of the 5' terminal part of the 1.95 kb mRNA, reading 5'AAGCG- (complementary strand). The mRNA therefore starts with A1120 (see Fig. 2). The upstream leaderpart has 51 bases, what is about in line with the size of the protected fragment seen in

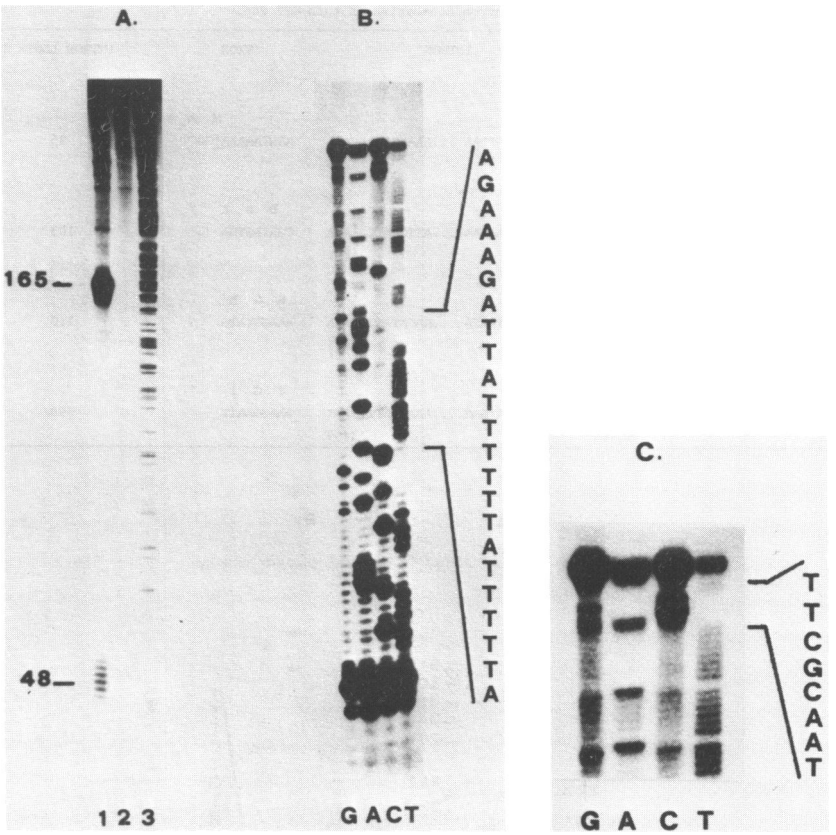

Fig. 4. Analysis of the 5'-terminal part of the 1.95 kb mRNA.
A. S1 nuclease protection analysis. DNA probe A was a 1.4 kb fragment (EcoRI-HinfI) which was ^{32}P-labelled using a M13 recombinant as template (synthesis of the coding strand, pos. 1424-2. Analysis of S1-resistant RNA:DNA hybrids (22) on 6% acrylamide gel, 8 M urea. Lane 1, protected fragments; lane 2, control with tRNA; lane 3, size marker; numbers on the margin are in bases. B. Dideoxy sequencing by primer extension; the sequence complementary to the RNA around the splice junction exon I - leader is shown. C. Enlargement of top part of B, with the sequence of the 5' end.

panel A (48 bases). The three base discrepancy can be explained by the high AT content which favors unstably hybridized termini, allowing the S1 nuclease to attack both ends. In conclusion we find that the leader has a total length of 59 bases (51 + 8) and is interrupted by an AT-rich intron (see Fig. 2) 95 bases long. To the best of our knowledge, this represents the first definition of a spliced untranslated leader of a chloroplast mRNA.

Table 1. Exon-intron boundaries in tufA-ORF 206.

	EXON	INTRON	EXON	INTRON LENTH (b)
			M A	
LEADER - EXON 1	TCTAATAAA	ATGAGTTAATTAA....AAAAGAAAACAAA	AAATAAAAATGGCT	95
	1170	1265		
	Q V D		D S E	
EXON 1 - EXON 2	CCAAGTAGA	ATAAGCTTAAAAA....ATTCCATCAAAAA	CGATAGTGA	103
	1698	1801		
	A I E		K G M	
EXON 2 - EXON 3	GCAATAGAA	GTGTCGTTTAATA....AACATATTGGAAA	AAAGGTATG	110
	2531	2641		
	K D I		E G I	
EXON 3 - EXON 4	AAAGACATA	GTAAAAGAATGAG....AAATTATTTTAAT	GAAGGAATT	298
	3161	3459		

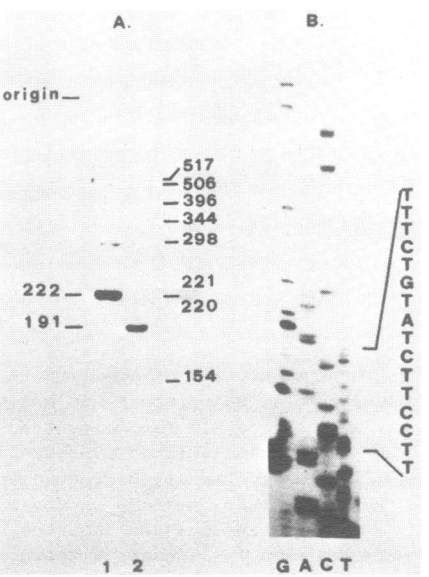

Fig. 5. Analysis of the 3'-terminal part of the 1.95 kb mRNA.
A. S1 nuclease protection analysis. Lane 1, probe B (HaeIII-HinfI fragment, pos. 3742-3391); Lane 2, probe C (HaeIII-RsaI fragment, pos. 3742-3491); chloroplast RNA was hybridized to the single strand DNA probes inserted in M13 (23). The protected hybrids were analysed on 6% acrylamide gel, 8 M urea after treatment with alkali. The single strand DNA fragments were transferred electrophoretically to nylon membranes and autoradiographically analysed by hybridisation with ^{32}P-labelled (nick-translation) fragment Eco·Q. Numbers on margin are bases. B. Dideoxy sequencing by primer extension. The autoradiogram shows the sequence complementary to RNA around the exon III - exon IV splice junction.

A

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ORF184                                MTWRSEH*WIEL*TGSR**SNFCWAFILF    29
ORF206   MNLRDINNMTLSKNENIKAKQKQINLPKILRQEIKENNKIIKWFYNIVML    50

         **SL***L**T***L*R***S*FPPQQ*******LV*S***IA*LFI*SY    79
         LGGIGFLIVGISSYIGNNLIYFLDASEIIFFPQGITMCFYGTCGILFSIN    100

         LWCT*SWN**S**DR*DRKEGIVC*F*W*FP***RR*FLRFLI***QSV*    129
         QISIILNGVGEGYNEFNKELNLMTIYRKGKQGKNSDINITYSLKDIEGIR    150

         **V*EGISARRVLYMDIRGQGSIPLTRTDE*LTPREIEQ*A*-*L*Y**R    178
         IEIKNEYFNVKQNVFLRIKDKNDLPIIQLSNPIKISDLEKQASEIASFLN    200

         ***EVF
         VPIKGY
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B

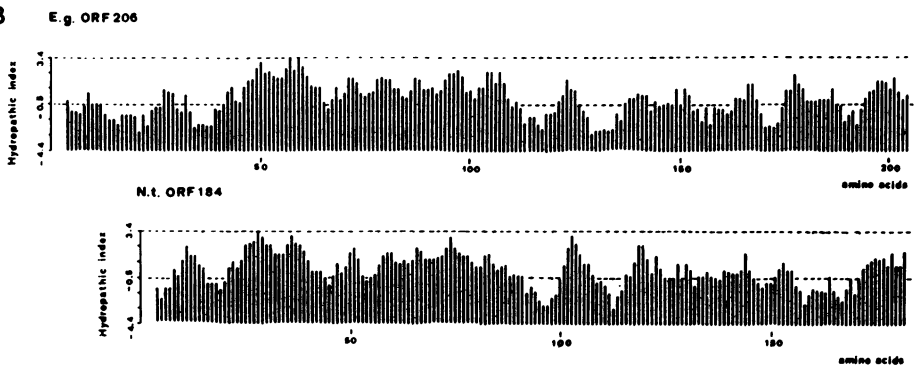

Fig. 6. Comparison of decoded ORF206 with tobacco ORF184 (3).
A. Amino acid sequences. Asterisks mark identy, - marks codon deletion in ORF184. B. Hydropathy plots (38). The two plots are aligned with respect to the 3' end. Window size was 6 aminoacids, using the PRPLOT program kindly provided by Dr. Nager, IFM, Basle. E.g., Euglena gracilis; N.t., Nicotiana tabacum.

3. The 3' terminal part : evidence for a split ORF206

We knew that the 1.95 kb mRNA extends well beyond the tufA coding region carrying in addition one, possibly two ORFs. To establish the 3' end of the mRNA and to clear the question regarding the ORF, we made S1 nuclease protection experiments using DNA probes B and C (see Fig. 1). In Fig. 5A we show the results. DNA probes B and C, as defined in the legend to Fig. 5, yielded protected fragments of 222 and 191 bases, respectively. Furthermore, we sequenced the exon IV - exon III junction by primer extension (Fig. 5B). The information obtained from both kinds of experiments permits us (1) to exactly position the intron within the ORF region (Fig. 2, Table 1); (2) to define a single ORF region and (3) to place the 3' end of the mRNA 39 positions after the ORF stop codon (pos. 3781).

4. The ORF206

The maximal length of the split ORF region is 206 codons. The putative protein has a calculated mol. weight of 23,500 with a polarity index of 46. We have no evidence for this ORF to be translated but the following facts are noteworthy : 1) A computer search revealed that ORF206 is significantly homologous with the decoded ORF184 of tobacco (pos. 62630; 3). As seen in Fig. 6A the putative Euglena protein matches with the tobacco protein in 62/184 positions. Quite important, sequence homology is found on either side of the splice junction and about equal sequence homology is also obtained with the equivalent ORF184 of Marchantia (24). Incidently, the N-terminal tripeptide MNL of Euglena ORF206 coincides exactly with that of Marchantia ORF184. The hydropathy plots of ORF206 and tobacco ORF184 are given in Fig. 6B. The sequel of hydrophobic domains of about equal length is similar in both putative proteins. 2) We checked for codon bias in the usage of isoaccepting tRNA species. The pyrimidine over purine ratio at the third position is 0.46 and therefore close to that of the tufA gene (0.49). It is interesting to see that the tufA, rps7, rps12 (1,18) and rpl20 (25) have a ratio around 0.5, while the light induced psbC (26) and psbA (8) and rbcL (7) genes coding for proteins involved in photosynthesis have a ratio of about 3. Gingrich and Hallick (7) have already noticed this striking difference and suggested the possibility that this might reflect a mechanism of translational regulation.

5. Introns

In Table 1 we list the sequences around the junction sites of the four introns which split the tufA-ORF206 gene. Hallick et al. (2) proposed for the 5'-termini of chloroplast introns the consensus sequence 5'-GTGYGY or a variant with five of the six bases. Except intron 3, none of these introns closely follows this rule. The only invariant position is a T at position 2. Even less conserved are the 3'-termini of the introns which were proposed (2) to read, -ATTTTAT-3'. Again the only invariant position is the penultimate A, however, the pyrimidine track is lacking (exception, intron 4). Introns 1,2, 3 are short, very rich in AT with an excess of A over T. According to Keller and Michel (27), some of the Euglena chloroplast introns qualify for group II introns capable of undergoing a specific folding pattern. Introns 1, 2, 3 have no such folding capacity; only intron 4 may be folded in such a way as to yield loop V and VI of group II introns (not shown). Presently we lack

information about splicing mechanisms in Euglena chloroplasts but as previously suggested (26) chloroplast intron excision and splicing may have features in common with the nuclear catalytic splicing process. If so we would expect a branching box near the intron 3' end. Circular forms (lariats ?) were seen in E. gracilis psbA transcripts (28). The CATA element seen in all four cases near the 3' end may serve as branch box (consult Fig. 2).

DISCUSSION

1. The tufA-ORF206 transcription unit

In E. coli the tufA gene is part of the str operon which contains the rps12 - rps7 - fus - tufA gene cluster (30). On Euglena chloroplast DNA we observe a somewhat similar arrangement (rps12 - rps7 - tufA - ORF206). However, previous and present data strongly suggest that the rps12 - rps7 genes are co-transcribed independently from the tufA - ORF206 region. Under the prevailing physiological growth and harvesting conditions the 1.95 kb mRNA is at least 30-100 times more abundant than the 1.2 kb mRNA (rps12+7) (consult legend to Fig. 3) although both gene products are involved in protein synthesis. Of course, our results do not exclude the possibility that both regions are transcribed with equal efficiency but differential post-transcriptional events may lead to the remarkable difference in the steady state concentration. Unfortunately, promoter regions for Euglena gracilis chloroplast mRNAs are not yet defined, and nothing is known about promoter qualities unlike the situation with higher plant chloroplast mRNAs (e.g. 31).

The 3'-terminal non coding part extends for 39 positions and contains inverted repeats which can be folded in a perfect AT rich stem of 13 bases. This structural element may be involved in transcription termination. A similarly short termination region (26-28 positions) was also found on the rbcL mRNA (7). However, in that case, no potential stem-loop structure was observed.

2. Introns and leader qualities

The Euglena gracilis chloroplast genome is rich in split genes. Interestingly, the 50 or so physically mapped introns (32) seem to occur exclusively in protein coding genes, since none of the 23 sequenced tRNA nor any of the rRNA genes is split. In higher plant chloroplast DNA introns may occur in tRNA genes and protein coding genes (3) and for Chlamydomonas an intron was reported to occur in the large subunit rRNA gene (33). An intron in

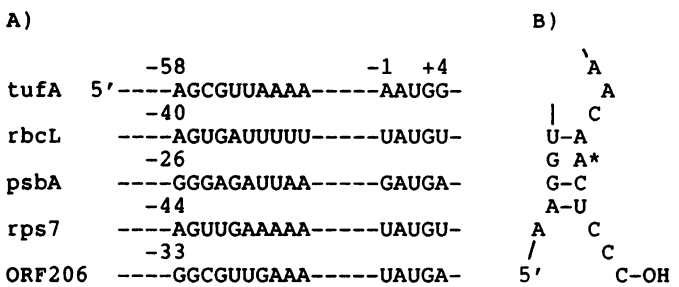

Fig. 7. Analysis of leader sequences of chloroplast mRNAs from Euglena gracilis.
A. Potential ribosome binding sites of leader sequences and positions relative to the start codon. B. Proposed secondary structure of the 3' end of 16S rRNA (29) which might interact with the mRNA. The asterisk marks base A replacing base C in E. coli 16S rRNA.

the untranslated leader of a mRNA is novel for chloroplast DNA, but such was observed in nuclear pre-mRNAs, e.g., in yeast genes coding for the large subunit ribosomal protein 29 (34) and subunit V of cytochrome oxidase (35). The function of such introns, if any, is presently unknown.

Chloroplast mRNAs are of prokaryotic nature. For instance they can be oligocistronic and may carry Shine-Dalgarno type sequences near the start codon. This latter statement seems to be correct for higher plant chloroplast mRNAs (3,5,6,24) but it does not hold for Euglena gracilis chloroplasts. We surveyed (Fig. 7) defined leader sequences searching for oligobase elements complementary to the 3' terminal region of 16S rRNA (9). We notice that complementary elements are absent near the start codon but they are located at variable distances further upstream. This may just be a statistical result with no functional significance. More important may be the observation that the sequence linking the putative "ribosome binding" site with the start codon is extremely rich in AU, never contains an AUG nor GUG triplet, although occasional Gs are found, and the region does not have the capacity to undergo stable foldings. Interestingly enough, the unspliced tufA leader contains an AUG which, however, is removed upon splicing. We analysed in the same way tentative leader regions of the Euglena chloroplast genes rps12 (18), rpl20 (25) and psaA (36) and obtained the same general result. Kosak (4) discusses other structural criteria of sequences around the start codon which may enhance the specificity of cistron recognition. It was postulated, e.g.,

that pyrimidines at position -1 and purines at position +4 stimulate ribosome binding. As seen in Fig. 7, no base preference is discernable at either position in Euglena gracilis mRNAs.

Certainly, the number of analysable mRNAs is too small to draw firm conclusions. But strong Shine-Dalgarno RNA:RNA interactions near the start codon cannot occur and for the moment we have no evidence for the upstream element to be involved in the translation initiation step. If so, it would mean that the ribosome (pre-initiation complex) scans the AU rich leader part until it recognizes the firts AUG, somewhat similar to the mechanism proposed for eukaryotes (4).

Spremulli and collaborators have studied translation initiation with Euglena gracilis chloroplasts (37). For instance the IF-2 (chloroplast)-directed binding of fMet-$tRNA_i^{Met}$ to chloroplast 30S ribosomal subunits is mRNA (AUG) dependent. Binding activity (same test) but using E. coli 30S subunits is comparably low, suggesting that translation initiation in Euglena chloroplasts does have features distinct from those of bacteria. According to our data ribosome recognition is one of these distinct features and using homogenous in vitro initiation translation systems with defined mRNAs will help to elucidate details of Euglena gracilis chloroplast translation.

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REFERENCES

1. Montandon, P.E. and Stutz,E. (1983) Nucleic Acids Res. 11, 5877-5892.
2. Hallick,R.B., Gingrich,J.C., Johanningmeier,U. and Passavant,C.W. (1985) in NATO ASI Series, van Vloten-Doting,L., Groot,G.S.P. and Hall,T.C. Eds., Vol. 83, pp. 211-220, Plenum Press, New York.
3. Shinozaki,K. et al. (1986) EMBO J. 5, 2043-2049.
4. Kosak,M. (1983) Microbiol. Rev. 47, 1-45.
5. McIntosh,L., Poulsen,L. and Bogorad,L. (1980) Nature, 288, 556-560.
6. Kirch,W., Seyer,P. and Herrmann,R.G. (1986) Curr. Genet. 10, 843-855.
7. Gingrich,J.C. and Hallick,R.B. (1985) J. Biol. Chem. 260, 16162-16168.
8. Keller,M. and Stutz,E. (1984) FEBS Lett. 175, 173-177.

9. Steege,D.A., Graves,M.C. and Spremulli,L.L. (1982) J. Biol. Chem. 257, 10430-10439.
10. Sagher,D., Grosfeld,H. and Edelman,M. (1976) Proc. Natl. Acad. Sci. USA 73, 722-726.
11. Ortiz,W., Reardon,E.M. and Price,C.A. (1980) Plant Physiol. 66, 291-294.
12. Hallick,R.B., Chelm,B.K., Gray,P.W. and Orozco,E.M. (1977) Nucleic Acids Res. 4, 3055-3064.
13. Müller,R., Slamon,D.J., Tremblay,J.M., Cline,M.J. and Verma,I. (1982) Nature 299, 640-644.
14. Maxam,A.M. and Gilbert,W. (1980) in Methods in Enzymology, Grossman,L. and Moldave,K. Eds, Vol. 65, pp. 499-560, Academic Press, New York.
15. Goldenberg,C.J. and Hauser,S.D. (1983) Nucleic Acids Res. 11, 1337-1348.
16. Rutti,B. (1982) Ph.D. thesis, University of Neuchâtel, Switzerland.
17. Passavant,C.W., Stiegler,G.L. and Hallick,R.B. (1983) J. Biol. Chem. 258, 693-695.
18. Montandon,P.E. and Stutz,E. (1984) Nucleic Acids Res. 12, 2851-2859.
19. Karabin,G.D. and Hallick,R.B. (1983) J. Biol. Chem. 258, 5512-5518.
20. Hu,N. and Messing,J. (1982) Gene 17, 271-277.
21. Rigby,P.W., Dickmann,M., Rhodes,C. and Berg,P. (1977) J. Mol. Biol. 113, 237-251.
22. Favaloro,J., Treisman,R. and Kamen,R. (1980) in Methods in Enzymology, Grossman,L. and Moldave,K. Eds., Vol. 65, pp. 718-749, Academic Press, New York.
23. Squires,C., Krainer,A., Barry,G., Shen,W.F. and Squires,C.L. (1981) Nucleic Acids Res. 9, 6827-6840.
24. Ohyama,K. et al. (1986) Nature 322, 572-574.
25. Manzara,T. and Hallick,R.B. (1987) Nucleic Acids Res. 15, 3927.
26. Montandon,P.E., Vasserot,A. and Stutz,E. (1986) Curr. Genet. 11, 35-39.
27. Keller,M. and Michel,F. (1985) FEBS Lett. 179, 69-73.
28. Koller,B., Clarke,J. and Delius,H. (1985) EMBO J. 4, 2445-2450.
29. Graf,L., Roux,E., Stutz,E. and Kössel,H. (1982) Nucleic Acids Res. 10, 6369-6381.
30. Jaskunas,S.R., Lindahl,L., Nomura,M. and Burgess,R.R. (1975) Nature 257, 458-462.
31. Gruissem,W. and Zurawski,G. (1985) EMBO J. 4, 3375-3383.
32. Koller,B. and Delius,H. (1984) Cell 36, 613-622.
33. Allet,B. and Rochaix,J.D. (1979) Cell 18, 55-60.
34. Mitra,G. and Warner,J.R. (1984) J. Biol. Chem. 259, 9218-9224.
35. Schneider,J.C. and Guarente,L. (1987) Nucleic Acids Res. 15, 3515-3529.
36. Manzara,T., Hu,J.X., Price,C.A. and Hallick,R.B. (1987) Plant Mol. Biol. 8, 327-336.
37. Gold,J.C. and Spremulli,L.L. (1985) J. Biol. Chem. 260, 14897-14900.
38. Kyte,J. and Doolittle,R.F. (1982) J. Mol. Biol. 157, 105-132.