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A point mutation in *Euglena gracilis* chloroplast $tRNA^{Glu}$ uncouples protein and chlorophyll biosynthesis

(porphyrin biosynthesis/$tRNA^{Glu}$ mutation/C_5 pathway/5-aminolevulinic acid)

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ABSTRACT **The universal precursor of tetrapyrrole pigments (e.g., chlorophylls and hemes) is 5-aminolevulinic acid (ALA), which in *Euglena gracilis* chloroplasts is derived via the two-step C_5 pathway from glutamate charged to $tRNA^{Glu}$. The first enzyme in this pathway, Glu-tRNA reductase (GluTR) catalyzes the reduction of glutamyl-$tRNA^{Glu}$ (Glu-tRNA) to glutamate 1-semialdehyde (GSA) with the release of the uncharged $tRNA^{Glu}$. The second enzyme, GSA-2,1-aminomutase, converts GSA to ALA. $tRNA^{Glu}$ is a specific cofactor for the NADPH-dependent reduction by GluTR, an enzyme that recognizes the tRNA in a sequence-specific manner. This RNA is the normal $tRNA^{Glu}$, a dual-function molecule participating both in protein and in ALA and, hence, chlorophyll biosynthesis. A chlorophyll-deficient mutant of *E. gracilis* (Y_9ZNalL) does not synthesize ALA from glutamate, although it contains GluTR and GSA-2,1-aminomutase activity. The $tRNA^{Glu}$ isolated from the mutant can still be acylated with glutamate *in vitro* and *in vivo*. Furthermore, it supports chloroplast protein synthesis; however, it is a poor substrate for GluTR. Sequence analysis of the tRNA and of its gene revealed a C56 → U mutation in the resulting gene product. C56 is therefore an important identity element for GluTR. Thus, a point mutation in the T loop of tRNA uncouples protein from chlorophyll biosynthesis.**

5-Aminolevulinic acid (ALA) is the universal precursor of porphyrins. In the phytoflagellate *Euglena gracilis*, as in lower and higher plants, ALA is formed in greening plastids from Glu-tRNA via the tRNA-dependent C_5 pathway (Fig. 1). In the first step, catalyzed by Glu-tRNA reductase (GluTR), the C1-carboxyl group of a glutamate esterified to its cognate tRNA is reduced to form glutamate 1-semialdehyde (GSA) with the concomitant release of the tRNA. Then the enzyme GSA-2,1-aminomutase (GSA-AM) catalyzes the exchange of the amino and carbonyl groups at positions C1 and C2 of GSA to form ALA (for review, see ref. 1). In plants and algae, the C_5 pathway is localized in the chloroplast. The enzymes involved (GluTR and GSA-AM) are encoded by the nucleus, and the tRNA cofactor is encoded by the chloroplast (refs. 2–4; N. Hori, A. M. Kumar, and D.S., unpublished data). Glu-tRNA is synthesized from glutamate and $tRNA^{Glu}$ by Glu-tRNA synthetase (GluRS), a key enzyme in protein synthesis (5, 6). Thus, Glu-tRNA is a dual-function molecule donating its activated amino acid for both ALA and protein synthesis. Consequently, $tRNA^{Glu}$ is recognized by at least three proteins, GluRS, the elongation factor EF-Tu, and GluTR.

The highly specific recognition of tRNA by different proteins is a fascinating area of study. The nucleotides in tRNA, termed identity elements, which are important for recognition by proteins, have been elucidated for a number of aminoacyl-tRNA synthetases. For *Escherichia coli* GluRS, positions in the acceptor stem and anticodon, as well as a modified uridine in the anticodon, have been shown to be involved in glutamate identity (7, 8). The close relationship of chloroplast and bacterial tRNAs and the observation that *E. gracilis* GluRS charges *E. coli* tRNA indicated the same may apply for *E. gracilis* GluRS. Much less is known about the elements for tRNA recognition by EF-Tu, and there is no knowledge about positions in $tRNA^{Glu}$ important for interaction with GluTR. If GluTR and GluRS employ different identity elements to recognize $tRNA^{Glu}$ and as the chloroplast genome of *E. gracilis* harbors only one $tRNA^{Glu}$ gene (9), it might be possible to isolate mutants containing an altered $tRNA^{Glu}$ that could still be charged but could no longer participate in the GluTR reaction. Hence, such mutants may not make sufficient amounts of ALA and be chlorophyll-deficient. Therefore, we searched for such a phenotype among nongreening mutants of *E. gracilis*.

One mutant with the desired phenotype, Y_9ZNalL (Y9), is orange and incapable of synthesizing chlorophyll or even protochlorophyll but still possesses chloroplast-specific structures (10). Ribulose-1,5-diphosphate carboxylase activity detected in cell extracts indicated the presence of functional chloroplast protein synthesis in this mutant (10). Thus, we investigated the ability of this mutant to form ALA and perform chloroplast protein synthesis and also determined the nucleotide sequence of the mutant $tRNA^{Glu}$. Here we show that a single base change makes the mutant $tRNA^{Glu}$ a poor substrate for GluTR.

MATERIALS AND METHODS

Strains and Growth Conditions. Cells of *E. gracilis*, var. *Bacillaris*, strain Z (wild-type) and of the Y9 mutant were grown in the light and harvested as described (10).

Isolation of DNA. *Euglena* DNA was prepared from frozen cells (0.2 g of wet weight, ≈2 × 10^8 cells). After resuspension in 5 ml of 0.15 M NaCl/0.1 M EDTA/1 mM Tris·HCl, pH 8.0, SDS and Pronase were added [final concentrations, 4% (wt/vol) and 100 μg/ml, respectively]. The suspension was gently shaken every 5 min while kept at 37°C for 30 min. After chilling on ice and addition of sodium perchlorate (final concentration, 1 M) the solution was extracted with an equal volume of chloroform/isoamyl alcohol, 24:1 (vol/vol) for 30 min. The aqueous phase was re-extracted with 1 vol of chloroform/isoamyl alcohol. The nucleic acids were precipitated by addition of absolute ethanol (2 vol), centrifuged, and

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Abbreviations: Y9, Y_9ZNalL; GluRS, glutamyl-tRNA synthetase; GluTR, Glu-tRNA reductase; GSA-AM, glutamate 1-semialdehyde-2,1-aminomutase; GSA, glutamate 1-semialdehyde; ALA, 5-aminolevulinic acid.

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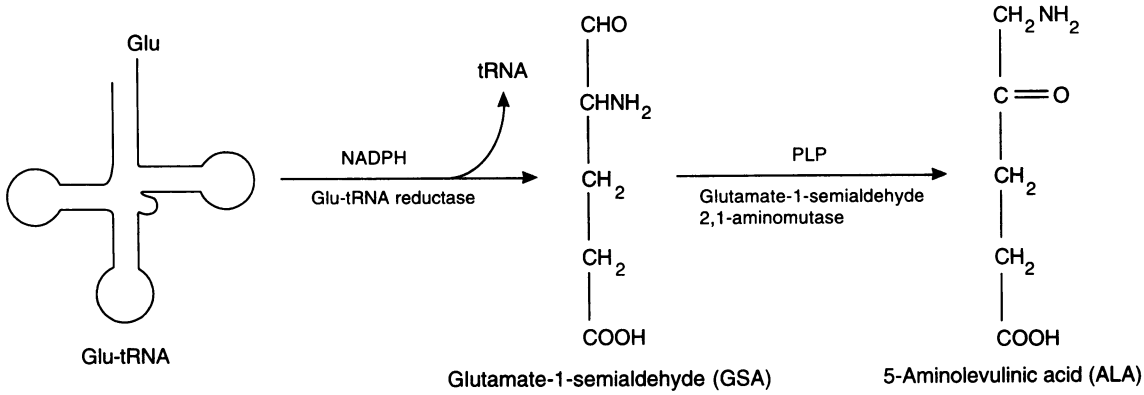

FIG. 1. The C_5 pathway of ALA formation. GluTR reduces normal Glu-tRNA^{Glu} to GSA with the release of intact tRNA^{Glu}. GSA is converted to ALA by GSA-AM.

dissolved in 0.1× standard saline citrate (SSC) (1× SSC is 0.015 M sodium chloride/0.0015 M sodium citrate). RNA was degraded by incubation with RNase T1 (100 units/ml) and RNase A (50 μg/ml) for 1 hr at 37°C. After precipitation with 2 vol of isopropanol the DNA was dissolved in 10 mM Tris·HCl/1 mM EDTA, pH 7.5.

PCR Cloning and Sequence Analysis of a DNA Fragment Carrying the Chloroplast tRNA^{Glu} Gene. As the DNA sequence surrounding the tRNA^{Glu} gene is known (9), primers were designed for amplification and cloning of the gene from wild-type and mutant *E. gracilis*. The primers (5′-CTGCGCTTTTAGATCAATTGGTAGAACGTAGG-3′ and 5′-GCGGGTAACGTGATTCGATCACGCAAC-3′) contained a *Sau*3A restriction site (underlined) to facilitate subcloning into a pBSK-*Bam*HI vector. The DNA sequence of the tRNA genes was determined on both strands by a modified dideoxynucleotide chain-termination method (11).

Northern Blot Analysis. Charged or uncharged total tRNA was isolated from *E. gracilis* as described (12, 13) and separated on an acidic 10% polyacrylamide/8 M urea gel (20 cm long, 1 mm thick). tRNA^{Glu} was detected by hybridization with the 5′-^{32}P-labeled tRNA^{Glu}-specific oligonucleotide 5′-GTGAAAGGGAGATGTCC-3′ in 6× SSC/10× Denhardt's solution/1 mM ATP/1% SDS/10 mM Na_2EDTA/poly(A) at 0.2 mg/ml and 40°C. The membrane was washed once in 6× SSC/0.05% SDS for 20 min at room temperature and then for 5 min at 50°C.

Cell Extracts. For preparation of S200 or S160 extracts *E. gracilis* cells were sonicated in 20 mM Hepes, pH 7.9/15 mM $MgCl_2$/2.5 mM dithiothreitol/0.2 mM phenylmethanesulfonyl fluoride/0.02 mM pyridoxal phosphate/20% (vol/vol) glycerol and centrifuged (14).

Immunoblotting. Proteins from S160 preparations were separated by electrophoresis (15) and transferred onto a nitrocellulose membrane (16). After blocking for 16 hr at 4°C with 10% nonfat dry milk in 50 mM Tris·HCl, pH 8.0/150 mM NaCl, the membrane-bound protein was incubated with antibodies against *Spinacia oleracea* CF1 ATP synthase β subunit (17). Anti-rabbit IgG horseradish peroxidase conjugate (Pierce) was used as secondary antibody, and the final antigen–antibody complexes were developed in Lumiglo (Kirkegaard & Perry Laboratories).

Partial Purification of GluTR from *E. gracilis* Wild-Type Cells. This was achieved by DEAE-cellulose chromatography of an S200 extract with a linear KCl gradient (20–600 mM). GluTR activity eluted between 150 mM and 250 mM KCl; the fractions were concentrated and stored in 20 mM Hepes, pH 7.9/15 mM $MgCl_2$/2.5 mM dithiothreitol/0.2 mM phenylmethanesulfonyl fluoride/0.02 mM pyridoxal phosphate/20% (vol/vol) glycerol.

ALA Formation from Glutamate. Assays (18) that included 1 μCi of [1-^{14}C]glutamate (59 mCi/mmol; 1 Ci = 37 GBq) and 1.5 mg of *E. gracilis* protein were done for 1 hr at 30°C. [1-^{14}C]Glu was used to preclude ALA formation by ALA-synthase via the Shemin pathway (19).

ALA Formation from Glutamyl-tRNA^{Glu}. [^{3}H]Glu-tRNA^{Glu} was prepared from total *E. gracilis* tRNA in the presence of 30 mM Hepes, pH 7.2/15 mM $MgCl_2$/20 mM KCl/1 mM [3,4-^{3}H]Glu (54 Ci/mmol)/5 mM ATP/2.5 mM dithiothreitol/30 units of barley chloroplast GluRS for 1 hr at 37°C. ALA synthesis was assayed in a total volume of 0.25 ml containing 6.9 mg of protein in 20 mM Hepes, pH 7.2/10 mM $MgCl_2$/20% (vol/vol) glycerol/1 mM dithiothreitol/5 mM levulinic acid/1 mM NADPH/0.1 mM pyridoxal phosphate/1 mM glutamate/4 μg of GSA-AM/126,000 cpm of [3,4-^{3}H]Glu-tRNA^{Glu} (specific activity of total tRNA, 4100 and 1600 cpm/μg for wild-type and Y9 mutant, respectively) at 30°C for 1 hr. The stabilities of the glutamyl linkage in mutant and wild-type Glu-tRNA were the same ($t_{1/2}$ = 13 min) in the above buffer. ALA formed was purified as described (18, 20). GluTR activity was completely abolished when precharged tRNA was preincubated with RNase A at 1.7 μg/ml for 15 min at 30°C before the addition of extract.

Purification of Chloroplast tRNA^{Glu} by Hybrid Selection. Plasmid DNA of individual clones containing the desired chloroplast tRNA^{Glu} gene (*Mse* I–*Alu* I subfragment of the tRNA gene cluster region in pBSK-*Sma*I vector) was heated at 65°C in 0.3 M NaOH for 40 min, neutralized with NaH_2PO_4 (final concentration, 0.5 M), and the DNA solution was further diluted with 1 vol of 20× SSC to a concentration of 80 μg/ml. DNA (500 μg) was bound to Schleicher & Schüll BA85 filters (ϕ = 2.5 cm) as described (21). Total tRNA (6 mg) in 5× SSC/50% (vol/vol) formamide/5× Denhardt's solution/0.2% SDS/1 mM EDTA was incubated in the presence of a DNA filter for 8 hr at 50°C. The filters were washed twice with 2× SSC at room temperature and twice with 2× SSC at 50°C. The bound tRNA^{Glu} was eluted by incubation in water at 80°C for 3 min.

Sequence Analysis of Chloroplast tRNA^{Glu}. The tRNA was digested with RNase T1. After removal of the 3′-phosphates with calf intestine phosphatase, RNA fragments were 5′-labeled with [γ-^{32}P]ATP (400 Ci/mmol) by polynucleotide kinase (22). Fingerprint analyses were done as described (22). After elution from the DEAE-cellulose thin-layer plate (23) most oligonucleotides were subjected to partial digestion with nuclease P1 followed by two-dimensional mobility-shift analysis (22).

RESULTS

tRNA^{Glu} from the Y9 Mutant Participates in Chloroplast Protein Biosynthesis *in Vivo*. Because we suspected that the

Y9 mutant phenotype may be caused by a mutation in the $tRNA^{Glu}$ we examined the competence of the Y9 mutant cells to charge this tRNA *in vivo*. The aminoacylated tRNA was isolated from Y9 cells, and charged chloroplast Glu-tRNA was determined by acid-gel Northern blot analysis with a chloroplast $tRNA^{Glu}$-specific probe (13). The results (Fig. 2*A*) show that Y9 $tRNA^{Glu}$ was fully charged, thus indicating correct *in vivo* aminoacylation by chloroplast GluRS. Although equal amounts of total RNA from wild-type and mutant cells were probed with a ^{32}P-labeled $tRNA^{Glu}$-specific oligonucleotide, Y9 cells contained only 15–20% as much Glu-tRNA as did the wild type. A Northern blot of uncharged tRNA (data not shown) confirmed this result; Y9 mutant cells contain a much reduced level (22%) of $tRNA^{Glu}$ compared with wild-type cells.

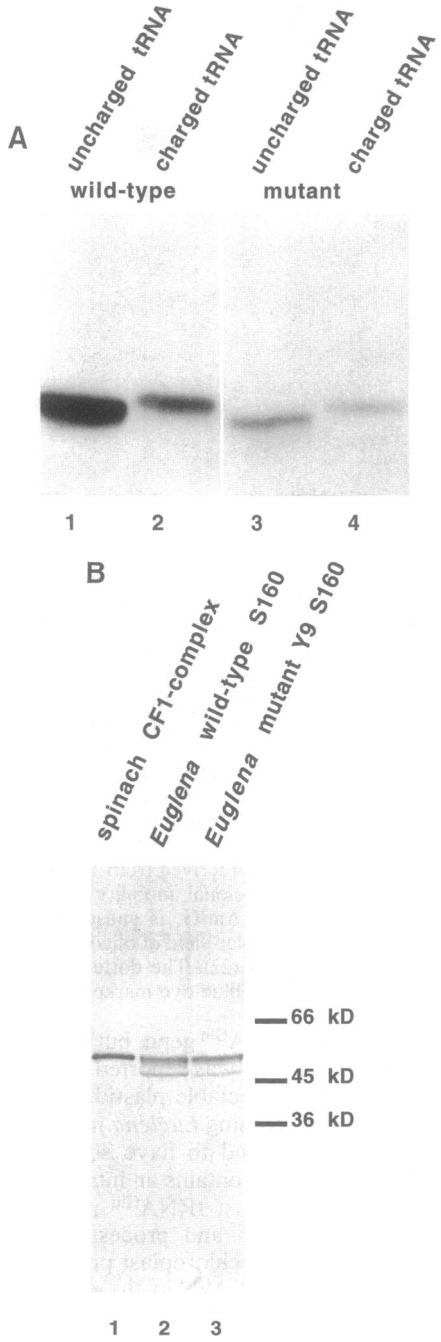

FIG. 2. Aminoacylation and protein synthesis in wild-type and mutant *E. gracilis*. (*A*) Electrophoretic separation of charged and uncharged $tRNA^{Glu}$. Lanes: 1, wild type (5 μg of total deacylated tRNA); 2, wild type (25 μg of total RNA isolated under nondeacylating conditions); 3, mutant (5 μg of total deacylated tRNA); 4, mutant (25 μg of total RNA isolated under nondeacylating conditions). The amounts of $tRNA^{Glu}$ and Glu-$tRNA^{Glu}$ were determined by densitometry. (*B*) Immunohybridization patterns of *E. gracilis* and *S. oleracea* chloroplast ATP synthase (CF1). Lanes: 1, 70 ng of purified spinach chloroplast ATP synthase (CF1) complex; 2, 30 μg of protein from an S160 extract from *E. gracilis* wild-type strain; 3, 30 μg from an S160 extract from *E. gracilis* Y9.

Is there protein biosynthesis in the chloroplasts of the Y9 mutant? The fact that intact chloroplast $tRNA^{Glu}$ is formed in the mutant strain (see above) points to the involvement of chloroplast RNA polymerase; some of its subunits are encoded in the chloroplast genome (24) and are synthesized in the chloroplast (25). Biochemical evidence for the occurrence of protein synthesis was obtained in early studies that demonstrated Rubisco activity in the Y9 mutant cells (10). We wanted to provide additional evidence for chloroplast protein synthesis by performing an immunoblot of an *E. gracilis* extract with a suitable protein. Examination of the *E. gracilis* chloroplast DNA sequence revealed that the β subunit of the ATP synthase CF1 complex is encoded in the organelle genome (24) and that the deduced amino acid sequence of this protein is 82% identical with the same protein from spinach (26). The molecular weights of the spinach and *Euglena* proteins are similar (53.9 vs. 52.1 kDa). By using the *S. oleracea* antibody (17), immunohybridizations revealed the presence of a strongly crossreacting protein in both wild-type and mutant extracts (Fig. 2*B*). We assume that the less intense, lower-molecular-weight band is due to proteolysis, as the amount of this protein (relative to the other band) varied in different extract preparations. These data strengthen the previous evidence for functional chloroplast protein synthesis in the mutant strain and hence for the competence of Y9 $tRNA^{Glu}$ to participate in this process.

$tRNA^{Glu}$ from the Y9 Mutant Cannot Support ALA Formation by the C_5 Pathway. Knowing that the mutant chloroplast $tRNA^{Glu}$ could support protein synthesis, we wanted to test its ability to function in ALA formation. Accordingly, we analyzed the tRNA-dependent conversion of radioactive glutamate to ALA. In these assays Glu-tRNA was generated *in situ* from uncharged tRNA and endogenous *E. gracilis* GluRS. GSA, the product of the GluTR reaction with Glu-tRNA, is then immediately converted to ALA by the action of GSA-AM contained in the S200 extract (18). Because the amount of $tRNA^{Glu}$ in the Y9 mutant was ≈20% of that found in wild-type cells (see above), we assayed at two tRNA concentrations, with the mutant total tRNA at 5-fold excess relative to wild-type tRNA; thus approximately equal amounts of charged Glu-tRNA were provided in the assays. When the tRNA source was wild type, extracts from both wild-type and mutant cells converted glutamate to ALA, although Y9 cell extracts were less active (Table 1). Therefore, GluRS and the two C_5 pathway enzymes GluTR and GSA-AM were present in wild-type and in mutant chloroplasts. With Y9 as the tRNA source, both mutant and wild-type extracts exhibited very low levels of ALA synthesis; Y9 $tRNA^{Glu}$, even when added in elevated amounts comparable to that of wild-type $tRNA^{Glu}$, supported <10% of the synthetic activity seen with wild-type tRNA.

Table 1. Glu → ALA conversion in *Euglena* cell extracts

tRNA source	tRNA amount, μg	pmol of ALA/mg of protein: Wild-type extract	pmol of ALA/mg of protein: Mutant extract
Wild type	10	1.9	0.6
	100	20.0	8.2
Y9 mutant	50	0.5	0.5
	500	2.0	0.9

As the assay with *in situ*-generated Glu-tRNA is not suited to measure initial velocities of GluTR, we used precharged [^{14}C]Glu-tRNA from wild-type and Y9 *Euglena* cells to assay partially purified GluTR from wild-type cells. In the presence of added *E. coli* GSA-AM, wild-type Glu-tRNA was converted to ALA at a rate of 0.041 pmol/hr per mg, whereas no measurable ALA synthesis was seen with the mutant tRNA under these conditions. These results showed that the mutant Y9 tRNA was incapable of serving efficiently in ALA formation and suggested that tRNA^{Glu} was mutated.

tRNA^{Glu} from the Y9 Mutant Harbors a Single Base Change. The results described above implied that a mutation in the tRNA^{Glu} gene was responsible for the inability of this tRNA to support ALA synthesis. Thus, we sequenced the chloroplast tRNA^{Glu} gene of the *E. gracilis* wild-type and Y9 mutant strains. As the nucleotide sequence of the cluster of six different tRNA genes, containing the tRNA^{Glu} gene, is known (9), we isolated the corresponding DNA fragment by PCR from both wild-type and Y9 mutant chloroplast genomes. In each case three independent PCR experiments were conducted and analyzed to reduce the possibility of PCR artifacts. As suggested above, the Y9 tRNA^{Glu} gene contained a point mutation, a C → T transition, at the highly conserved position 56 in the T loop of the corresponding tRNA molecule. The tRNA sequences, deduced from the gene sequences, of wild type and Y9 mutant are shown in Fig. 3. To establish that this mutation is also found in the corresponding tRNA, wild-type and mutant tRNAs were isolated by hybrid selection, digested with RNase T1, and end-labeled. The fingerprint patterns (Fig. 4) showed a distinct difference between mutant and wild-type tRNA. Spot 6 (Fig. 4 *Left*) in wild-type tRNA contained the oligonucleotide AUUCG derived from the T loop. This spot was missing in the Y9 fingerprint and instead appeared as AUUUG in spot 6 (Fig. 4 *Right*). This result clearly demonstrated the presence of the C56 → U mutation in Y9 tRNA^{Glu}.

DISCUSSION

In our survey of nongreening and greening *Euglena* mutants (W_{14}ZNalL, W_{14}ZNalL-A, W_{14}ZNalL-B, Y9 and P_{27}ZNalL, Nal^{r}_{80}ZNalL, respectively) we noticed that all mutants tested

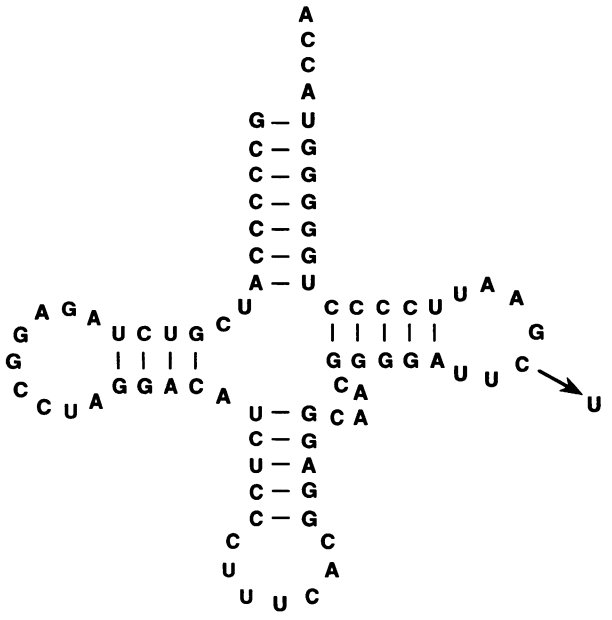

FIG. 3. Nucleotide sequences of the chloroplast tRNA^{Glu} from wild-type and Y9 mutant *E. gracilis*. The base change in Y9 is indicated by an arrow.

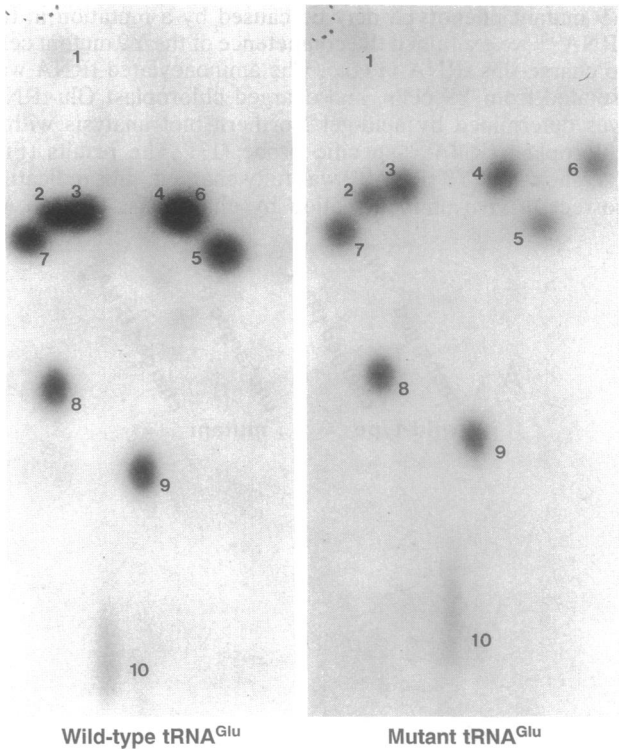

FIG. 4. RNase T1 fingerprint patterns of wild-type and Y9 mutant tRNA^{Glu}. Oligonucleotide fractionation was by high-voltage electrophoresis at pH 3.5 on cellulose acetate in the first dimension (from left to right) and by homochromatography in a 30 mM KOH "homomix" on DEAE-cellulose thin-layer plates at 65°C in the second dimension (from bottom to top) according to ref. 22. The oligonucleotides were identified by their position (23), by comparison with the DNA sequence (ref. 9, this work), and by the estimation of their molar yields. The numbers represent the RNase T1-oligonucleotides from wild type and Y9 mutant, respectively: 1, AG; 2, UACCA; 3, CCUAG; 4, UCUAG; 5, Am^{1}G (m^{1}G is 1-methylguanosine); 6, AUUCG (wild type), AUUUG (mutant); 7, CAACG; 8, CCCCCAUCG; 9, AAUUCCCCUG; 10, ACAUCUCCCUUUCACG. Sequences of oligonucleotides 2–7 (derived from Y9 tRNA) have been further verified by two-dimensional mobility-shift analysis. The mobility of oligonucleotide 5, Am^{1}G, is caused by a 2′,3′-cyclic phosphate end (27). The low molar yield of oligonucleotide 1, AG, is sometimes seen in fingerprints (22). The dotted outline marks the position of xylenecyanol FF, a blue dye marker.

contained a chloroplast tRNA^{Glu} gene, but only the Y9 strain harbored a mutation in it. It was reported earlier that strain W_{14}ZNalL is devoid of detectable plastid DNA (28). However, as with other nongreening *Euglena* mutants thought to be aplastidic and later found to have some plastid DNA sequences (29), W_{14}ZNalL contains an intact tRNA^{Glu} gene. Thus, the lack of chloroplast tRNA^{Glu} in this strain (30) implies that the expression and processing of this gene depend on some product of chloroplast protein synthesis.

Although the level of tRNA^{Glu} in the chloroplast of the mutant Y9 strain was only 20% of that of the wild-type organelle, we are convinced that the Y9 phenotype is not merely a function of the low abundance of this particular tRNA. A sufficient explanation of the phenotype is the *in vitro* demonstration that even in the presence of increased quantities of total tRNA (compensating for the decreased amount of tRNA^{Glu} seen in the mutant) very poor ALA formation was observed (Table 1). These conclusions are further corroborated by the initial rates of the GluTR reaction with wild-type and mutant Glu-tRNA^{Glu}, where the latter was unable to serve as a substrate for the wild-type enzyme.

The mutation lies in a highly conserved region of the tRNA gene and molecule. As the C56 → U mutation affects a

nucleotide directly involved in the maintenance of tRNA tertiary structure (31), it is possible that the Y9 $tRNA^{Glu}$ displays altered conformational stability. Such changes have been detected by electrophoresis in nondenaturing polyacrylamide gels at temperatures between 30°C and 60°C (32). A similar experiment separating total unlabeled tRNA from wild type and Y9 and probe-specific detection (see *Materials and Methods*) was done. Even at elevated temperatures (60°C) no difference was seen, indicating comparable stability of both wild-type and Y9 $tRNA^{Glu}$ (data not shown). However, as the base exchange may affect the rate of tRNA processing and the amounts of mature-size tRNAs (33, 34), a mutation in the Y9 $tRNA^{Glu}$ gene would be consistent with the reduced abundance of the corresponding tRNA in Y9 chloroplasts.

The finding of a single base alteration in the chloroplast $tRNA^{Glu}$ gene of the *Euglena* Y9 mutant resulting in a C56 → U change in the tRNA is consistent with all the biochemical and physiological data of this strain. Glutamylation *in vitro* and *in vivo* has been demonstrated, and chloroplast protein synthesis *in vivo* is occurring (ref. 10 and this work). As the mutant tRNA is a poor substrate for GluTR, there may not be enough ALA for chlorophyll formation. The orange phenotype is due to lack of chlorophyll, which normally masks the color of the carotenoids. It would be interesting to convert the Y9 mutant to wild-type phenotype by transformation with the wild-type $tRNA^{Glu}$ gene. This experiment would also examine the question whether additional mutational changes in the Y9 strain contribute to the phenotype. Unfortunately, transformation of *Euglena* has not yet been established.

It may have been a fortunate coincidence that we surveyed *Euglena* for mutations. This organism is known to use two pathways for ALA formation: the C_5 pathway in the chloroplast for the needs of chlorophyll synthesis and the Shemin pathway in the mitochondrion to provide ALA for heme synthesis (19). It is unlikely that in other organisms (e.g., higher plants) a similar mutation can be obtained, if heme formation relies entirely on ALA synthesis via the C_5 pathway of the chloroplast (35).

GluTR is a very important molecule—it competes with EF-Tu for Glu-tRNA and thus regulates the flux of glutamine into porphyrin synthesis. The mutation in the Y9 $tRNA^{Glu}$ has different consequences on GluTR and GluRS (and EF-Tu). The result defines C56 in $tRNA^{Glu}$ as a significant element for GluTR recognition and shows this position to be less important for GluRS or elongation factor EF-Tu recognition. Thus, the existence of this mutant is a colorful illustration of the notion that chloroplast $tRNA^{Glu}$ is a dual-function molecule (36) that links protein and chlorophyll biosynthesis, seemingly disparate areas of cell metabolism.

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