*INDUCTION OF EUGLENA TRANSFER RNA'S BY LIGHT**

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Abstract.—Exposure of dark-grown, wild-type *Euglena gracilis* to light induces the formation of at least three new chromatographic species of tRNA. Parallel studies with a bleached mutant (W_3BUL) of *Euglena* demonstrate that the induction of these new tRNA species is dependent upon the cell's ability to form chloroplasts and rule out the possibility that the new species arise from an effect of light on the tRNA's *per se*.

One of the current, unresolved issues in biology is the question of whether tRNA's play a regulatory role in normal cellular processes. These adaptor molecules are attractive candidates for involvement in regulation because numerous experimental observations indicate the appearance of new and/or modified tRNA's due to a variety of biological phenomena,[1–13] for example, phage infection, oncogenesis, and differentiation. Modifications of tRNA *in vivo* have also been observed during the apparently normal growth of *Escherichia coli*.[14] Theoretically, it is not difficult to envision a regulatory mechanism in which the availability of a single species of tRNA controls the translation of a group of polycistronic messenger RNA's by codon restriction, i.e., the translational requirement of a codon near the 5′ terminus for a specific tRNA.

The present studies with *Euglena gracilis* demonstrate the induction of new species of tRNA by light, under conditions which permit the development of functional chloroplasts. Mutant strains which lack chloroplast DNA and do not develop chloroplasts do not exhibit the phenomenon of light induction.

Experimental Procedures.—Strains: Euglena gracilis, wild-type strain B, and a UV-induced, bleached mutant derivative of strain B, W_3BUL, were used.

Growth conditions and media: Two types of media were used: (1) The heterotrophic medium consisted of 0.87% Difco *Euglena* broth. (2) The autotrophic medium was basically Hutner's medium as modified by Rigopoulos and Fuller[15]; it contained the following, in grams per liter: Na_2EDTA, 0.50; KH_2PO_4, 0.30; $MgSO_4 \cdot 7H_2O$, 0.50; $CaCO_3$, 0.06; $(NH_4)_2SO_4$, 2.00. Vitamin B_1 (0.6 mg/liter), vitamin B_{12} (0.005 mg/liter), and Hutner's metal mix[16] (1 ml/liter) were added and the pH was adjusted to 3.7 with concentrated HCl. Cells were grown either in the dark or under G.E. warm white fluorescent bulbs (at an intensity of ~1000 ft-c (~1×10^4 ergs/cm^2 per second)) in 12-liter, flat-bottom Florence flasks containing 10 liters of medium. Cultures were bubbled continuously at 25°C with 2% CO_2 in air.

Preparation of tRNA: Logarithmically grown *Euglena* cells were harvested in a Sharples centrifuge at 4°C and immediately suspended in 4 ml (per gram wet weight) of: 0.01 *M* magnesium acetate; 0.01 *M* Tris-HCl buffer, pH 7.5; 0.001 *M* EDTA; 0.01 *M* β-mercaptoethanol; and 1% sodium lauryl sulfate (British Drug Houses, Ltd., Poole, England). Cells were disrupted by passage through a Gaulin press at 10,000 psi, an equal volume of phenol was added, and the preparations were stirred vigorously overnight at 4°C. Following centrifugation, the aqueous phase was precipitated twice with 75% ethanol and the tRNA isolated by DEAE column chromatography as described previously (essentially the method of Holley *et al.*[17]).

Preparation of aminoacyl-RNA synthetases: Wild-type cells in the logarithmic phase of growth in autotrophic medium (in the light) were harvested in a Sharples centrifuge at 4°C and suspended in 0.2 *M* KPO_4 buffer (pH 7.5) containing 0.01 *M* β-mercaptoethanol and 10% glycerol (4 ml/gm wet weight cells). Cells were disrupted by passage through a Gaulin press at 10,000 psi and the homogenate was centrifuged at 10,000 × *g* for 30 min and 70,000 × *g* for 2 hr to remove cellular debris and ribosomes, respectively. The soluble extract was then passed over a DEAE-cellulose column (equilibrated with the same buffer) to remove nucleic acids,[18] and the protein was precipitated by the addition of $(NH_4)_2SO_4$ crystals to a final concentration of 3.0 *M*. After standing overnight at ~3°C, the precipitate was collected by centrifugation at 10,000 × *g* for 30 min. The ammonium sulfate precipitates may be stored at 0°C for at least several months without loss of enzyme activity. Ammonium sulfate and phosphate were removed prior to use by passage through Sephadex G-25 which had been equilibrated with the appropriate buffer. For preparation of an unfractionated synthetase preparation, the equilibrating buffer consisted of 0.01 *M* Tris-HCl buffer (pH 7.5), 0.1 *M* KCl, 0.01 *M* β-mercaptoethanol, and 20% glycerol, in which aliquots may be frozen and stored at −20°C without loss of activity.

Measurement of tRNA and aminoacyl-RNA synthetases: The acylation reaction mixture contained (in addition to enzyme and tRNA) per ml: 50 μmoles of HEPES (*N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid, Calbiochem Corporation) buffer (pH 7.5); 0.5 μmole of ATP; 10.0 μmoles of magnesium acetate; 5.0 μmoles of β-mercaptoethanol; 1.0 μc of ^{14}C- or ^{3}H-amino acid; and 500 pmoles of each of the other ^{12}C-amino acids. Reactions were performed at 30°C. Radioactive aminoacyl-RNA was assayed by a modification[19] of the filter paper disk method of Bollum.[20]

Preparation of aminoacyl-tRNA: The acylation reaction was allowed to proceed for 10 or 15 min as described above and then made to 0.1 *M* potassium acetate (pH 5.0). An equal volume of phenol (equilibrated to pH 5.0 with the same buffer) was added, and the preparation was shaken for 5 min and centrifuged. The aqueous phase was precipitated with ethanol, and the precipitate collected either by centrifugation or filtration on Millipore filters (HAWP). The radioactive aminoacyl-RNA's were then dissolved in 0.01 *M* sodium acetate buffer (pH 4.5) containing 0.35 *M* NaCl, 0.01 *M* magnesium acetate, 0.001 *M* EDTA, and 0.01 *M* β-mercaptoethanol.

Chromatography: The reversed-phase system described by Weiss and Kelmers[21] was used. Columns were 225 × 1.5 cm. For chromatographic comparisons, approximately

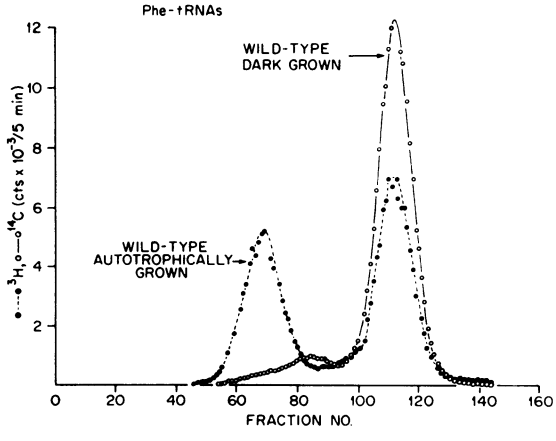

FIG. 1.—Elution profiles of the Phe-tRNA's (acylated *in vitro* with either ^{14}C- or ^{3}H-phenylalanine) of *Euglena* grown autotrophically (●--●) and heterotrophically (O—O). 10-ml aliquots were eluted (and collected) from the reversed-phase column (225 × 1.5 cm) with a linear gradient of NaCl from 0.4 *M* (1000 ml) to 0.6 *M* (1000 ml) in 0.01 *M* sodium acetate buffer (pH 4.5) containing 0.01 *M* magnesium acetate, 0.001 *M* EDTA, and 0.01 *M* β-mercaptoethanol. The columns were run at ~23°C (room temperature) at a flow rate of 1.8 ml/min. 2 ml of 50% TCA and 0.2 mg of carrier ribosomal RNA were added to each fraction; they were stirred, cooled to 4°C for ~15 min, and the precipitates collected on Millipore filters. The filters were dried under vacuum at 30°C and counted in toluene containing 0.4% 2,5-bis-[2-(5-tert-butylbenzoxazolyl)]-thiophene in a liquid scintillation spectrometer.

equal counts per minute of ^{14}C- and ^{3}H-labeled aminoacyl-tRNA's were applied to the columns. Chromatography was as described in Figure 1 and yielded 85–90% recovery of acid-insoluble counts applied to the columns.

Results and Discussion.—These studies were designed to determine whether formation of new species of tRNA is associated with light-induced chloroplast development. *Euglena* was the organism chosen for several reasons: (1) It can be grown heterotrophically in the dark on medium containing a carbon source such as glutamic acid (*Euglena* broth); (2) with all other factors remaining constant, exposure to light induces the formation of functional chloroplasts; (3) it can be grown as a photoautotroph in the light in the absence of a carbon source other than CO_2; and (4) bleached mutants which lack chloroplast structure and chloroplast DNA are available.

A chromatographic comparison of the phenylalanine acceptor RNA's from autotrophic§ and heterotrophic§ or dark-grown cells may be seen in Figure 1. It is apparent that the two growth conditions result in major differences in the cellular phenylalanine tRNA complement in that there is a major species (Phe-

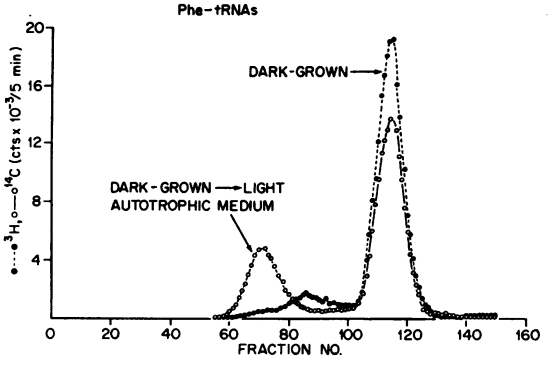

FIG. 2.—Elution profiles of *in vitro* acylated Phe-tRNA's. tRNA was isolated from two types of cells for acylation and reversed-phase cochromatography: (●--●) *Euglena* cultured for 18 days in the dark heterotrophically; (○—○) *Euglena* grown 18 days in the dark heterotrophically and then transferred to autotrophic medium and grown in the light for 3 days. Chromatography was as described in Fig. 1.

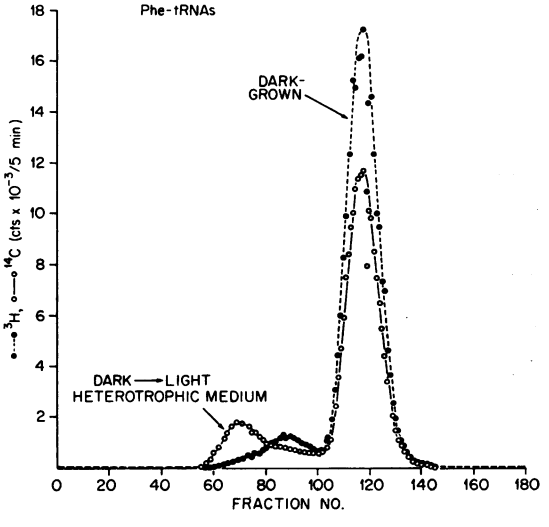

FIG. 3.—Elution profiles of *in vitro* acylated Phe-tRNA's. tRNA was isolated from two types of cells for acylation and cochromatography. (●--●) *Euglena* cultured for 18 days in the dark on heterotrophic medium, and (○—○) *Euglena* grown 18 days in the dark on heterotrophic medium, then transferred to the light for 3 days in the same medium.

tRNA I) in the autotrophic cells which is not detectable in the dark-grown cells. The tRNA's used for Figure 1 were isolated from cells which had been grown for several months under their particular regimen. The transition in the Phe-tRNA complement, however, may also be seen in cells after short (Fig. 2) periods of exposure to conditions which induce the photosynthetic apparatus. In these experiments, the cells were grown heterotrophically in the dark for 18 days and then transferred to either fresh broth or autotrophic medium and cultured for 3 days (~6 generations) in the light. Maximal induction of Phe-tRNA I is observed (Fig. 2) after transfer to autotrophic medium, although it is clearly present (Fig. 3) in those cells transferred to broth and grown in the light. The lower level of induction in broth was not completely unexpected, in view of the fact that certain photosynthetic enzymes are partially repressed by the presence of organic metabolites.[22]

The bleached mutants of *Euglena* afford a convenient control for the effect of light on the tRNA's of *Euglena*. Strain W_3BUL is a UV-induced, bleached derivative of wild-type B (used in these studies) that does not contain detectable chloroplast DNA and cannot develop even rudimentary chloroplast structure.[23] As seen in Figure 4, continuous growth of W_3BUL in the light does not alter the pattern of Phe-tRNA's; W_3BUL grown in the light is identical to wild-type grown in the dark (Fig. 1). It appears, therefore, that the alterations in the tRNA patterns are directly related to chloroplast development and not some other light-related phenomenon.

In contrast to the induction of Phe-tRNA I during the transition from heterotrophy to photoautotrophy, species II undergoes an apparent repression (Figs. 1–4). This diminution in Phe-tRNA II is not as striking as the appearance of Phe-tRNA I for two reasons: Its chromatographic position overlaps with species I, and it is a relatively minor species. Nevertheless, species II is present in detectable amounts only in cells which are restricted to heterotrophic growth either by culture in the dark (Fig. 3) or mutation (Fig. 4). An interesting possibility is that species II seen in heterotrophic cells represents a small amount of precursor to the species I of photoautotrophs. Precursor, or incompletely modified forms of tyrosine tRNA which lack the 2-methylthio and ^6N-(γ,γ-dimethylallyl) substitutions on the adenosine adjacent to the anticodon, have been identified[24]

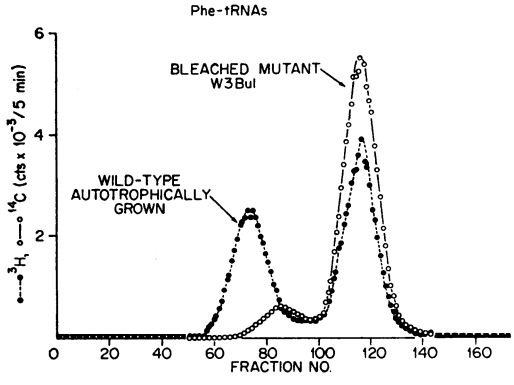

FIG. 4.—Elution profiles of *Euglena* Phe-tRNA's from wild-type and the bleached mutant W_3BUL. tRNA's were isolated from wild-type (●--●) grown autotrophically in the light and W_3BUL (O—O) grown on *Euglena* broth in the light and acylated with either ^{14}C- or ^3H-phenylalanine. Chromatography was as in Fig. 1.

in the tRNA's from phage $\phi 80$ dsu_{III}^{+}-infected *E. coli*. Such a precursor relationship between species I and II would imply that the role of light in the induction of species I is to trigger the chemical modification or "maturation" of species II.

The acceptor activity of Phe-tRNA's I, II, and III is unaffected by exposure to 95°C for 5 min in 0.01 *M* EDTA (pH 7.5) followed by quick cooling. This

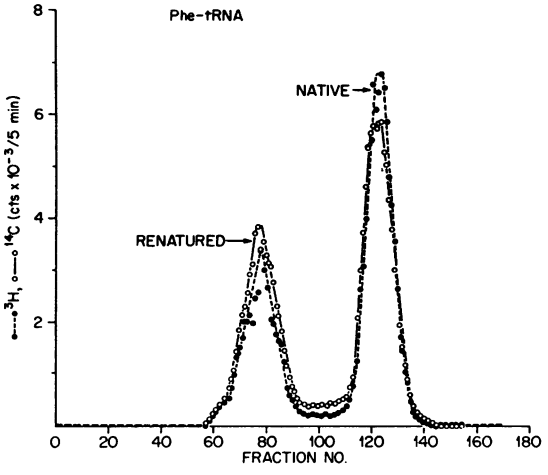

FIG. 5.—Elution profiles of native (●-●) and renatured (O—O) Phe-tRNA's from autotrophically grown *Euglena*. Native refers to the state of the tRNA's as they are when isolated (see *Experimental Procedures*) and acylated. Renatured tRNA's were incubated in 0.01 *M* magnesium acetate, 0.001 *M* EDTA, and 0.01 *M* Tris-HCl buffer (pH 8.0) at 60°C for 5 min and quick-cooled prior to acylation.

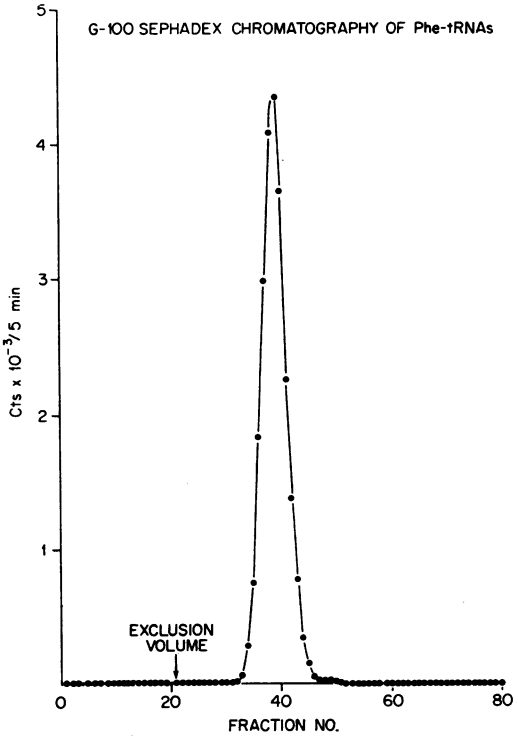

FIG. 6.—Gel-filtration chromatography of Phe-tRNA's. tRNA from autotrophically grown, wild-type cells was acylated and prepared for reversed-phased column chromatography as described in *Experimental Procedures*. An 0.5-ml sample was applied to columns (1.2 × 100 cm) of Sephadex G-100 equilibrated with: 0.01 *M* magnesium acetate (pH 4.5), 0.01 *M* β-mercaptoethanol, 0.001 *M* EDTA, and 0.35 *M* NaCl. 2-ml fractions were collected, and the acid-insoluble radioactivity was collected on Millipore filters as described in Fig. 1.

indicates that the three species consist of intact polynucleotide strands and that none is a fragment or an enzymatically nicked version of one of the others. Similar results have been obtained from renaturation studies. Renaturation[25] in the presence of Mg^{2+} at 60°C leads to no detectable alteration in chromatographic properties (Fig. 5). Furthermore, gel filtration shows that the tRNA's exist in the monomeric form. This is evidenced by the single symmetrical elution peak from Sephadex G-100 (Fig. 6) which separates monomers from dimers.[26, 27] Thus, the chromatographic separation of these species does not appear to result from different physical states or an aggregation phenomenon. The results all point to the interpretation that the light-induced Phe-tRNA is a chemically unique species.

It has recently been reported[28] by Yegian and Stent that the rates of *in vitro* aminoacylation differ greatly for the various isoaccepting species of *E. coli* isoleucine tRNA. We have observed no significant differences in the acylation kinetics for the *Euglena* phenylalanine tRNA's, and cochromatography of the Phe-tRNA's after 2 and 20 minutes *in vitro* acylation time revealed no differences in their elution profiles. Phe-tRNA's I and III have also been rechromatographed, and neither species I nor III gives rise to the other upon recycling through the reversed-phase columns.

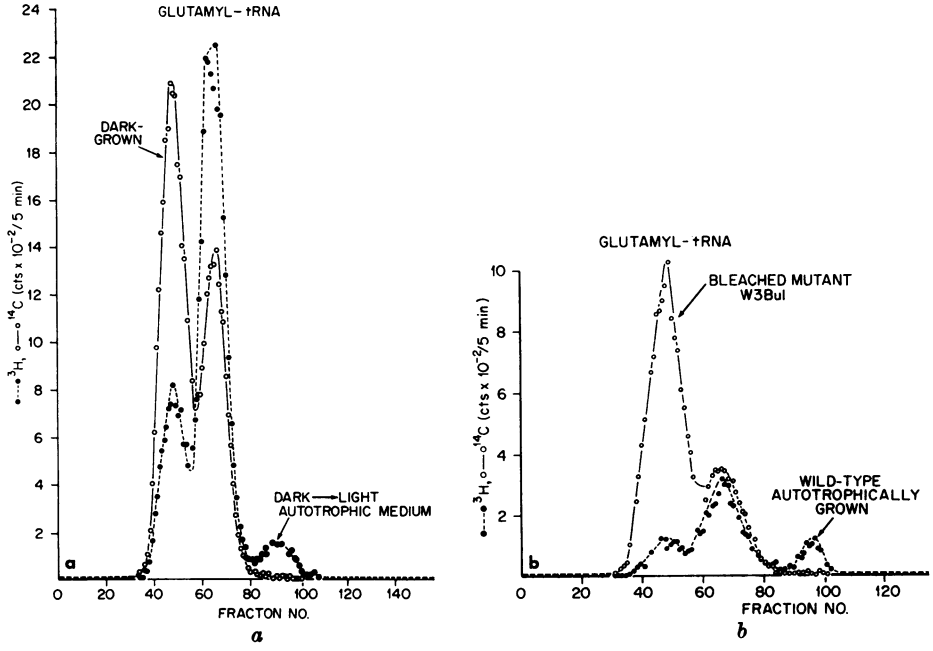

FIG. 7.—Elution profiles of *Euglena* glutamyl-tRNA's. tRNA's were isolated from the cells grown as indicated and acylated with either ^{14}C- or ^{3}H-glutamic acid. *Dark → light* indicates cells cultured in the dark for 18 days heterotrophically and then shifted to the light and autotrophic medium for 3 days' growth before tRNA isolation: (*a*) cochromatography of Glu-tRNA's from dark-grown (18 days) cells (O—O) and from dark → light cells (●--●); (*b*) cochromatography of Glu-tRNA's from autotrophically grown wild-type (●--●) and W_3BUL (O—O) grown in the light. For details of chromatography, see Fig. 1.

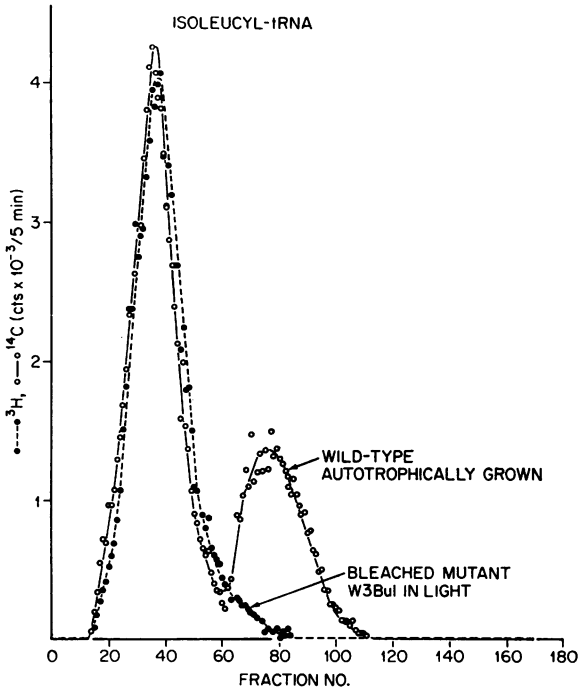

FIG. 8.—Elution profiles of *Euglena* isoleucyl-tRNA's from autotrophically grown wild-type cells and from W_3BUL grown in the light were acylated with ^{3}H- and ^{14}C-isoleucine, respectively, and chromatographed as described in Fig. 1.

Chromatographic analyses of other amino acid-specific tRNA's from *Euglena* have revealed additional examples of light-induced, chromatographically unique tRNA species. For example, Figure 7*a* shows that shifting dark-grown cells to photoautotrophic conditions results in a third chromatographic species of glutamyl-tRNA. This new peak is also induced in broth-grown cells by exposure to light, although, as with Phe-tRNA I, to a lesser extent. The third glutamyl-tRNA is absent in W_3BUL, whether grown in the light or dark (Figure 7*b*), an observation again analogous to the results with Phe-tRNA I. Figure 8 shows one further example: an isoleucyl-tRNA found only in autotrophically grown cells. Gel-filtration chromatography (Sephadex G-100) of both the glutamyl- and isoleucyl-tRNA preparations indicated that both were completely in the monomeric form. Chromatographic comparisons between the arginyl- and leucyl-tRNA's of light- and dark-grown cells failed to reveal significant differences.

The experiments described in this report demonstrate that light has a profound effect upon the tRNA complement of wild-type *Euglena*. New chromatographic species of phenylalanine, glutamic acid, and isoleucine tRNA's are observed as a result of photosynthetic growth. Light does not induce the appearance of these new tRNA's in the bleached mutant W_3BUL; it appears, therefore, that their induction is dependent upon the cell's ability to form chloroplasts and to grow photosynthetically. Since it has been shown[29–32] that mitochondria contain tRNA's which are distinct from those found in the cell's cytoplasm, it is tempting to believe that the light-induced species of tRNA observed here are

chloroplast tRNA's and that they are essential for the synthesis of chloroplast protein. This concept is currently under investigation.

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§ Autotrophic or photoautotrophic refers to growth on medium containing no carbon source other than CO_2; heterotrophic growth refers to growth on *Euglena* broth containing the utilizable carbon source, L-glutamic acid.

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