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On the Similarity Between the tRNAs of Organelles and Prokaryotes

(mitochondria/chloroplasts/*Euglena gracilis*/*Neurospora crassa*/fluorescent bases)

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ABSTRACT **Fluorescence studies with organelle transfer RNAs separated from their cytoplasmic counterparts revealed that phenylalanine tRNA from *Euglena* chloroplasts or *Neurospora* mitochondria does not contain a fluorescent "base Y." In contrast, cytoplasmic phenylalanine tRNA from *Euglena* and cytoplasmic tRNA from *Neurospora* were found to contain fluorescent bases. The fluorescence-emission spectra of *Neurospora* cytoplasmic tRNAs and those of the related ascomycete *Saccharomyces cerevisiae* were observed to be quite different. The results support the generalization that eukaryotic tRNAs contain a fluorescent base, but indicate that their respective organelle tRNAs do not. They also indicate a striking parallelism between organelle and prokaryotic tRNAs.**

The origin of cellular organelles has long been an issue of discussion among biologists and biochemists. The notion that mitochondria and chloroplasts evolved from endosymbiotic prokaryotic cells has received much attention (see, for example, refs. 1–5), largely as a result of their apparent autonomous replication (6–11) and their DNA content (5, 7, 8, 10–13). In the past few years, this concept has gained considerable impetus from studies detailing translation within these organelles. Briefly stated, organelle protein synthesis is mechanistically analogous to that of the bacterial systems (1–5, 14–21) (e.g., drug sensitivity and chain initiation) and, in this regard, is strikingly different from cytoplasmic protein synthesis. In addition, the transfer RNAs, aminoacyl-tRNA synthetases, and ribosomes of organelles differ from their cytoplasmic counterparts (5, 22–29). In earlier reports from this laboratory (27–29), the identification of organelle tRNAs was based on the observation that chromatographically distinct tRNAs are present in isolated organelles and, in the case of chloroplasts, on the fact that *Euglena* chloroplast tRNAs are (*a*) induced by light and (*b*) absent in bleached mutants of *Euglena* that contain no chloroplast structure or chloroplast DNA.

In this report, a further parallel between the translational apparatus of organelles and prokaryotes is presented. Neither chloroplast nor mitochondrial tRNAs (from *Euglena* and *Neurospora*, respectively) contain "base Y" (29, 30), a fluorescent base that appears to be unique to eukaryotic tRNAs (30–33). "Base Y" is of particular interest, because it resides adjacent to the 3′ adenosine of the anticodon of eukaryotic phenylalanine tRNAs (32, 34) and is necessary for normal codon recognition (35, 36).

Abbreviation: BD-cellulose, benzoylated DEAE-cellulose.

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MATERIALS AND METHODS

Strains. Euglena gracilis var. *bacillaris* and *Neurospora crassa*, wild-type strain OR23-1a, were used.

Preparation of Neurospora Mitochondrial and Cytoplasmic Fractions. Neurospora mitochondria were obtained by the zonal centrifugation procedure of Barnett and Brown (28), as described by Epler *et al.* (19). The cytoplasmic fraction was prepared as described (19). Mitochondrial tRNAs prepared by these procedures contain very little (if any) contamination by cytoplasmic tRNAs.

Preparation of tRNA. Euglena cells in the logarithmic growth phase 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)–1 mM EDTA–0.01 M 2-mercaptoethanol–1% sodium dodecyl 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 shaken vigorously for 1 hr. After centrifugation, the aqueous phase was extracted two additional times with phenol and 5–6 times with chloroform–isopentyl alcohol (24:1). The final aqueous phase was precipitated twice from 75% ethanol (containing 0.1 M NaCl); the tRNA was isolated by DEAE cellulose column chromatography (essentially the method of Holley *et al.*, ref. 37). tRNA from mitochondrial and cytoplasmic fractions of *Neurospora* was prepared similarly. tRNAs were deacylated in 0.5 M Tris·HCl buffer (pH 7.5) for 45 min at 23°C before application to benzoylated DEAE-cellulose (BD-cellulose) chromatography columns.

Benzoylated DEAE-Cellulose Chromatography. BD-cellulose chromatography was by a modification of the procedure of Gillam *et al.* (38). The column (2 × 30 cm) was washed with 2.0 M NaCl (1–2 liters) until the A_{260} of the eluate was less than 0.25 units /ml. It was then equilibrated with 0.01 M Tris·HCl buffer (pH 7.5), containing 0.01 M magnesium acetate and 0.02% (w/v) sodium azide (to inhibit bacterial growth). Deacylated tRNA [in 0.01 M Tris·HCl (pH 7.5), containing 0.01 M magnesium acetate] was applied to the column and washed with 400–500 ml of the Tris–magnesium acetate–sodium azide buffer. The tRNAs were then eluted

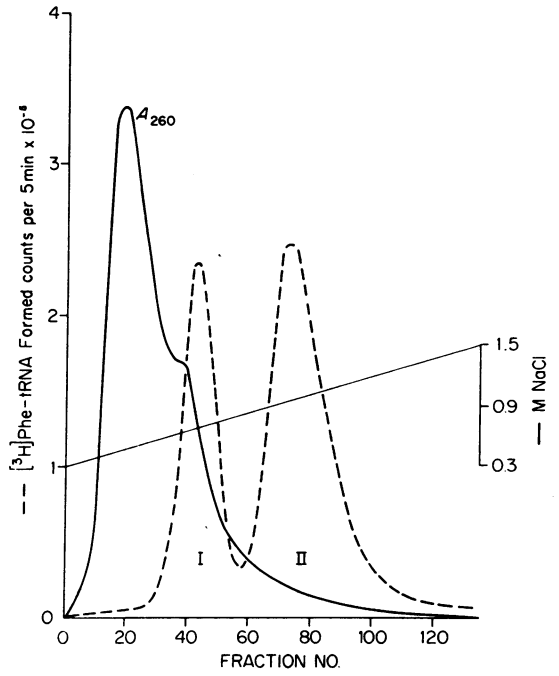

FIG. 1. Benzoylated DEAE-cellulose chromatography of otosynthetically grown *Euglena* phenylalanine tRNAs. About 2 0 mg of deacylated tRNA was chromatographed and assayed for phenylalanine acceptance as described in *Methods*. Peaks I and II represent chloroplast $tRNA^{Phe}$ and cytoplasmic $tRNA^{Phe}$, respectively.

with a linear gradient from 0.3 to 1.5 M NaCl [containing 0.01 M Mg^{++} and 0.01 M Tris (pH 7.5)]. The tRNA from individual fractions was collected by ethanol precipitation and centrifugation. Phenylalanine acceptance was determined by the filter-paper disc method of Bollum (39) as described (40).

Poly (U)-Directed Polyphenylalanine Synthesis. An *Escherichia coli* S30 fraction was prepared according to the procedure of Nirenberg (41); the incorporation of [^{3}H]Phe from [^{3}H]Phe-tRNA into protein was followed by a modification of the

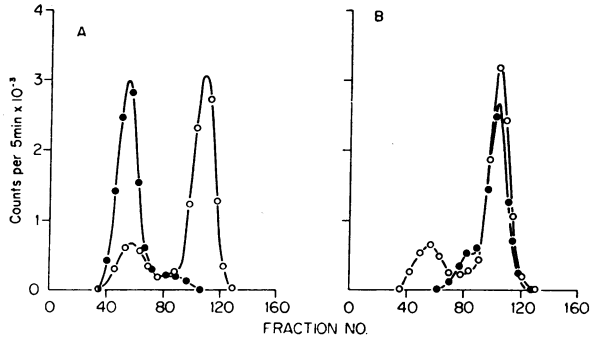

FIG. 2. Reversed-phase chromatography of chloroplast $tRNA^{Phe}$ (*A*) and cytoplasmic $tRNA^{Phe}$ (*B*). tRNA from BD-cellulose fractions containing phenylalanine tRNAs I and II (see Fig. 1) were individually acylated with [^{3}H]Phe (●——●) and co-chromatographed (see *Methods*) with unfractionated tRNA (acylated with [^{14}C]Phe, O——O) from photosynthetically grown cells. ●——●, tRNA fraction I (*left*), fraction II (*right*); first peak (O——O), inducible chloroplast $tRNA^{Phe}$; second peak (O——O), constitutive cytoplasmic $tRNA^{Phe}$.

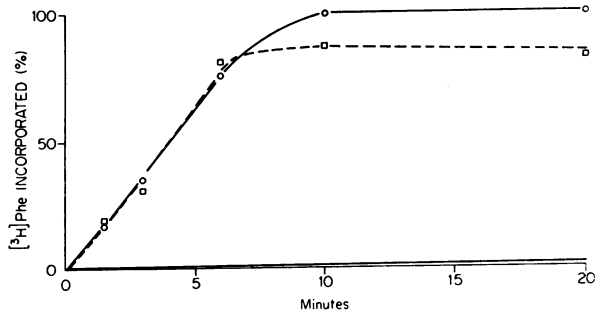

FIG. 3. Participation of *Euglena* chloroplast and cytoplasmic phenylalanine tRNAs in poly(U)-directed polyphenylalanine synthesis. *In vitro* polyphenylalanine synthesis was performed as described in *Methods*, by the use of the two phenylalanine tRNAs separated by BD-cellulose chromatography (see Fig. 1). O——O, cytoplasmic [^{3}H]Phe-$tRNA^{Phe}$; □- - □, chloroplast [^{3}H]Phe-$tRNA^{Phe}$;——, poly(U)$_n$ controls.

procedure of Kan *et al.* (42). Each milliliter of reaction mixture contained: 100 μmol Tris·HCl buffer (pH 7.8)-14 μmol magnesium acetate-50 μmol KCl-30 μmol GTP-6 μmol 2-mercaptoethanol-7.5 μmol phosphoenolpyruvate-16 μg phosphoenolpyruvate kinase (EC 2.7.1.40)-40 μmol of a mixture of each of the 20 L-[^{12}C]amino acids-0.05 ml of preincubated *E. coli* S30 fraction (this concentration gave

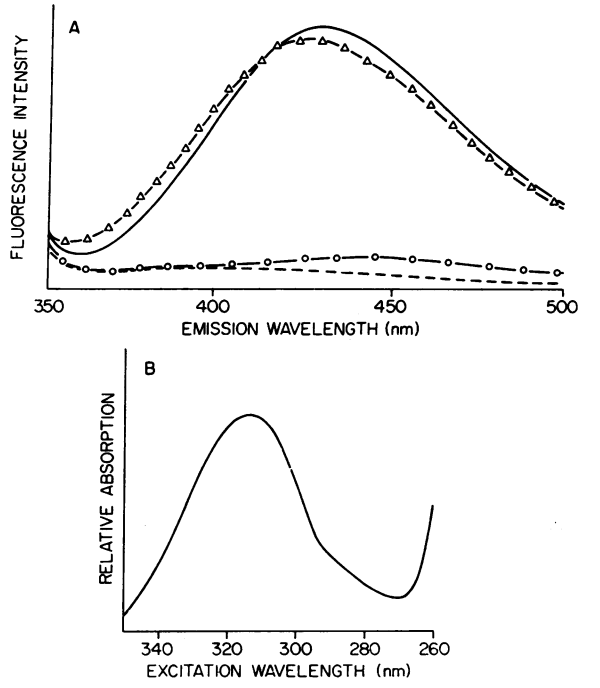

FIG. 4. Uncorrected fluorescence emission (*A*) and excitation (*B*) spectra of *Euglena* cytoplasmic (——) and chloroplast (- - -) phenylalanine tRNAs and *E. coli* (O——O) and yeast unfractionated (Δ—Δ) tRNAs. Emission spectra were obtained as described in *Methods* at the following tRNA concentrations: *E. coli*, 5.5 A_{260}/ml; yeast, 44 A_{260}/ml; *Euglena* chloroplast $tRNA^{Phe}$, 6.2 A_{260}/ml; cytoplasmic $tRNA^{Phe}$, 5.0 A_{260}/ml. The excitation spectrum shown for cytoplasmic *Euglena* $tRNA^{Phe}$ was monitored at 430 nm, and the tRNA concentration was 5.5 A_{260}/ml.Chloroplast $tRNA^{Phe}$ and cytoplasmic $tRNA^{Phe}$ represent tRNA from peaks I and II, respectively (BD-cellulose Phe acceptor peaks, Fig. 1).

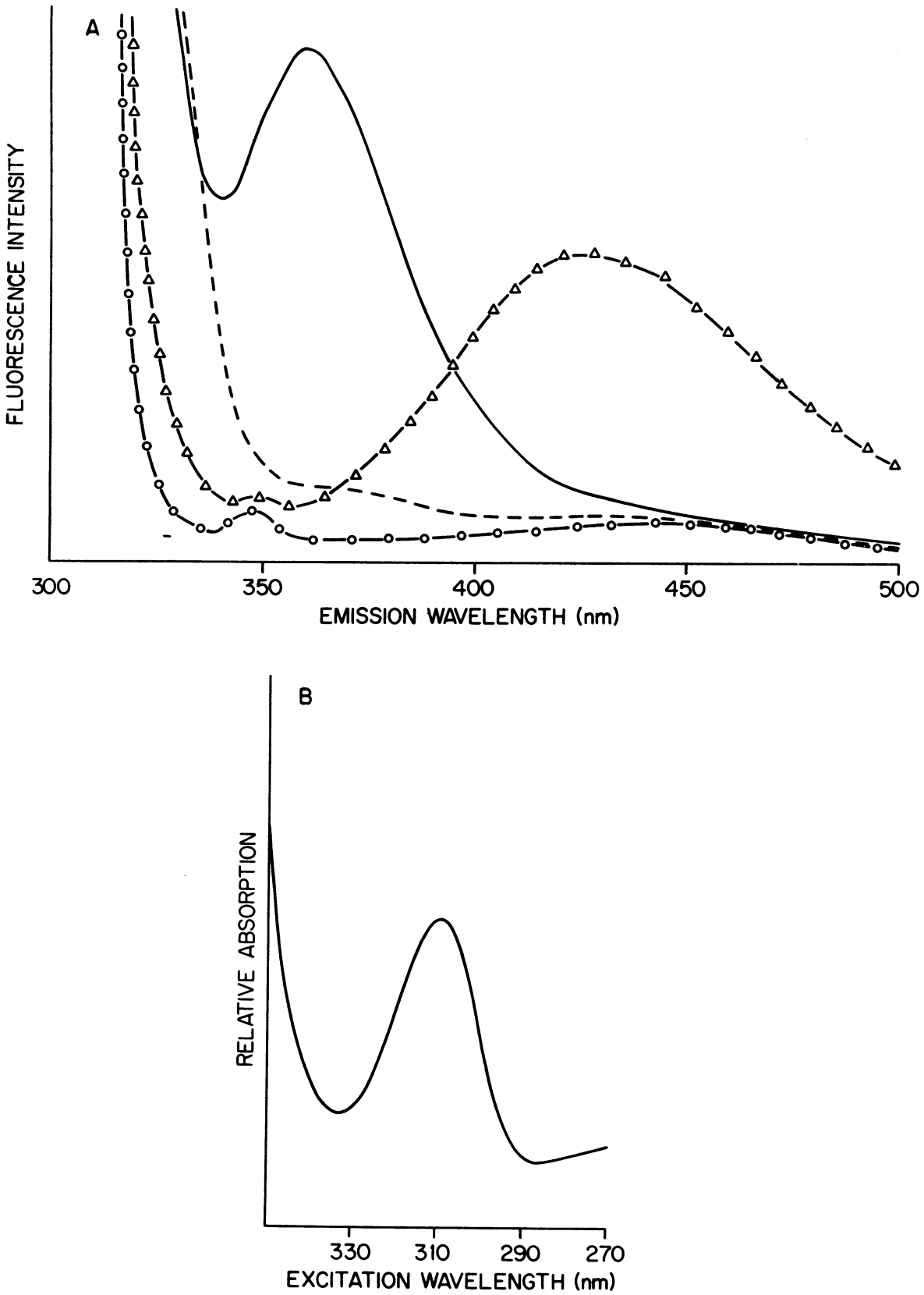

FIG. 5. Uncorrected fluorescence emission (*A*) and excitation (*B*) spectra of *Neurospora* cytoplasmic (——), *Neurospora* mitochondrial (- - -), yeast (Δ—Δ), and *E. coli* (O—O) tRNAs. Conditions were as described in *Methods* and the tRNAs were used at the following concentrations: *E. coli*, 5.5 A_{260}/ml; yeast, 44 A_{260}/ml; *Neurospora* mitochondrial, 18 A_{260}/ml; *Neurospora* cytoplasmic, 17.5 A_{260}/ml. The excitation spectrum shown for *Neurospora* cytoplasmic tRNA was monitored at 360 nm, and the concentration of tRNA was 17.5 A_{260}/ml.

the optimal incorporation). The reaction mixture was incubated for 10 min at 37°C, then 1–2 × 10^5 cpm of [^{3}H]Phe-tRNA was added together with 200 μg of [^{12}C]aminoacyl-tRNAs, acylated with a mixture of 19 [^{12}C]amino acids (except phenylalanine). Two A_{260} units of poly(U) were added at time zero. 0.2-ml aliquots were removed and dispersed into 8 ml of 10% trichloroacetic acid at 4°C. After 15 min, the samples were heated to 95°C for 20 min; the acid-insoluble material was collected on GF/C glass filter discs. The discs were then washed twice with 10% trichloroacetic acid and once with 70% ethanol containing 0.05 M NaCl, then dried; their radioactivity was determined in a liquid-scintillation spectrometer.

Reversed-Phase Chromatography. Reversed-phase column chromatography was as described (40).

Fluorescence Studies. The tRNAs were dissolved in 0.01 M Tris·HCl buffer (pH 7.5), containing 0.01 M magnesium acetate and 0.1 M NaCl; the emission and excitation spectra were obtained on a spectrofluorimeter as described by Longworth and Rahn (43). The emission spectra were obtained after excitation at 312 nm, and the excitation spectra of *Euglena* cytoplasmic tRNA^{Phe} and *Neurospora* cytoplasmic tRNA were monitored at 430 and 360 nm, respectively.

RESULTS AND DISCUSSION

For the present studies, the cytoplasmic and chloroplast phenylalanine tRNAs from *Euglena* (cytoplasmic tRNA^{Phe} and chloroplast tRNA^{Phe}) were separated by benzoylated DEAE-cellulose column chromatography (Fig. 1). The two species were identified by chromatography of the individual tRNA^{Phe}s on reversed-phase columns (Fig. 2), which were originally used to identify chloroplast tRNAs (27). Peaks I and II from BD-cellulose columns correspond, respectively, to the light-inducible chloroplast tRNA^{Phe} and the constitutive cytoplasmic tRNA^{Phe} (see ref. 27). Both cytoplasmic tRNA^{Phe} and chloroplast tRNA^{Phe} participate in poly(U)-directed polyphenylalanine synthesis (Fig. 3). The small (about 15%) difference in transfer from chloroplast tRNA^{Phe} reflects the fact that under the conditions of the experiment (pH 7.8, 37°C for 10 min), about 15% of the aminoacyl bonds of chloroplast [^{3}H]Phe-tRNA are cleaved spontaneously, whereas cytoplasmic [^{3}H]Phe-tRNA is completely stable.

The fluorescence emission and excitation spectra of the cytoplasmic and chloroplast phenylalanine tRNAs are shown in Fig. 4. From these spectra, it is apparent that chloroplast tRNA^{Phe}, like *E. coli* tRNA^{Phe}, does not contain a fluorescing base, whereas the fluorescence spectrum of cytoplasmic tRNA^{Phe} (emission maximum, 430 nm) is essentially indistinguishable from that of unfractionated yeast tRNA, which is known to contain "base Y" (30, 34).

As with *Euglena* chloroplast tRNA, *Neurospora* mitochondrial tRNA does not fluoresce upon excitation at 312 nm, where *Neurospora* cytoplasmic tRNA does (Fig. 5). In contrast to the *Euglena* cytoplasmic tRNA^{Phe}, however, the fluorescence spectrum (emission maximum, 360 nm) of *Neurospora* cytoplasmic tRNA is quite different from that of yeast tRNA, suggesting that the two fluorescent bases are not identical in these two closely related fungi. The mitochondrial tRNAs used in these studies have also been shown to participate in synthetic polyribonucleotide-directed ribosome binding, and thus are capable of recognition of normal codons (44).

In a recent communication (45), Fink *et al.* concluded that the tRNA^{Phe} of rat-liver mitochondria does contain "base Y" (or a related fluorescent base). Their conclusion was based on the observation that uncharged mitochondrial tRNA^{Phe} from the rat liver elutes from BD-cellulose in the "ethanol region" rather than in the aqueous "NaCl region". The "base Y"-containing cytoplasmic tRNA^{Phe} also elutes in the "ethanol region"; it is assumed that the hydrophobicity on BD-cellulose is indicative of the presence of "Y-base". The observations reported here show that uncharged *Euglena* cytoplasmic tRNA^{Phe}, which contains a fluorescent "base Y", does not require ethanol for elution from BD-cellulose. (When acylated with phenylalanine, however, *Euglena* cytoplasmic tRNA^{Phe} and chloroplast tRNA^{Phe} do require ethanol for elution.) Thus, the elution profiles from BD-cellulose are not a convincing evidence for the presence or absence of "base Y".

The results presented here indicate that organelle tRNAs, like the tRNAs of prokaryotes, lack a fluorescent base and that the presence of a "Y" base in eukaryotes is restricted to cytoplasmic tRNA.

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