Gene, 17 (1982) 337–339
Elsevier Biomedical Press

Short Communications

Location of a phenylalanine tRNA gene on the physical map of the *Euglena gracilis* chloroplast genome

(tRNA gene; phenylalanine tRNA; hybridization)

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(Received March 4th, 1982)
(Accepted March 10th, 1982)

SUMMARY

A $tRNA^{Phe}$ from *Euglena gracilis bacillaris* chloroplasts was purified and hybridized with restriction fragments of chloroplast DNA. From the hybridization pattern, the location of the corresponding gene on the physical map of the chloroplast genome was determined.

The protein-synthesizing system of the chloroplast contains ribosomal RNAs and transfer RNAs coded in the chloroplast genome. The nucleotide sequence of the phenylalanine tRNA from the chloroplast of *E. gracilis* strain *bacillaris* was the first tRNA sequence from an organelle to be determined (Chang et al., 1976). Rather few organellar tRNAs have been sequenced (Sprinzl and Gauss, 1982), but a knowledge of the general structure (i.e., without modifications) may be obtained from the gene sequence. The genes for $tRNA^{Ile}$ and $tRNA^{Ala}$, located in the spacer region between 16S and 23S genes in *Euglena* chloroplast DNA (Keller et al., 1980) have been sequenced (Graf et al., 1980; Orozco et al., 1980). The tRNA gene sequence can be determined more easily than the sequence of the corresponding RNA. However, it is important to verify by hybridization that the gene corresponds to a specific tRNA appearing in vivo rather than identify the gene by its presumed anticodon sequence alone.

The physical map of chloroplast DNA of strain *bacillaris* including the location of rRNA genes and regions hybridizing with total tRNA has been presented (El-Gewely et al., 1981; Hallick, 1982). However, other than the genes in the rRNA gene set, hybridization of a specific tRNA with a specific locus in the *Euglena* chloroplast DNA has not been reported. We report here the precise location of the $tRNA^{Phe}$ gene in the chloroplast DNA, as determined by blot hybridization of the purified $tRNA^{Phe}$ with restriction fragments of chloroplast DNA.

Chloroplast DNA was isolated as described (Lomax et al., 1977). Plasmid DNA was isolated by a modification of the procedure reported earlier (El-Gewely and Helling, 1980) using a CsCl step gradient in a vertical rotor. In brief, 1.7 ml of DNA solution containing ethidium bromide (380 μg/ml) was layered over 2.7 ml of saturated CsCl

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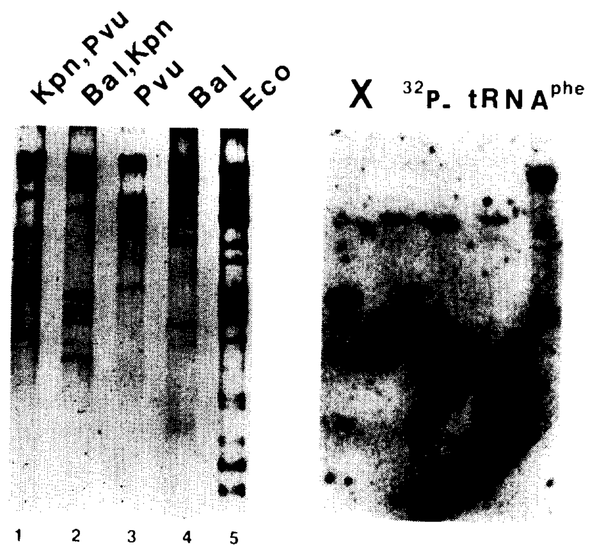
in a 4.4 ml tube. Centrifugation was carried out in the TV865 rotor (Dupont-Sorvall) at 20°C for 4 h at 55000 rev./min. The lower band containing covalently closed circular DNA was removed and purified as described (El-Gewely and Helling, 1980). The DNA was digested with restriction endonucleases, the resulting fragments were electrophoresed through agarose, and the separated fragments were transferred to nitrocellulose filters (El-Gewely et al., 1981).

The tRNA^{Phe} was purified from chloroplasts and labelled with ^{32}P at the 5′-end (Chang et al., 1976; Silberklang et al., 1979). Approx. 1×10^6 cpm (by Cérenkov counting) of the tRNA (0.25 to 0.4 μg) were used for hybridization with the filter-bound DNA in the presence of unlabelled rRNA and the hybridizing bands were visualized by fluorography (El-Gewely et al., 1981).

None of the following cloned segments of chloroplast DNA showed hybridization with tRNA^{Phe}: *Ava*-G; *Bam*-E, F; *Eco*-I, J, L, M, N, O, P, R, S, T, U, V, X, 2A; *Sal*-C. However, after total chloroplast DNA was treated with site-specific endonucleases, in each instance a single resulting DNA fragment showed hybridization with the tRNA (Fig. 1). When the locations of these fragments were compared, it was found that in every case the hybridizing fragment included a common segment. This segment is located between *Bal*I and *Pvu*II cleavage sites 660 base pairs apart (Fig. 1; El-Gewely et al., 1981), and is not among the cloned chloroplast DNA fragments tested. Although the locus was determined using chloroplast DNA from strain *bacillaris*, it is likely that the gene is present at the equivalent position in strain Z. The gene is located in fragment *Eco*-A of strain *bacillaris*, and hybridization with *Eco*RI-digested Z strain DNA shows the tRNA gene to be in either *Eco*-A or *Eco*-B of that strain (Fig. 1) as expected.



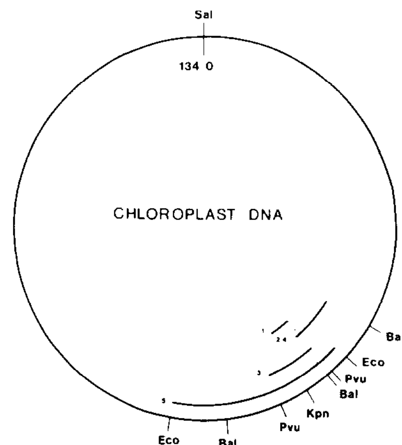


Fig. 1. Hybridization of ^{32}P-labeled tRNA^{Phe} with specific segments of *Euglena* chloroplast DNA. Left: the agarose-gel electrophoretic pattern of DNA treated with the designated endonucleases. Right: fluorograms showing the specific DNA segments hybridizing with the tRNA. Below: the locations of the hybridizing DNA fragments on the physical map of the chloroplast DNA. Chloroplast DNA in samples 1–4 came from strain *bacillaris*; the DNA in sample 5 came from strain Z.

Knowledge of the exact map location will allow the tRNA^{Phe} gene to be cloned and its structure to be determined, together with the sequences flanking the structural gene and responsible for control of its expression. The tRNA genes may play roles in controlling expression of other genes. In particular genes for tRNAs or pseudotRNAs are associated with initiation of transcription of chloroplast rRNAs (Tohdoh et al., 1981; Schwarz et al., 1981; Hallick, 1982; El-Gewely, M.R., Helling, R.B. and Dibbits, J., in preparation), the major transcripts from human mitochondrial genes are punctuated by tRNAs (Ojala et al., 1980), and in *E. coli* transcripts of several genes for proteins are initiated from tRNA genes (Hudson et al., 1981). It will be interesting to see if the tRNA genes of the chloroplast play any role in the expression of adjacent genes for proteins.

ACKNOWLEDGEMENTS

This work was supported by NIH grant GM25565, and by the U.S. Energy Research and Development Administration under Contract with Union Carbide Corporation, M.R.E.-G. is on leave from Alexandria University Science Center for Advancement of Postgraduate studies.

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Communicated by H.G. Zachau.

NOTE ADDED IN PROOF

A $tRNA^{Phe}$ has been mapped at the same position in strain Z (M. Kuntz, personal communication).