Location of the Single Gene for Elongation Factor Tu on the Euglena gracilis Chloroplast Chromosome*  
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The structural gene for elongation factor Tu (EF-Tu) has been mapped by heterologous hybridization to a 2900 base pair sequence of Euglena gracilis Klebs Strain Z Pringsheim chloroplast DNA within the EcoRI fragment, Eco N. The hybridization probes were obtained from a HhaI restriction fragment containing internal sequences to Escherichia coli EF-Tu, located in the tufA gene locus, and from an EcoRI restriction fragment of chloroplast DNA from the eukaryotic algae Chlamydomonas reinhardtii, containing the 3' end of the chloroplast EF-Tu gene. This is the second identified protein gene locus to be mapped on the E. gracilis chloroplast genome, and the first using prokaryotic DNA as a probe.

Although integrated in the algal cell, Euglena gracilis chloroplasts retain control over the synthesis of several chloroplast proteins via a plastid protein synthesizing system. All RNA's involved in chloroplast protein synthesis are likely encoded in the chloroplast genome. To date, ribosomal RNAs (Hallick, 1982), and tRNA (Orozco et al., 1976; Orozco and Hallick, 1982) gene loci, and from the large subunit of ribulose-1,5-bisphosphate carboxylase (Steigler et al., 1982) have been mapped on the E. gracilis chloroplast genome.

Elongation factor Tu promotes the binding of aminoacyl tRNA to ribosomes during protein synthesis (Pedersen et al., 1976). EF-Tu's have been isolated from chloroplasts of spinach (Tiboni et al., 1978) and, more recently, from E. gracilis (Beck and Spremulli, 1982). There are several reports that some chloroplast elongation factors, including EF-Tu, are encoded in chloroplast DNA (Brittenberger et al., 1979; Ciferri et al., 1979; Fox et al., 1980). Most recently, a 7.5-kbp BamHI/SalI fragment of spinach chloroplast DNA has been tentatively identified as the site of the EF-Tu coding locus. There is preliminary evidence that this spinach chloroplast EF-Tu gene can be functionally expressed in Escherichia coli transformants.

The EF-Tu gene has previously been mapped only for chloroplast DNA from C. reinhardtii (Watson and Surzycki, 1982). In this communication, we report the location of the EF-Tu gene with respect to the restriction endonuclease map of E. gracilis chloroplast DNA, and the cloning of the gene.

EXPERIMENTAL PROCEDURES

Materials and Chemicals—Restriction endonucleases were obtained from New England Biolabs, Beverly, MA. and Bethesda Research Laboratories, Gaithersburg, MD. Agarose, type II, was purchased from Sigma. (α-32P)ATP (specific activity: 600 Ci/mmol) was obtained from New England Nuclear. DNA Polymerase I was purchased from New England Biolabs, Beverly, MA. Cellulose nitrate filters (BA 85) were obtained from Schleicher and Schuell, Keene, NH. All other chemicals were of reagent grade or better.

Chloroplast and Recombinant Plasmid DNAs—E. gracilis chloroplast DNA was isolated as previously described (Gray and Hallick, 1977). The plasmid pT1 containing 691 bp of the 3' end of the E. coli EF-Tu gene on a 1.1-kbp Smal restriction fragment cloned in Smal digested pMB8, was generously provided by Dr. A. Furano. The plasmid pCH16 (Rochaix, 1978) containing the 3' end of the C. reinhardtii chloroplast EF-Tu (Watson and Surzycki, 1982) was generously provided by Dr. J. Watson and Dr. S. Surzycki. Plasmid pEZC3 containing EcoRI fragment Eco N from E. gracilis chloroplast DNA was isolated from a shotgun library containing EcoRI and EcoRI/PvuI restriction fragments of E. gracilis chloroplast DNA. Fragments were ligated into pBR225 by standard procedures. E. coli C600 strain LS1078 (bdhR: hasM, Su-2, gal-66, strR, ILV-6, thr-1, Thr-) was transformed as described by Dagert and Erlich (1979). Plasmid DNAs were prepared from mid-log phase cultures following amplification by addition of chloramphenicol to 240 mg/liter. Plasmid DNA was purified following the procedure of Guerry et al., (1973).

Preparation of Hybridization Probes—Extraction of plasmid DNA restriction fragments previously separated by electrophoresis through agarose gels was performed by passing the excised gel fragment through a 15-gauge hypodermic needle into 10 ml of 0.1 M sodium acetate, 0.1% sodium dodecyl sulfate, pH 6.5, and gently agitating the mixture overnight at 37°C. Excess agarose was removed by centrifuging for 10 min at 24,000 × g. DNA was subsequently precipitated from the supernatant and collected by centrifugation. Purified DNA fragments used as EF-Tu probes were radioactively labeled to a specific activity of ≥10⁶ cpm/μg using [α-32P]ATP. Incorporation of the label was accomplished by utilizing the DNA repair activity of E. coli DNA polymerase I (Cheln and Hallick, 1976; Rigby et al., 1977).

Gel Electrophoresis of DNA and Heterologous Hybridization—Restriction endonuclease digestion products of chloroplast DNA were electrophoresed through agarose gels as described by Orozco and Hallick (1982). Transfer of DNA to cellulose nitrate filters was performed as described by Rushlow et al., (1980).

Heterologous hybridizations using specific EF-Tu probes were carried out as described by Steigler et al., (1982). Hybridization conditions were 15% formamide and 37°C for the E. coli probe, and 20% formamide and 37°C for the C. reinhardtii probe. Following hybridization, the filters were washed four times in 6 × SSC (SSC: 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0) at room temperature, blotted dry, and exposed to X-ray film at -60°C with an intensifying screen.

RESULTS

The location of the EF-Tu gene on E. gracilis chloroplast DNA was determined by using specific EF-Tu probes from E. coli DNA and C. reinhardtii chloroplast DNA. The E. coli probe is a 621-bp internal HhaI restriction fragment beginning at position 531 and ending at position 1152 at the 3' end of the 1182-bp coding locus (Yokota et al., 1980; An and Friesen, 1980). The C. reinhardtii probe is a 1200-bp EcoRI restriction fragment, designated either R03 (Rochaix, 1978), or R25reviewed in part by the payment of page charges. This article must be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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* The abbreviations used are: EF-Tu, elongation factor Tu; bp, base pair; kbp, kilobase pair.
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(Watson and Surzycki, 1982), which contains the 3' end of the gene and other chloroplast DNA, possibly coding for tRNAs (Malnoe and Rochaix, 1978).

The radioactively labeled E. coli and C. reinhardii probes were hybridized to cellulose nitrate filter blots of EcoRI or SmaI restriction digests of pCR1G, and to an SmaI restriction digest of pT1. The E. coli probe hybridized specifically to C. reinhardii chloroplast DNA fragment R03 (R25'); the C. reinhardii probe hybridized to the SmaI (EF-Tu) insert in pT1 (Fig. 1), in agreement with the report of Watson and Surzycki (1982).

The location of the EF-Tu gene on E. gracilis chloroplast DNA was determined by hybridizing the above probes to cellulose nitrate filter blots of EcoRI or HindIII restriction digestion products of E. gracilis chloroplast DNA. The results of these experiments are presented in Fig. 2. In each case, specific hybridization was obtained to the 2900-bp EcoRI fragment Eco N, and to the 3600-bp HindIII fragment Hind 11 (Fig. 2, lanes 1 and 3, and 2 and 4, respectively). These are overlapping restriction fragments. The location of the EF-Tu coding locus with respect to a restriction map of E. gracilis chloroplast DNA is presented in Fig. 3.

Fig. 3. Restriction endonuclease cleavage map of the 145-kbp E. gracilis chloroplast DNA. The maximum boundary of the 3' end of the EF-Tu coding DNA is shown by the shaded box. Other previously mapped chloroplast genes are also shown.

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Fig. 4. Restriction endonuclease map of the E. gracilis EF-Tu coding region. a, detailed cleavage map of the Eco Q-Eco N-Eco J' region. b, and c, cleavage maps based on digestion of chloroplast DNA with EcoRI and HindIII, respectively. d, maximum boundary of the region hybridizing with the E. coli and C. reinhardii EF-Tu probes. Fragment sizes are in kbp.

Fig. 2. Location of the E. gracilis chloroplast EF-Tu gene by Southern hybridization. Left, ethidium bromide staining pattern of chloroplast DNA restriction fragments after electrophoresis on 0.8% agarose gels. Lanes: 1 and 3, HindIII cut chloroplast DNA; lanes 2 and 4, EcoRI cut chloroplast DNA. Right, corresponding autoradiograms after hybridization with EF-Tu DNA probes from E. coli (A), or C. reinhardii (B). The hybridizing fragments are named to the right of the figure as described in the text. Fragment size estimates are in kbp.

Fig. 1. Hybridization of 32P-labeled EF-Tu probes to plasmid DNAs. Left, ethidium bromide staining pattern of restriction fragments after electrophoresis on 1% agarose gels. Lanes: 1, SmaI cut pCR1G; 2, EcoRI cut pCR1G; 3, SmaI cut pT1. Right, corresponding autoradiograms after blotting to cellulose nitrate filters and hybridization with the 621-bp probe internal to the EF-Tu gene and other chloroplast DNA, possibly coding for tRNAs (Watson and Sunycki, 1982), which contains the 3' end of the gene and other chloroplast DNA, possibly coding for tRNAs (Malnoe and Rochaix, 1978).

Restriction endonuclease map of the E. gracilis EF-Tu coding region. a, detailed cleavage map of the Eco Q-Eco N-Eco J' region. b, and c, cleavage maps based on digestion of chloroplast DNA with EcoRI and HindIII, respectively. d, maximum boundary of the region hybridizing with the E. coli and C. reinhardii EF-Tu probes. Fragment sizes are in kbp.

Fig. 3. Restriction endonuclease cleavage map of the 145-kbp E. gracilis chloroplast DNA. The maximum boundary of the 3' end of the EF-Tu coding locus is shown by the shaded box. Other previously mapped chloroplast genes are also shown.

Restriction endonuclease map of the E. gracilis EF-Tu coding region. a, detailed cleavage map of the Eco Q-Eco N-Eco J' region. b, and c, cleavage maps based on digestion of chloroplast DNA with EcoRI and HindIII, respectively. d, maximum boundary of the region hybridizing with the E. coli and C. reinhardii EF-Tu probes. Fragment sizes are in kbp.

Karabin and R. B. Hallick, unpublished observation.
The *C. reinhardtii* probe also gave a strong hybridization signal to Hind 2, and to Eco V (Fig. 2). Eco V is an 1100-bp fragment internal to Hind 2 (Orozco and Hallick, 1982). Approximately 80% of the Eco V DNA sequence is known (Holdingsworth and Hallick, 1982). This DNA encodes a cluster of five tRNA genes, but not a protein coding locus. *C. reinhardtii* chloroplast DNA fragment R03 (R25') is known to hybridize with tRNA (Malnoe and Rochaix, 1978). Therefore, we believe the hybridization of R03 (R25') to Eco V and Hind 2 is most likely due to complementary sequences between *E. gracilis* and *C. reinhardtii* chloroplast tRNA genes.

**DISCUSSION**

The DNA coding locus for elongation factor Tu of *E. gracilis* chloroplasts has been identified by heterologous hybridization using DNA probes from another algal chloroplast and from a prokaryotic organism. This is the first protein gene to be mapped on the *E. gracilis* chloroplast genome using a prokaryotic DNA as a probe.

The EF-Tu gene is located in the large, single copy region of the genome. This region also contains coding loci for several tRNAs (Orozco and Hallick, 1982) and the 32,000-dalton photogene. These genes are within a 25-kbp region of the genome designated Pst C. Of the 15 kb of transcripts of Pst C, at least 6 kb are developmentally regulated (Chelm et al., 1979). In addition, Rutti et al. (1981) have demonstrated, by hybrid arrested and hybrid selection translation using a rabbit reticulocyte lysate, that the Eco N restriction fragment of *E. gracilis* chloroplast DNA specifically interacts with the mRNA of a chloroplast stromal polypeptide of 53,000 daltons. The EF-Tu gene is located in the large, single copy region of the genome. This region also contains coding loci for several tRNAs (Orozco and Hallick, 1982) and the 32,000-dalton photogene. These genes are within a 25-kbp region of the genome designated Pst C. Of the 15 kb of transcripts of Pst C, at least 6 kb are developmentally regulated (Chelm et al., 1979). In addition, Rutti et al. (1981) have demonstrated, by hybrid arrested and hybrid selection translation using a rabbit reticulocyte lysate, that the Eco N restriction fragment of *E. gracilis* chloroplast DNA specifically interacts with the mRNA of a chloroplast stromal polypeptide of 53,000 daltons. In addition to this polypeptide, two other polypeptides of approximately 35,000 and 40,000 daltons are produced under hybrid selected conditions using Eco N. It is not known if any of these *in vitro* translation products are related to EF-Tu. The 2.9-kbp Eco N is large enough to encode both the putative 1.2-kbp gene for EF-Tu and a 53,000-dalton polypeptide. It is, however, not large enough to encode the EF-Tu gene and the three polypeptides of 53,000, 40,000 and 35,000 daltons. We would predict that either one or more of the translation products described by Rutti et al. is an EF-Tu polypeptide, or that one or both of the minor translation products of 40,000 and 35,000 daltons are premature translation termination products of the 53,000-dalton mRNA.

Since our preliminary studies on the location of the EF-Tu coding region is currently based on Southern hybridization data, the possibility exists that positive signals may be due to hybridization of the probes to a nonfunctional pseudogene, or to related sequences within a gene of different function. In order to resolve these questions and to firmly establish the location and functionality of the EF-Tu gene in the *E. gracilis* chloroplast chromosome, we are currently performing DNA sequence analysis of the EF-Tu coding region, and characterizing the expression, biological activity, and developmental regulation of the EF-Tu gene. In addition, we are examining the possibility of using probes from prokaryotic DNA sequences as a general method for locating specific eukaryotic gene coding regions.

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