Euglena gracilis Chloroplast EF-Ts

EVIDENCE THAT IT IS A NUCLEAR-CODED GENE PRODUCT

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Extracts of Euglena gracilis cells contain high levels of elongation factor (EF)-Ts (EF-Ts dec) activity which can be assayed by measuring the rate of exchange of GDP with Escherichia coli EF-Tu-GDP. The appearance of EF-Ts activity in Euglena gracilis cells is light-stimulated, suggesting that the EF-Ts is required for chloroplast function. However, based on experiments with a mutant of Euglena gracilis lacking chloroplast DNA, as well as studies on the effect of antibiotics on EF-Ts synthesis, it is concluded that the EF-Ts dec gene is nuclear-coded.

In chloroplasts, many of the protein synthesis components are thought to be similar to those of prokaryotes. This conclusion has come mainly from studies showing that (i) antibiotics active against bacterial ribosomes inhibit chloroplast protein synthesis (1-4); (ii) initiation of protein synthesis uses Met-tRNA (4); (iii) initiation factors from Escherichia coli are active with spinach ribosomes (5); and (iv) highly purified elongation factors Tu and G from spinach chloroplasts (EF-Tu,1, EF-G,1) (6) and EF-G,3 from Euglena gracilis (7, 8) can function with E. coli ribosomes. Detailed studies remain to be done comparing the physical and chemical properties of purified chloroplast and E. coli elongation factors. Of considerable interest also is the location of the genes for the chloroplast elongation factors. In spinach, EF-Tu,3 and EF-G,3 are coded by chloroplast DNA (9), whereas Euglena gracilis EF-G is nuclear-coded (8). To better understand the nature of the chloroplast elongation factors and the regulation of their synthesis, we have initiated studies on the purification of chloroplast elongation factors from Euglena gracilis. The present report describes the presence of EF-Tu,3 in Euglena extracts and presents evidence that it is a nuclear-coded gene product.

MATERIALS AND METHODS

Euglena gracilis Klebs' var. bacillaris Cori (Euglena B) and the mutant strain W.BUL were obtained from W. E. Barnett and J. Schiff, respectively. EF-Tu was purified from extracts of E. coli as described previously (10). [3H]GDP (15 Ci/mmol) was purchased from Amer sham.

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† The abbreviations used are: EF, elongation factor; chi, chloroplast; chloramphenicol, d-(-)-threo-2,2-dichloro-N-[β-hydroxy-α-(hydroxymethyl)-p-nitrophenethyl]acetamide.

For experiments to examine the effect of light on EF-Ts synthesis, cells were grown in a volume of 3 liters, in the dark, to a density of 0.5 to 1.0×10⁶ cells/ml in a Difco Euglena broth medium. The cells were centrifuged at 2500 rpm for 15 min and then gently suspended in 3 liters of phototrophic medium, pH 3.5 (11). The suspension was divided and a portion was incubated in the dark, whereas a second aliquot was exposed to white light (400 ft-l) provided by fluorescent lights and a spotlight. Both incubations were carried out at 27°C. At various times, the cells were removed and harvested by centrifugation (15 min at 3000×g). The cells were washed with 40 ml of a buffer containing 20 mM each of Tris/Cl, pH 7.4, MgCl₂, and NH₄Cl (Buffer A) and then suspended in 2 ml of a buffer containing 50 mM Tris/Cl, pH 7.8, 0.1 mM EDTA, 50 mM NH₄Cl, 5 mM MgCl₂, 5 mM β-mercaptoethanol, and 10% glycerol (Buffer B). The cells were disrupted by sonication for 2×30 s in a Branson sonicator. The suspension was centrifuged for 10 min at 30,000×g and the supernatant was centrifuged at 150,000×g for 2 hr.

EF-Ts activity was measured by the ability of this factor to catalyze, at 0°C, an exchange of [3H]GDP with GDP bound to E. coli EF-Tu (EF-Tu-GDP) (12). The incubations contained in a total volume of 50 μl: 50 mM Tris/Cl, pH 7.4, 10 mM MgCl₂, 50 mM NH₄Cl, 5 mM dithiothreitol, 25 pmol of E. coli EF-Tu-GDP, 2.5×10⁻⁶ M [3H]GDP, (200 cpm/pmol), and cell extract containing up to 15 units of EF-Ts activity. The incubations were performed for 5 min at 0°C and were stopped by the addition of 3 ml of cold Buffer A and rapid filtration through a nitrocellulose filter. The filter was washed with 6 ml of Buffer A and dissolved in scintillation fluid (13) and the radioactivity was determined. A unit of EF-Ts activity is defined as the amount of protein required to catalyze the exchange of 1 pmol of [3H]GDP with GDP bound to EF-Tu under the conditions used. Specific activity is units/mg of protein. All values were corrected for any GDP binding in the Euglena extract in the absence of E. coli EF-Tu. Protein was determined by the method of Lowry et al. (14), using bovine serum albumin as standard.

The reaction mixture for EF-Tu activity was similar to that for the EF-Ts assay except that the incubations were done at 37°C for 10 min in the absence of E. coli EF-Tu-GDP.

RESULTS AND DISCUSSION

EF-Ts Activity in Euglena Extract—The most convenient procedure to assay for EF-Ts activity takes advantage of the ability of EF-Ts to catalyze an exchange of GDP with GDP bound to EF-Tu (EF-Tu-GDP). The EF-Ts activity in Euglena extracts could be assayed using E. coli EF-Tu-GDP. As seen in Fig. 1, a cell-free extract had high levels of EF-Ts activity which was proportional to the amount of extract added. The activity in these extracts was often greater than 20,000 units/ml with a specific activity of 1500 to 2000. This is comparable to the specific activity of EF-Ts (3400 units/mg) observed in crude E. coli extracts (15).

Effect of Light on EF-Ts Formation—Fig. 2 shows the effect of light on the EF-Ts activity in Euglena extracts. In these experiments, dark-grown Euglena cells were placed in a phototrophic medium and incubated for up to 96 hr in the dark or in light (see "Materials and Methods"). As seen in Fig. 2, exposure of the cells to light resulted in a large increase in the EF-Ts specific activity over the time course of the experiment. Cells kept in the dark showed no increase in EF-Ts activity with time. Since it is known that the synthesis of chloroplast proteins can be regulated by light (whether coded by nuclear or chloroplast DNA) the above results suggest that the observed EF-Ts activity is due to chloroplast EF-Ts (EF-Ts dec).

Evidence That EF-Ts Is Nuclear-Coded—To determine whether the EF-Ts dec gene is chloroplast- or nuclear-coded, a mutant of Euglena (W.BUL) that is lacking chloroplast
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Table 1

EF-Ts activity in wild type and W:BUL Euglena cells

The cells were grown in the dark and then suspended in phototrophic medium as described in the text. The cells were divided and then incubated for 48 h under the conditions shown in the Table. The preparation of the cell-free extracts and the assay for EF-Ts are described under "Materials and Methods." Cycloheximide and chloramphenicol concentrations were 10 and 100 μg/ml, respectively.

| Incubation conditions | Wild type | W:BUL |
|-----------------------|-----------|-------|
| Light                 | 1625      | 441   |
| Dark                  | 152       | 72    |
| Light + cycloheximide | 328       |       |
| Light + chloramphenicol | 1643     |       |

to be coded by chloroplast genes (9), E. gracilis EF-Gₜₚ is nuclear-coded (8). The present study indicates that EF-Tₜₚ in this organism is also nuclear-coded and that the synthesis of this factor, similar to that observed with EF-G from Euglena (8), is stimulated by light.

Further evidence suggesting that the EF-Tₜₚ gene is located in the nucleus was obtained by the use of antibiotics. If cytoplasmic ribosomes are the site of EF-Tₚₚ synthesis, cycloheximide would inhibit its synthesis, whereas chloramphenicol should inhibit EF-Tₚₚ synthesis, if it occurs on chloroplast ribosomes. As also shown in Table 1, cycloheximide resulted in an 80% inhibition of EF-Tₚₚ synthesis, while chloramphenicol had no effect.

Although EF-Tₚₚ and EF-Gₜₚ have been purified from spinach chloroplasts (6, 16) and EF-Gₜₚ from E. gracilis extract (7), EF-Tₚₚ activity has not been previously identified. Tiboni et al. (6) did report that some of the EF-Tₚₚ in spinach extracts was present as an EF-T complex (presumed to be an EF-Tₚₚ-EF-Ts complex), but no evidence for EF-Ts activity was presented.

Although the spinach factors EF-Tₚₚ and EF-Gₜₚ appear

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