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Extensive sequence homology in the DNA coding for elongation factor Tu from *Escherichia coli* and the *Chlamydomonas reinhardtii* chloroplast

(*tuf* gene/heterologous hybridization probe)

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ABSTRACT **Considerable DNA sequence homology can be detected between the *Escherichia coli* genes coding for translational components and *Chlamydomonas reinhardtii* chloroplast DNA. Labeled chloroplast DNA was found to hybridize to restriction fragments of the transducing phage λ*fus*3 that code for elongation factor Tu. The chloroplast probe also reacts with fragments coding for ribosomal proteins carried by this phage. The region homologous to the elongation factor genes was located on the physical map of the chloroplast genome by probing restriction fragments of chloroplast DNA with cloned fragments, labeled *in vitro*, carrying the *E. coli* elongation factor Tu genes.**

Chloroplasts contain complete translational and transcriptional systems distinct from those used in expression of nuclear genes. Some elements of the translational machinery in this organelle have been shown to be encoded by the chloroplast genome. The genes for rRNAs, tRNAs, and perhaps some ribosomal proteins (r proteins) (1, 2) are located in chloroplast DNA (cpDNA). There is some evidence suggesting that at least some of the plastid elongation factors are also synthesized in the organelle and encoded in organelle DNA (3–5). The chloroplast translational system has been shown to have structural and functional similarities with the prokaryotic system. For example, there is extensive nucleotide sequence homology between the rRNA (6, 7) and tRNA (8) genes of chloroplasts and bacteria. Also, there is evidence showing that the elongation factors (EFs) of *Escherichia coli* and plastids are functionally exchangeable (4). Moreover, low levels of immunological homology exist, at least in some cases, between bacterial and chloroplast EFs (9) and some r proteins of the small ribosomal subunit (10).

An understanding of the control of gene expression in chloroplasts would be greatly facilitated by knowledge of the organization of the genes coding for proteins involved in this process. Identification of such genes usually involves preparing antibodies to a specific purified protein and then using the antibody in identifying the DNA fragment that codes for the protein. This is very laborious, so we sought to develop alternative methods. Because of the similarities between the bacterial and chloroplast translational systems mentioned above, we reasoned that there may be enough homology between the respective coding sequences to permit identification of the corresponding chloroplast genes in heterologous DNA·DNA hybridizations.

Our approach was to first identify which, if any, bacterial genes are homologous to cpDNA (using a labeled cpDNA probe) and then use these genes to map the corresponding sequences on the chloroplast genome. As convenient sources of a large number of well-characterized *E. coli* genes, we chose the specialized transducing phages λ*fus*3 (11) and λ*rif*^{d}18 (12) and recombinant plasmids derived from them. The DNA segments carried by λ*fus*3 and λ*rif*^{d}18 between them account for ≈70 kilobases of the bacterial chromosome and carry genes for RNA polymerase α, β, and β′ subunits, EF Tu, EF G, rRNAs, tRNAs, and 31 r proteins (13). By using DNA dot hybridizations (14), we established reaction conditions under which the labeled cpDNA reacts with transducing phage DNA but not with wild-type phage or plasmid vector sequences. This is necessary to eliminate cross-hybridization between promoters, ribosome binding sites, or termination sequences present on the vectors. Very similar sequences are present on cpDNA (15, 16). These conditions were then used for Southern hybridizations (17) with labeled cpDNA probes and blotted restriction fragments of the phage DNAs. The experiments determined which of the genes on these phages share homology with cpDNA. Cloned phage fragments were then hybridized to Southern blots of cpDNA, thereby locating these sequences in the chloroplast genome. The results presented here locate the chloroplast EF Tu gene, which shows extensive homology with the *E. coli* EF Tu gene. We also show that some homology exists between cpDNA sequences and λ*fus*3 fragments containing the following *E. coli* genes: the α subunit of RNA polymerase and ribosomal proteins S4, S11, S13, L2, L4, L22, and L23. These results indicate that our basic premise was correct and raise the possibility that this approach may be useful in quickly identifying chloroplast genes other than those studied here. A portion of this work has been reported in preliminary form (18).

MATERIALS AND METHODS

Materials. Restriction enzymes *Bam*HI and *Eco*RI were obtained from Miles; all other restriction enzymes, *E. coli* DNA polymerase I, T4 DNA ligase, T4 polynucleotide kinase, and bacteriophage λ cI857S7 DNA (referred to as λ) were from Bethesda Research Laboratories. All were used as recommended by the supplier. DNase I, RNase A, and RNase T1 were obtained from Sigma; calf intestine alkaline phosphatase was from Boehringer Mannheim. [α-^{32}P]dCTP (400 Ci/mmol; 1 Ci = 3.7 × 10^{10} becquerels) was from Amersham. [γ-^{32}P]ATP (6000 Ci/mmol) was prepared as described (19).

Purification of DNAs. *Chlamydomonas reinhardtii* cpDNA was prepared from strain CW15(+) as described (20). Specialized λ transducing phages were obtained from S. R. Jaskunas or were purified from the appropriate lysogen after thermal induction as described (21). Phage DNA was extracted essentially according to Miller (21). Plasmid DNA was isolated from cells by an alkaline-detergent method (22) after either growth to saturation or amplification with chloramphenicol. After treatment

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Abbreviations: cpDNA, chloroplast DNA; r protein, ribosomal protein; EF, elongation factor; rDNA, DNA complementary to rRNA.

with RNase A and RNase T1, repeated phenol and chloroform extractions, and dialysis, plasmid DNA was separated from chromosomal DNA by acidic phenol extraction (23) followed by repeated ethanol precipitation. All DNAs were stored at 4°C in 10 mM Tris·HCl, pH 7.5/1 mM EDTA.

Construction of Recombinant Plasmids. The *Eco*RI fragments of λ*fus*3 were mixed with *Eco*RI-cleaved pBR325 (24) (mass ratio, 4:1) and the mixture was incubated with T4 DNA ligase. The ligation reaction mixture was then used to transform (25) *E. coli* strain DG-75 (26). Colonies harboring recombinant plasmids were selected by plating first on ampicillin medium and then on chloramphenicol medium. One such plasmid, found to carry the 8.5% fragment by restriction analysis, was called pLF8.5 and is used here. The 18.6% *Eco*RI fragment of λ*rif*^{d}18 was cloned in the vector pSF2124 (27) and was obtained from S. R. Jaskunas as pAB80. The λ*fus*3 *Sma* I fragment encoding the 3′ half of the *tufA* gene, cloned into pMB9, was obtained from A. Furano (28) as pT1.

DNA·DNA Hybridizations. DNA dot filters were prepared as described (15). Filters were incubated prior to hybridization at 42°C in hybridization buffer (0.48 M NaCl/0.060 M sodium citrate/0.08 M sodium phosphate, pH 6.8)/double strength Denhardt's solution (29) containing denatured calf thymus DNA at 40 μg/ml and the concentration of formamide used in the subsequent hybridization. The treated filters were incubated at 42°C for 30–36 hr with [^{32}P]cpDNA probe in hybridization buffer and formamide as indicated in Fig. 2. The probe for dot hybridization was *Hae* III restriction fragments of cpDNA phosphorylated at 5′ ends with [γ-^{32}P]ATP as described (30) except that calf intestine alkaline phosphatase was used. All probes were ethanol precipitated with *E. coli* tRNA as carrier, dissolved in hybridization buffer/formamide, and denatured by boiling (5 min). After hybridization, the filters were washed twice with hybridization buffer at 42°C containing formamide at the level used in the hybridization. The filters were then washed once at room temperature with hybridization buffer/formamide and once with hybridization buffer. The filters were dried, mounted, and exposed to Kodak XAR-5 x-ray film using intensifying screens (Picker or DuPont Cronex) at −70°C. For Southern blot experiments, DNAs were digested with the appropriate restriction enzyme, and the digests were subjected to agarose gel electrophoresis (20, 31). The DNA fragments were then transferred to nitrocellulose sheets (17) and treated as described above. The blots were incubated with labeled probes prepared by nick-translation with [α-^{32}P]dCTP, *E. coli* DNA polymerase I, and DNase I (32, 33), washed, and autoradiographed as described above, except that 35% formamide was used throughout. cpDNA probes were used at 1×10^6 cpm/ml and plasmid probes were used at 2×10^5 cpm/ml.

RESULTS

Our approach for identifying cpDNA sequences homologous to bacterial genes carried by specialized transducing phages and recombinant plasmids derived from them is discussed in the Introduction. The physical and genetic structures of the relevant portions of the phages, λ*fus*3 and λ*rif*^{d}18, and of the plasmids used (pLF8.5, pT1, pAB80) are shown in Fig. 1 (34–36). The appropriate reaction conditions were established by using heterologous DNA·DNA dot hybridizations. The stringency of hybridization was varied by changing the formamide concentration in the reaction, which is analogous to changing the hybridization temperature (37). The results indicate that hybridizations carried out with 30% formamide at 42°C or at ≈61°C without formamide (37) fulfill the stringency criterion discussed above (Fig. 2). At this level of formamide, the [^{32}P]cpDNA probe does not react with λ or pBR325 but does react with λ*fus*3

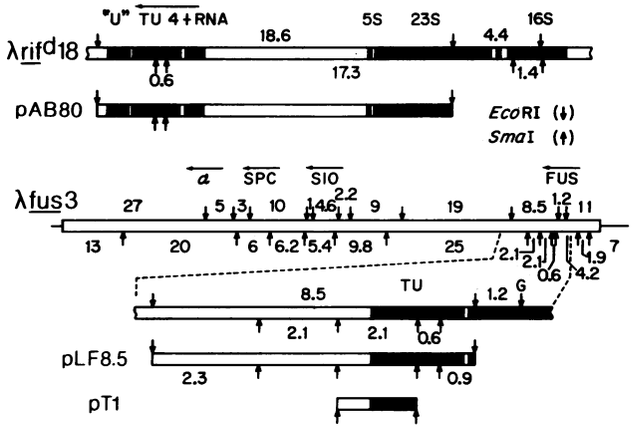

FIG. 1. Maps of portions of the λ*fus*3 and λ*rif*^{d}18 genomes and regions carried on recombinant plasmids. ▮▮, Coding region for the gene indicated above the phage map; α, SPC, S10, and FUS represent the pertinent operons carried by λ*fus*3; →, direction of transcription of operons. EF Tu genes carried by λ*fus*3 and λ*rif*^{d}18 are *tufA* and *tufB* loci, respectively. Numbers represent sizes of restriction fragments in % λ units (1% λ equals ≈490 base pairs). See refs. 34–36.

and λ*rif*^{d}18 DNAs (Fig. 2). Moreover, the results described below show that the hybrid duplexes formed are even more stable than the dot hybridizations indicate, in that the following experiments were done at a formamide concentration of 35%. In fact, we have obtained results using 40% formamide that are qualitatively identical to those shown in Fig. 4 (data not shown).

The transducing phage DNA fragments responsible for the hybridization were identified by using Southern hybridizations (Fig. 3). Restriction fragments of λ*fus*3, pLF8.5, and pT1 DNAs were transferred to nitrocellulose and hybridized with nick-translated [^{32}P]cpDNA. The labeled probe hybridizes most intensely to the *Eco*RI 9–8.5% doublet (Fig. 3*C*) and to the *Sma* I fragments that overlap the 8.5% fragment (*Sma* I 4.2%, 2.1%, and 0.6%; Fig. 3*F*). Electrophoresis of similar digests on 1% agarose gels, in which the 9–8.5% doublet is clearly resolved, shows that the cpDNA probe reacts with the 8.5% fragment but not with the 9% fragment (data not shown). This is confirmed by hybridization to an *Eco*RI/*Sma* I digest of pLF8.5 (Fig. 3*D*). *Sma* I digestion of this fragment produces five subfragments, and only those overlapping the coding sequences for the EF Tu gene (*tufA*) apparently react with the probe (0.9%, 0.6%, and 2.1% fragments; Fig. 3*D*). The *Sma* I 0.6% fragment from pLF8.5 (Fig. 3*D*), λ*fus*3, (Fig. 3*F*), and λ*rif*^{d}18 (not shown) is composed entirely of EF Tu gene sequences. Of the two 2.1% fragments produced by *Sma* I digestion of whole λ*fus*3 DNA, the one encoding the 3′ end of the *tufA* gene is carried by pT1, and this fragment, which overlaps *tufA*, reacts with the cpDNA probe (Fig. 3*E*).

We have also noted hybridization of cpDNA to λ*fus*3 fragments other than those coding for EF Tu (e.g., *Eco*RI 5% and 4.6%; see Fig. 3*C*). These fragments are derived from the α and

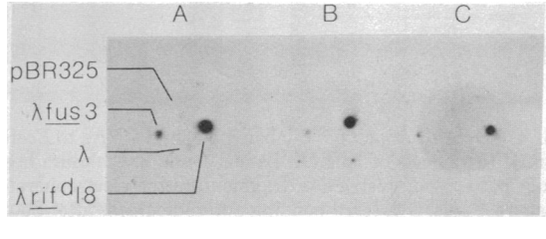

FIG. 2. Hybridization of [^{32}P]cpDNA to DNA dot filters using 20% (*A*), 30% (*B*), and 50% (*C*) formamide. Each DNA dot contained 1 μg of DNA.

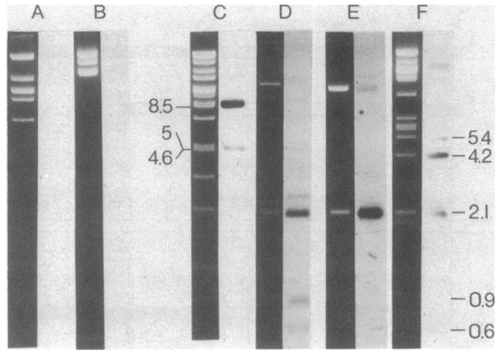

FIG. 3. Hybridization of [^{32}P]cpDNA to restriction fragments of phage and plasmid DNAs. (*Left*) Ethidium bromide staining pattern after fractionation on 1.5% agarose gels. (*Right*) Corresponding autoradiograms after Southern blotting and hybridization. Lanes: A, *Eco*RI-cut λ; B, Sma I-cut λ; C, *Eco*RI-digested λ*fus*3; D, *Eco*RI/*Sma* I-digested pLF8.5; E, *Sma* I-digested pT1; F, Sma I-digested λ*fus*3. Fragments showing hybridization that are not partial digestion products are indicated.

S10 r protein operons, respectively, and code for eight r proteins and the α subunit of RNA polymerase. These results, as well as hybridizations to the *rpoBC* operon carried by λ*rif*^{d}18, will be the subject of a separate communication.

The identity of the cpDNA fragments homologous to the *E. coli tuf* genes was determined by hybridization of *tuf* gene probes to cpDNA. cpDNA was digested with either *Eco*RI or *Bam*HI and then fractionated on agarose gels and transferred to nitrocellulose. The Southern blots were hybridized with nick-translated pLF8.5, pT1, or pAB80 (Fig. 4). Plasmid pT1, carrying the 3′ end of the *tufA* gene, hybridizes to cpDNA band *Eco* 25 (Fig. 4*A*, lane 2). The pLF8.5 probe, carrying the entire *tufA* gene, hybridizes to *Eco* 25 as well as to band *Eco* 1 (lane 3). Similar results are obtained with the pAB80 probe, containing the *tufB* gene (lane 4). However, in the latter case, additional hybridization to the *Eco* 3 band is also observed. This is due to the presence of *E. coli* 23S rRNA coding sequences on pAB80, which are homologous to 23S rDNA on cpDNA (see Fig. 5). The results of experiments in which *Bam*HI fragments of cpDNA were used are shown in Fig. 4*B*. Both truncated (pT1) and intact (pLF8.5) *tufA* genes hybridize to fragment *Bam* 5 (lanes 2 and 3). The *tufB* gene, carried by pAB80, also hybridizes to this fragment, and the DNA complementary to the rRNA

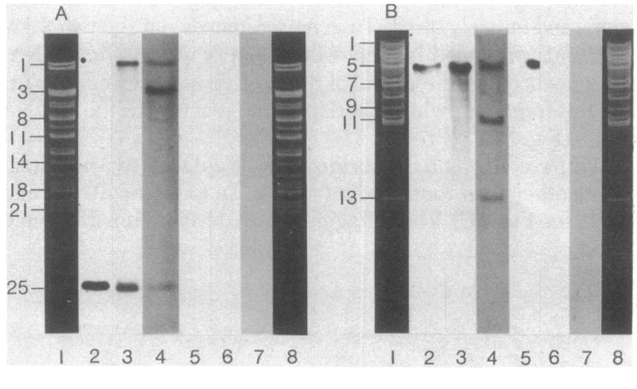

FIG. 4. Hybridization of ^{32}P-labeled plasmid DNAs to *Eco*RI (*A*) or *Bam*HI (*B*) digests of cpDNA. Lanes: 1 and 8, ethidium bromide staining patterns of restriction fragments after electrophoresis on 0.8% agarose gels; 2–7, corresponding autoradiograms after Southern blotting and hybridization with pT1 (lane 2), pLF8.5 (lane 3), pAB80 (lane 4), pMB9 (lane 5), pBR325 (lane 6), or pSF2124 (lane 7) DNAs. Numbers on left are selected fragment numbers (20). The radioactive spot in lane 5 of *B* is a background spot and not a hybridization signal.

(rDNA) on the plasmid hybridizes to fragments *Bam* 11 and *Bam* 13 (lane 4). Further, when total *E. coli* chromosomal DNA is used as nick-translated probe, hybridization is observed to the *Eco* 1 and 25 and *Bam* 5 fragments, as well as to all rDNA-containing fragments (data not shown).

cpDNA band *Eco* 25 is composed of two distinct fragments originating from widely separated regions on the chloroplast chromosome (see Fig. 5). Therefore, it is impossible to determine precisely the location of *tuf* sequences by *Eco*RI digestion alone. This is resolved by *Bam*HI analysis. The *tuf* gene probes hybridize to the *Bam* 5 fragment, thus, the fragment of band *Eco* 25 showing homology must be *Eco* 25′ (see Fig. 5; refs. 38–40). Fragment *Bam* 8 overlaps fragment *Eco* 25 and does show homology with some portion of the bacterial DNA insert carried by pAB80. This bacterial DNA segment probably resides between the 5S RNA gene and the four tRNA genes located 5′ to the *tufB* locus (see Fig. 1; unpublished data). Four *Eco*RI cpDNA fragments are contained entirely within *Bam* 5 but have not been ordered with respect to one another (38). The fact that the entire *E. coli tuf* gene probe hybridizes to fragments *Eco* 1 and *Eco* 25′, while the 3′-terminal half of the gene reacts only with *Eco* 25′, suggests that the *Eco* 1 and *Eco* 25′ fragments are adjacent to one another on the cpDNA restriction map (Fig. 5) and that the chloroplast *tuf* gene spans a single *Eco*RI site. We conclude that the gene is transcribed from fragment *Eco* 1 toward fragment *Eco* 25′.

DISCUSSION

The data presented here show that cpDNA contains a gene homologous to the *E. coli tuf* genes. This conclusion is based on heterologous DNA·DNA hybridizations between *E. coli* genes and cpDNA. Our reaction conditions were selected to be stringent enough not to allow hybridization between cpDNA and vector sequences. This was determined with DNA dot hybridizations and various formamide concentrations. The *tuf* gene

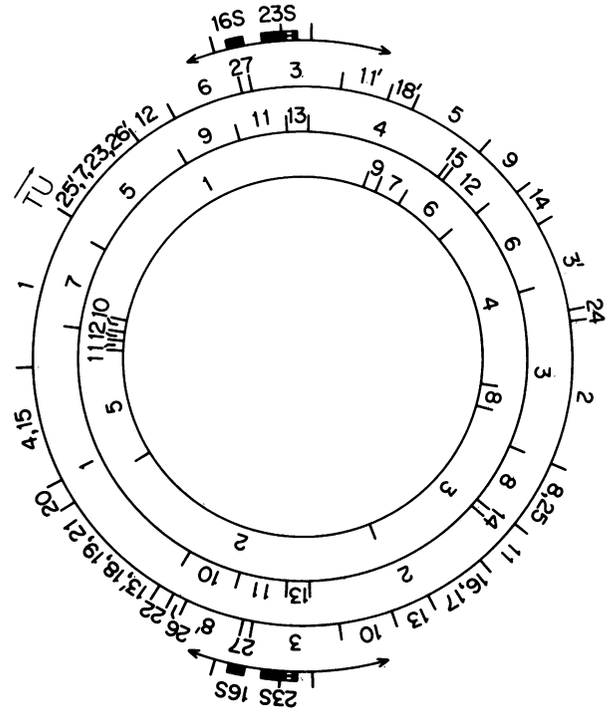

FIG. 5. Restriction map of *C. reinhardtii* cpDNA (38, 39) indicating positions of rDNA-containing inverted repeats (38). Inner circle, *Bgl* II map; middle circle, *Bam*HI map; outer circle, *Eco*RI map. TU, EF Tu gene; →, direction of transcription. Numbers refer to bands as described in ref. 20.

hybrid duplexes we observe can form at a formamide concentration of 30–40%, which corresponds to a temperature of 61–67°C without formamide (37). Based on hybridization intensity, gene size, and reiteration frequency of the restriction fragments, we estimate the level of homology between bacterial and chloroplast *tuf* genes to be approximately the same as, if not higher than, the homology between the rRNA genes. Our hybridization conditions are comparable with those of others using heterologous probes (41, 42). A similar approach has been useful in identifying nitrogen fixation genes among prokaryotes (41, 42). Our work probably represents the first demonstrated case of interkingdom hybridization involving protein-coding genes.

That the *tuf* genes of *E. coli* and the *C. reinhardtii* chloroplast are homologous is clearly shown by Fig. 3. The pattern of [^{32}P]cpDNA hybridization to phage and plasmid restriction fragments shows that the homology extends throughout the entire length of the *tufA* gene. Using three different *E. coli tuf* gene probes, we determined the location of the chloroplast EF Tu gene by hybridization to blots of cpDNA restriction digests that have known maps. Since the chloroplast *tuf* gene overlaps an *Eco*RI site, the direction of transcription of the gene was inferred from differential hybridization patterns (Fig. 4). Transcription proceeds from fragment *Eco* 1 toward fragment *Eco* 25′ and is therefore in the same direction as the closest rRNA operon. As the gene is not located within the rDNA-containing inverted repeats and spans an *Eco*RI site, we conclude that there is one EF Tu gene per chloroplast chromosome. This is in contrast to the situation in the *E. coli* genome, which contains duplicate *tuf* genes (13). The cellular functions necessitating this situation must not occur in the *C. reinhardtii* chloroplast. It is known that the *tuf* genes of a wide variety of prokaryotes are conserved (43), in particular, their COOH-terminal regions. Synthesis of both EF Tu and EF G by isolated spinach plastids has been observed (3), suggesting that both are chloroplast encoded. However, a different situation exists in *Euglena* chloroplasts, in which EF G and EF Ts may be nuclear encoded (4, 5). Interestingly, we found no detectable homology between cpDNA and the *E. coli* EF G gene (see Fig. 3*C*). It may be that the gene is nuclear encoded in *Chlamydomonas* or that the homology of the chloroplast gene is too low to be detected under our hybridization conditions. These two possibilities cannot as yet be distinguished. Finally, is the newly defined chloroplast *tuf* gene expressed? Although not directly approached in the present work, we previously found (20) that this region of cpDNA is extensively transcribed *in vivo* throughout the cell cycle. It seems likely that some of the transcripts observed previously are derived from the *tuf* gene.

We also find homology between cpDNA and some part(s) of both the α and S10 operons. Mapping of cpDNA regions hybridizing with the S10 and *rpoBC* operons (unpublished results) indicates that these genes, like the *tuf* gene, do not lie within the inverted repeats. The S10 region maps between the closest inverted repeat and the *tuf* gene. This, together with data concerning possible tRNA coding sequences (44) in this area of the chloroplast genome, suggests to us a possible clustering of genes coding for components of the protein synthetic machinery. Our demonstration of sequence homology between the EF Tu genes of *E. coli* and cpDNA of *C. reinhardtii* is in accord with the widely held hypothesis that chloroplasts have a prokaryotic origin.

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