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Protein Synthesis Elongation Factors Tu and Tu·Ts from *Caulobacter crescentus*: Sensitivity to Kirromycin and Activity in Qβ Replicase

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The protein synthesis elongation factors Tu and Ts are responsible for binding aminoacyl-transfer ribonucleic acid (RNA) to the ribosome. In addition, they perform an undefined function, as the EF-Tu·Ts complex, in the RNA phage RNA replicases. In an effort to obtain insight into these two apparently unrelated roles, we purified the elongation factors from *Caulobacter crescentus* and compared them to the analogous *Escherichia coli* polypeptides. Although most physical and functional characteristics were found to be similar, significant differences were found in the molecular weight of EF-Ts and relative affinities of guanine nucleotides, sensitivity to trypsin cleavage, and rate of heat denaturation of EF-Tu. The antibiotic kirromycin was active with EF-Tu from both bacterial species. When *C. crescentus* EF-Tu·Ts was substituted for the *E. coli* elongation factors in Qβ phage RNA replicase, an enzyme capable of apparently normal RNA synthetic activity was formed.

The protein synthesis elongation factors EF-Tu and EF-Ts from *Escherichia coli*, which catalyze the enzymatic binding of aminoacyl-tRNA to ribosomes, have been the subject of much recent investigation (reviewed in references 15 and 17). EF-Tu is a particularly interesting polypeptide. In *E. coli* it has been found to be present in the cell in very large amounts (12, 14) and to be associated with the membrane (14). Recently, a new antibiotic, kirromycin, has been described that specifically interacts with EF-Tu (23). It permits EF-Tu to express GTPase activity in the absence of ribosomes (9) and prevents the conformational transitions of EF-Tu necessary for recycling on the ribosome (10, 24). In addition, both EF-Tu and EF-Ts are found as components of the RNA phage RNA replicase enzymes (6).

In an effort to determine the function of the elongation factors in phage Qβ replicase, the endogenous EF-Tu and EF-Ts have been replaced with elongation factors which exhibit altered protein synthetic activity. There appears to be a great deal of flexibility in the enzyme's requirement for EF-Tu and EF-Ts since each protein synthetic function of the elongation factors can be eliminated independently without loss of RNA synthetic activity (4, 7, 8). It has also been shown that in the RNA synthesis reaction the elongation factors act in the EF-Tu·Ts complex form (7, 8). These studies have led to the conclusion that EF-Tu and EF-Ts probably do not perform functions in Qβ replicase derived directly from their protein biosynthetic functions (7, 8).

Recently, we have screened several bacterial species for the presence of proteins which can replace *E. coli* EF-Tu·Ts in Qβ replicase (unpublished data). Among the bacterial species which had such an activity, *Caulobacter crescentus*, a dimorphic, gram-negative bacterium, seemed the most promising on which to base a more detailed study. Filer and Furano (11) have recently shown that while the portion of the gene which codes for the C-terminal region of EF-Tu is highly conserved between *E. coli* and *C. crescentus*, the remainder of the gene has diverged significantly. Thus, we felt that *C. crescentus* EF-Tu was likely to be sufficiently different from *E. coli* EF-Tu to result in the formation of an altered enzyme when inserted in Qβ replicase, in place of *E. coli* EF-Tu. Furthermore, since *Caulobacter* sp. has been heavily studied in recent years, particularly in regard to its developmental cycle (18), a characterization of the protein synthesis elongation factors from this organism should provide information relevant to other studies.

In this paper, we describe the purification of the elongation factors from *C. crescentus* and characterize them with respect to several known properties of *E. coli* EF-Tu and EF-Ts. In addition, we show that they can substitute for the homologous elongation factors in Qβ replicase.

MATERIALS AND METHODS

Materials and reagents used were as described in previous publications from this laboratory (3–5). Qβ replicase was purified as described previously and contained only the four polypeptide chains (S1, II, EF-Tu, and EF-Ts) as demonstrated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (3).

Purification of elongation factors. EF-Tu and EF-Ts from both *E. coli* and *C. crescentus* were purified by the following procedure developed by Daniel Smith of this laboratory from the procedures described by Arai et al. (1) and Furano (12, 13). Buffers used were as follows. Buffer A contained 20 mM Tris-hydrochloride (pH 7.5), 10 mM Mg $(OAc)_2$, 0.1 mM EDTA, 5 mM 2-mercaptoethanol, 3 mM phenylmethylsulfonyl fluoride, and 2 μg of DNase I per ml. Buffer B is buffer A (without phenylmethylsulfonyl fluoride and DNase) with 0.25 M sucrose. Buffer C is buffer B without sucrose. All procedures were carried out at 4°C.

Forty-five grams of frozen cells was broken with glass beads in a chilled, water-jacketed Waring blender for 15 min in the presence of 45 ml of buffer A. The liquid was decanted from the beads and subjected to centrifugation at 12,000 × *g* for 20 min. The supernatant was then subjected to further centrifugation at 31,000 × *g* for 30 min, followed by centrifugation at 100,000 × *g* for 190 min to remove the ribosomes. The 100,000 × *g* supernatant was then extracted twice with solid $(NH_4)_2SO_4$ (37 and 73% saturated). The material insoluble in 73% saturated $(NH_4)_2SO_4$ was collected by centrifugation, resuspended in buffer B, dialyzed against buffer C plus 0.14 M KCl, and chromatographed on a diethylaminoethyl (DEAE)-Sephadex A-50 column (2.5 by 22.5 cm). The column was developed with a nine-column-volume linear gradient of 0.16 to 0.4 M KCl in buffer C. The *E. coli* EF-Tu·Ts and EF-Tu were found to elute at 0.18 M and 0.22 M KCl, respectively, while the *C. crescentus* EF-Tu·Ts and EF-Tu eluted at 0.19 and 0.21 M KCl, respectively. The EF-Tu-containing fractions were pooled, precipitated by $(NH_4)_2SO_4$ (70% saturated), collected by centrifugation, resuspended in buffer B plus 10 μM GDP, and chromatographed on a Sephadex G-100 column (2.5 by 58 cm) equilibrated in buffer C plus 0.12 M KCl and 10 μM GDP. The column was developed with a one-column volume of the same buffer. The EF-Tu-containing fractions were pooled, dialyzed against buffer C plus 20% (vol/vol) glycerol, 0.15 M KCl, and 10 μM GDP and chromatographed on a hydroxylapatite column (0.9 by 8.8 cm) equilibrated in the same buffer. The column was developed with a 20-column-volume linear gradient of 0 to 0.6 M $(NH_4)_2SO_4$ in buffer C plus 20% (vol/vol) glycerol and 10 μM GDP. The *E. coli* EF-Tu eluted at 0.12 M $(NH_4)_2SO_4$, while *C. crescentus* EF-Tu eluted at 0.17 M $(NH_4)_2SO_4$. The polypeptide appeared to be pure as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 1).

The EF-Tu·Ts-containing fractions from the DEAE-Sephadex column were extracted twice with $(NH_4)_2SO_4$ (50 and 70% saturated). The 70% precipitate was collected by centrifugation, resuspended in buffer B, and chromatographed on a Sephadex G-100 column (2.5 by 58 cm) equilibrated in buffer C plus 0.14 M KCl. The column was developed with a one-column volume of the same buffer. The EF-Tu·Ts-containing fractions (20 ml) were pooled, diluted with 5 ml of buffer C plus 60% (vol/vol) glycerol, and loaded onto a hydroxylapatite column (0.9 by 4.5 cm) equilibrated in buffer C plus 20% glycerol. The column was developed with a 20-column-volume linear gradient of 0 to 0.6 M $(NH_4)_2SO_4$ in buffer C plus 20% glycerol. *E. coli* EF-Tu·Ts eluted at 0.25 M $(NH_4)_2SO_4$, while the *C. crescentus* EF-Tu·Ts eluted at 0.18 M $(NH_4)_2SO_4$. Although small amounts of contaminating polypeptides were present in both EF-Tu·Ts preparations (Fig. 1), they were judged to be sufficiently pure for the experiments reported here.

Nucleotide binding. GDP and GTP binding to EF-Tu was measured by the binding of ^3H-labeled nucleotides to nitrocellulose filters as described previously (3). Reaction volumes were 100 μl and ^3H-labeled nucleotide concentrations were 3 μM, unless otherwise noted. GTP binding experiments were performed in the presence of 5 μg of pyruvate kinase per ml and 5 mM phosphoenolpyruvate to ensure conversion of all contaminating GDP to GTP. Assay mixtures were incubated for 10 min at 30°C before filtration through 6-mm Schleicher and Schuell B6 filters.

EF-Ts assays. EF-Ts was quantitated by measurement of exchange between GDP bound to EF-Tu and free [^3H]GDP as described previously (6). Assay mixtures (as above) containing EF-Ts or EF-Tu·Ts at various concentrations were incubated at 0°C with [^3H]GDP. Reactions were initiated by addition of EF-Tu·GDP and, after a 5-min incubation at 0°C, the mixtures were filtered and counted as described above.

GTPase activity. To assay for GTPase, 32 pmol of EF-Tu mixed with [^3H]GTP (2.2×10^{-5} M, 10.8 Ci/mM) in 40 μl of binding buffer in the absence or presence of kirromycin was incubated for 10 min at 37°C. Reactions were stopped by the addition of an equal volume of 30 mM EDTA, and a 3-μl sample of each was applied to polyethyleneimine thin-layer chromatography sheets (Polygram 300, Brinkmann). The samples were developed with 1.5 M KH_2PO_4 (pH 3.4). The sheets were dried, and the 2-cm-wide column above each application spot was cut into 1-cm segments. These segments were inserted into scintillation vials and counted with toluene-Omnifluor in a liquid scintillation counter. The positions of GDP and GTP were determined by comparison with control spots.

RESULTS

Physical properties. EF-Tu and EF-Tu·Ts isolated from *E. coli* and from *C. crescentus* are compared in Fig. 1. Although we could not distinguish between the two EF-Tu's by electrophoresis in sodium dodecyl sulfate-polyacrylamide gels, we estimate that the *C. crescentus* EF-Ts is about 15% larger than the homologous polypeptide from *E. coli*. In other experiments (not shown) *C. crescentus* EF-Tu and EF-Tu·Ts did not cross-react on Ouchterlony immunodiffusion plates with antisera prepared against these proteins from *E. coli*.

Nucleotide binding. *E. coli* EF-Tu binds ca.

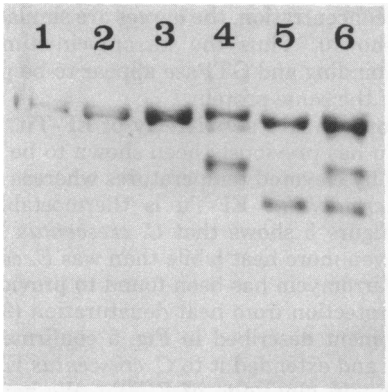

FIG. 1. *Polyacrylamide gel electrophoresis of EF-Tu and EF-Tu·Ts. Elongation factors were subjected to electrophoresis on a sodium dodecyl sulfate-10% polyacrylamide slab gel by the method of Weber and Osborn (20). Lanes: (1) 0.6 μg of C. crescentus EF-Tu; (2) 0.6 μg of E. coli EF-Tu; (3) 0.6 μg of each EF-Tu; (4) 1.5 μg of C. crescentus EF-Tu·Ts; (5) 3.0 μg of E. coli EF-Tu·Ts; (6) 1.5 μg of C. crescentus EF-Tu·Ts and 3.0 μg of E. coli EF-Tu·Ts.*

1 mol of GDP or GTP per mol of protein and binds GDP 100-fold more tightly than GTP (17). To determine the relative strengths of GDP and GTP binding to the *C. crescentus* EF-Tu, we mixed EF-Tu·GDP with [^{3}H]GDP (3 μM) and increasing concentrations of unlabeled GTP as competitor (Fig. 2). With *E. coli* EF-Tu, we found the GTP to be a better competitor than expected on the basis of a 100-fold differential in the binding of the two nucleotides. This result is probably explained by a small GDP contamination of the GTP. When the same experiment was performed with *C. crescentus* EF-Tu, the GTP competitor was found to be less effective. When we plotted the data by the method of Dixon to obtain inhibitor constants (not shown), we calculated that with *C. crescentus* EF-Tu, GTP competed about 3.5 times less effectively than with *E. coli* EF-Tu. Thus the ratio of GDP to GTP binding was significantly greater with the *C. crescentus* elongation factor. A control experiment in which GTP was used as competitor with mixed EF-Tu preparations from the two species demonstrated that the difference was not due to the presence of an endogenous competitor (not shown). A 1,000-fold excess of ATP or ADP did not compete with [^{3}H]GDP binding to *C. crescentus* EF-Tu, as has been previously demonstrated for *E. coli* EF-Tu (6, 17).

EF-Ts activity. EF-Ts can be assayed in vitro by measurement of exchange of GDP bound to EF-Tu with free [^{3}H]GDP at 0°C. Since 1 mol of EF-Ts can catalyze the exchange of 35 mol of GDP in 5 min, the assay can be performed with EF-Tu·Ts as the source of EF-Ts without interference from the EF-Tu component of EF-Tu·Ts (21). Figure 3 presents the data from exchange assays performed with increasing concentrations of *E. coli* and *C. crescentus* EF-Tu·Ts with either 0.6 μg of *E. coli* (Fig. 3A) or *C. crescentus* (Fig. 3B) EF-Tu·GDP. The *C. crescentus* EF-Tu·Ts was found to be equally effective with homologous or heterologous EF-Tu. On the other hand, the *E. coli* EF-Tu·Ts showed significant activity only with *E. coli* EF-Tu. When purified *E. coli* EF-Ts was tested in this assay, it was also found to be effective only with *E. coli* EF-Tu (not shown).

Kirromycin stimulation of GDP/GTP binding and GTPase activity. The antibiotic kirromycin has been shown to stimulate exchange at 0°C between EF-Tu-bound GDP and free GDP and GTP, and to allow EF-Tu to cleave GTP in the absence of ribosomes (4, 23). In this section, we demonstrate that these activities were also found when *C. crescentus* EF-Tu was treated with kirromycin. In Fig. 4, GDP binding, GTP binding, and GTPase are plotted as a function of kirromycin concentration. The three curves are quite similar, each reaching a plateau at ca. 10 μM kirromycin. These results are comparable to those previously found with *E. coli* EF-Tu (4, 23). We have also found that when GTP binding and GTPase activity of *C. crescentus* EF-Tu are plotted as a function of

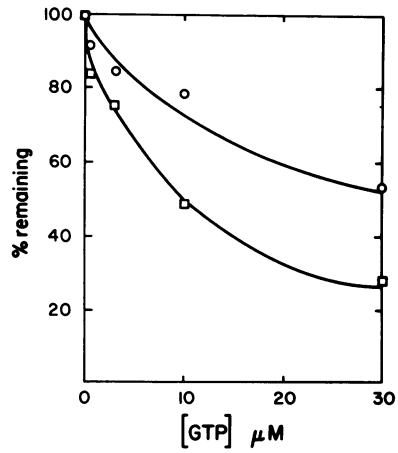

FIG. 2. *EF-Tu-dependent binding of [^{3}H]GDP: competition by GTP. GDP binding to E. coli (□) or C. crescentus (○) EF-Tu in the presence of the indicated concentrations of GTP was measured as described in the text (GDP concentration, 3 μM). The data are presented as the percentage of binding without competitor (11.7 pmol bound by 13 pmol of C. crescentus EF-Tu, and 8.9 pmol bound by 13 pmol of E. coli EF-Tu).*

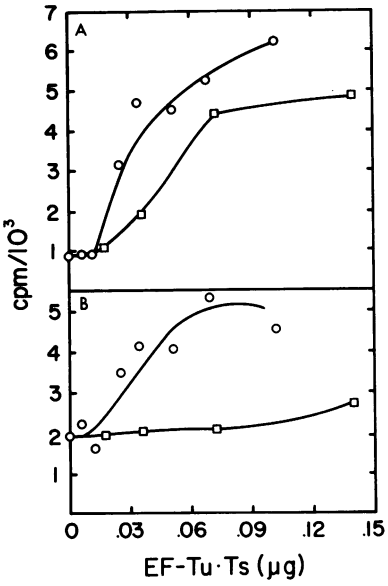

FIG. 3. *EF-Ts activity of EF-Tu·Ts preparations with homologous and heterologous EF-Tu. EF-Ts activity was measured as described in the text using the amounts of EF-Tu·Ts shown as a source of EF-Ts. A 0.68-μg amount of EF-Tu was added in each case. (A) E. coli EF-Tu; (B) C. crescentus EF-Tu. □, E. coli EF-Tu·Ts; ○, C. crescentus EF-Tu·Ts.*

GTP concentration, the curves are similar (data not shown). Thus the kirromycin-stimulated GTP binding and GTPase appear to be properties of the same protein.

Temperature sensitivity of EF-Tu. *E. coli* EF-Tu has previously been shown to be inactivated by elevated temperatures whereas *B. stearothermophilus* EF-Tu is thermostable (17, 22). Figure 5 shows that *C. crescentus* EF-Tu was even more heat labile than was *E. coli* EF-Tu. Kirromycin has been found to provide partial protection from heat denaturation (9). The experiment described in Fig. 5 confirmed that result and extended it to *C. crescentus* EF-Tu.

Trypsin cleavage of EF-Tu. We have previously reported that kirromycin increases the rate at which trypsin cleaves EF-Tu·GDP, yielding a 39,000-dalton, C-terminal fragment (4). To compare *E. coli* and *C. crescentus* EF-Tu with respect to this property, we cleaved both, in the presence and absence of saturating kirromycin, for various lengths of time. The trypsin-treated preparations were electrophoresed on sodium dodecyl sulfate-polyacrylamide gels, stained and scanned, and the amount of protein at the 45,000- (uncleaved EF-Tu) and 39,000-dalton positions was determined. The results, plotted as the percentage of total protein at the 39,000-dalton position, are shown in Fig. 6. They demonstrate that the *C. crescentus* EF-

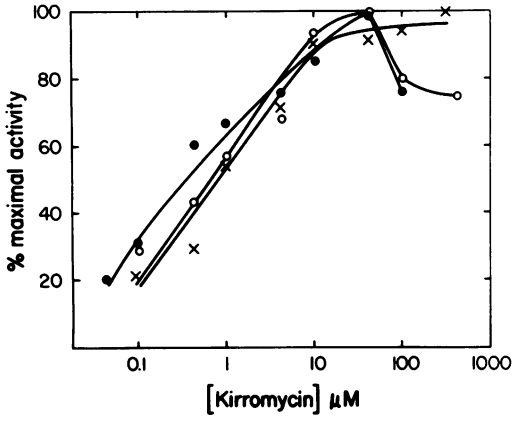

FIG. 4. *Kirromycin stimulation of guanine nucleotide binding and GTPase activity. GDP and GTP binding by C. crescentus EF-Tu (13 pmol/assay) was measured at 0°C, as described in the text, in the presence of the indicated concentrations of kirromycin. Maximal binding was 10.8 pmol of GDP and 10.0 pmol of GTP per assay. GTPase activity of C. crescentus EF-Tu (32 pmol/assay) was measured as a function of kirromycin concentration as described in the text. Maximal activity represented 35% of the GTP converted to GDP in 10 min at 40°C. ○, GDP binding; ●, GTP binding: ×, GTPase.*

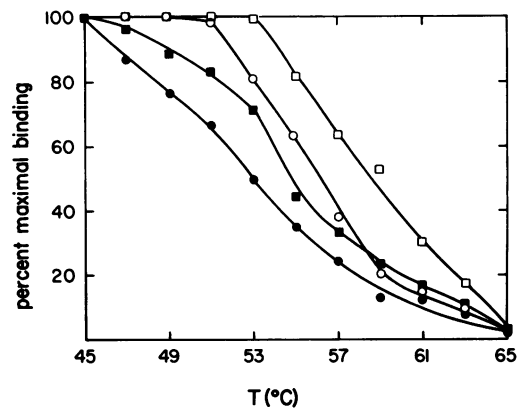

FIG. 5. *Temperature inactivation of EF-Tu in the presence and absence of kirromycin. EF-Tu·GDP from E. coli (□, ■) or C. crescentus (○, ●) was incubated for 1 min at the temperatures shown without (closed symbols) or with (open symbols) 20 μM kirromycin. [³H]GDP was added to 3.0 μM, and the samples were incubated for 10 min at 30°C. They were filtered and counted as described in the text. The data are presented as the percentage of control samples incubated for 1 min at 30°C (8.0 pmol bound by 13 pmol of E. coli EF-Tu; 11 pmol bound by 13 pmol of C. crescentus EF-Tu).*

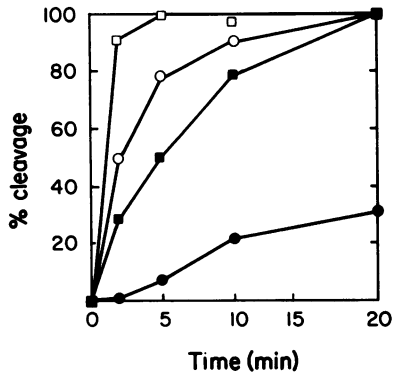

FIG. 6. *Trypsin cleavage of EF-Tu. A 25-μl solution containing 7.5 μg of EF-Tu from E. coli (□, ■) or C. crescentus (○, ●) in the presence (open symbols) or absence (closed symbols) of 20 μM kirromycin was mixed at 0°C with trypsin at a final trypsin concentration of 12 μg/ml. At the times shown, 5-μl samples were removed to tubes containing 5 μl of 0.25-μg/ml soybean trypsin inhibitor. The samples were subjected to electrophoresis on 10% polyacrylamide-sodium dodecyl sulfate gels by the method of Laemmli (16). The gels were stained with Coomassie brilliant blue and scanned at an absorbance at 600 nm on a Varian Superscan III spectrophotometer. The only bands seen were at the 45,000-dalton position (uncleaved EF-Tu) and at the 39,000-dalton position, the first cleavage product (4). The peaks from the gel scans were cut out and weighed. The data are plotted as the percentage of the total in the two peaks that are found at the 39,000-dalton position.*

Tu was cleaved more slowly than was the *E. coli* EF-Tu and that, as with the *E. coli* polypeptide, the rate of cleavage was increased when kirromycin was present (Fig. 6). Thus, the antibiotic produced similar conformational changes with EF-Tu from the two species.

Function of *C. crescentus* EF-Tu and EF-Tu·Ts in Qβ replicase. Using a previously described denaturation-renaturation procedure, we can replace the EF-Tu or EF-Tu·Ts in Qβ replicase with exogenous elongation factors (5). When Qβ replicase is denatured with 8 M urea, and subsequently diluted into a renaturation buffer at 0°C, enzymatic activity is recovered extremely slowly because EF-Tu renatures slowly. If native EF-Tu or EF-Tu·Ts is present in the renaturation buffer, then renaturation is rapid and the initial rate is directly dependent upon the exogenous EF-Tu concentration (5). All of the reconstituted Qβ replicase contains the exogenous EF-Tu, since the endogenous EF-Tu remains denatured during the course of the experiment. To determine whether EF-Tu or EF-Tu·Ts from *C. crescentus* could function in Qβ replicase, we denatured the enzyme and renatured it in the presence of increasing concentrations of elongation factors from either *E. coli* or *C. crescentus*. The resulting data (Fig. 7) clearly show that the *C. crescentus* EF-Tu·Ts could stimulate the renaturation of denatured Qβ replicase and in fact did so at even lower concentrations than did the *E. coli* EF-Tu·Ts. On the other hand, *C. crescentus* EF-Tu stimulated renaturation very poorly compared to *E. coli* EF-Tu. For EF-Tu to stimulate the rate of Qβ replicase renaturation, the exogenous EF-Tu must be able to form an effective complex with the endogenous EF-Ts. Since we found that *E. coli* EF-Ts would not catalyze GDP exchange with *C. crescentus* EF-Tu (Fig. 3), we presume that the failure of the *C. crescentus* EF-Tu to stimulate Qβ replicase renaturation was due to its inability to form a functional complex with the *E. coli* EF-Ts. Because *C. crescentus* EF-Tu·Ts stimulated renaturation of Qβ replicase much more effectively than did *C. crescentus* EF-Tu, both polypeptides must have been capable of functioning in the RNA synthesis reaction. We have also found that the enzyme renatured with the *C. crescentus* EF-Tu·Ts is active in the Qβ-RNA-dependent replication reaction (not shown).

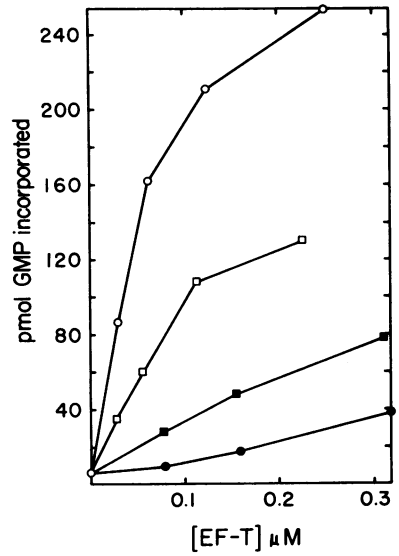

FIG. 7. *Stimulation of Qβ replicase renaturation by EF-Tu and EF-Tu·Ts. Qβ replicase at 4.6 μM was denatured, incubated for 1 h at 21°C, diluted in renaturation buffer, and incubated for 3.5 h at 0°C in the presence of the indicated amounts of E. coli EF-Tu·Ts (□), C. crescentus EF-Tu·Ts (○), E. coli EF-Tu (■), or C. crescentus EF-Tu (●). The procedure used has been described previously (5). Samples were then assayed for polycytidylic acid-dependent polyguanylic acid polymerase activity (3).*

DISCUSSION

Renewed interest has recently been generated in the study of the protein synthesis elongation factors EF-Tu and EF-Ts because they appear to be multifunctional proteins. In addition to its integral role in the protein biosynthetic apparatus of *E. coli*, EF-Tu has been shown to be associated with the membrane (14). It may be involved in the control of rRNA synthesis (19), and it may be related to eucaryotic actin because it can form filaments and paracrystalline arrays (2, 25). It has also been shown to be abundant, comprising about 5% of the cellular protein (12–14). EF-Tu and EF-Ts have also been shown to be subunits of Qβ replicase (6).

In this study, we have isolated and characterized these polypeptides from *C. crescentus*, a dimorphic, gram-negative bacterium. We have found both similarities to and differences from the analogous *E. coli* polypeptides. The molecular weights of the EF-Tu's were found to be the same, but the *C. crescentus* EF-Ts was somewhat larger than the *E. coli* EF-Ts. Our finding that the *C. crescentus* elongation factors did not cross-react immunologically with those from *E. coli* is somewhat surprising in light of the fact that the genes for EF-Tu from the two species have been found to be quite similar (11). This may be partially due to the fact that EF-Tu is a weak antigen (unpublished data) or to our use of the Ouchterlony immunodiffusion technique. A more sensitive assay might demonstrate an immunological relationship between the EF-Tu or EF-Ts from the two species.

Functionally, the similarities between the elongation factors from the two species were more striking than the differences. Both EF-Tu's bound GDP much more tightly than GTP (and excluded other nucleoside di- and triphosphates), but the *C. crescentus* EF-Tu had a 3.5-fold-greater GDP/GTP binding ratio. Both EF-Tu's exchanged bound with free GDP slowly at 0°C, and both EF-Ts's increased the rate of this exchange; but only the *C. crescentus* EF-Ts could catalyze GDP exchange with heterologous EF-Tu. Kirromycin appeared to act identically with the two EF-Tu's; it increased the rate of GDP and GTP exchange at 0°C, induced a ribosome-independent GTPase, inhibited heat inactivation, and increased the accessibility of the peptide bond in EF-Tu most sensitive to cleavage by trypsin. In the absence of kirromycin, both EF-Tu's were inactivated by heating, but the *C. crescentus* EF-Tu was more temperature sensitive. Both polypeptides were cleaved to form a 39,000-dalton fragment by trypsin, but the *E. coli* EF-Tu was cleaved more rapidly. Thus, although the two EF-Tu preparations showed the same general properties, their responses in most assays were quantitatively different. We do not know whether the *C. crescentus* EF-Tu or EF-Ts could substitute for *E. coli* polypeptides in protein biosynthesis. The fact that they can do so, however, in Qβ replicase is evident from Fig. 7.

Recent experiments from our laboratory have shown that EF-Tu can be chemically or enzymatically altered in a wide variety of ways without interfering with its ability to function in Qβ replicase (4, 7, 8); since inhibitors of EF-Tu·Ts complex formation do prevent the production of active enzyme, the elongation factors must act in the complex form in RNA synthesis (7, 8). Our results with *C. crescentus* EF-Tu and the EF-Tu·Ts complex provide further support for this conclusion. *C. crescentus* EF-Tu was able to effectively stimulate the rate of Qβ replicase renaturation only in the presence of its homologous EF-Ts, presumably because *C. crescentus* EF-Tu and *E. coli* EF-Ts do not form a functional EF-Tu·Ts complex. This result demonstrates that both *C. crescentus* polypeptides were able to function effectively in Qβ replicase in spite of the fact that this EF-Tu·Ts complex differs physically and immunologically from that of *E. coli*. This result, taken together with earlier findings, strongly suggests that whatever function the EF-Tu·Ts complex performs in Qβ replicase, the requirement for it to have a particular molecular configuration is not terribly stringent.

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