# Localization of the Elongation Factor Tu Binding Site on *Escherichia coli* Ribosomes†

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ABSTRACT: Fluorescent techniques were used to study binding of peptide elongation factor Tu (EF-Tu) to *Escherichia coli* ribosomes and to determine the distances of the bound factor to points on the ribosome. *Thermus thermophilus* EF-Tu was labeled with 3-(4-maleimidylphenyl)-4-methyl-7-(diethylamino)coumarin (CPM) without loss of activity. In the presence of Phe-tRNA and a nonhydrolyzable analogue of GTP, 70S ribosomes bind the CPM–EF-Tu [$K_b = (3 \pm 1.2) \times 10^6$ $M^{-1}$] causing a decrease of CPM fluorescence. Binding of CPM–EF-Tu to 50S subunits was at least 1 order of magnitude lower than with 70S ribosomes, and binding to 30S subunits could not be detected. Reconstituted 70S ribosomes containing either S1 labeled with fluoresceinmaleimide or ribosomal RNAs labeled at their 3′ ends with fluorescein thiosemicarbazide were used for energy transfer from CPM–EF-Tu. The distances between CPM–EF-Tu bound to the ribosomes and the 3′ ends of 16S RNA, 5S RNA, 23S RNA, and the closest sulfhydryl group of S1 were calculated to be 82, 70, 73, and 62–68 Å, respectively.

Peptide elongation factor Tu (EF-Tu)[1] with GTP promotes codon-directed binding of aminoacyl-tRNA to bacterial ribosomes (Kaziro, 1978; Weissbach, 1980; Parmeggiani & Sander, 1981). An EF-Tu·GTP binary complex or an aminoacyl-tRNA·EF-Tu·GTP complex can be formed in the absence of ribosomes. GTP hydrolysis and release of EF-Tu·GDP occur after codon-directed binding of the ternary complex into the A or aminoacyl-tRNA binding site on ribosomes. The free amino acid amino group of aminoacyl-tRNA thus bound into the A site functions as the acceptor for the nascent peptide chain that is transferred from the tRNA in the P or peptidyl ribosomal site during the peptidyltransferase reaction. Although the A and P sites have long been defined functionally (Bretscher & Marcker, 1966), little is known of their physical characteristics or location on the ribosome. Immunoelectron microscopy, protein–protein and protein–RNA cross-linking, fluorescence measurements, and other methods have been applied to evaluate the structure of the ribosome [for review see Lake (1980)]. Portions of both the A and P sites are located on the 50S as well as the 30S subunit (Ofengand, 1980). The structure of each of the subunits has been reported, but there are many discrepancies between different models, for instance the spatial relationships between large and small subunits (Ofengand, 1980). The 3′ end of the 16S RNA (Zimmermann, 1980) is located in the central part of the platform of the 30S subunit. The center of mass of the highly elongated S1 molecule, as determined by neutron scattering, is centrally located in the 30S subunit between S3, S6, and S9 (Sillers & Moore, 1981). The 3′ end of the 5S RNA is located in the central protuberance of the 50S subunit (Stöffler-Meilicke et al., 1981). The corresponding region of the 23S RNA is below the L7/L12 stalk on the noninterfacing surface of this subunit (Stöffler-Meilicke et al., 1981). The distance between the 3′ ends of the 5S RNA and 23S RNA is too far to be measured accurately by fluorescence techniques but is greater than 65 Å (Odom et al., 1980).

*Escherichia coli* EF-Tu contains 393 amino acids and three cysteine residues, two of which can be modified with sulfhydryl reagents under nondenaturing conditions (Laursen et al., 1981). Alkylation of Cys-81 results in loss of aminoacyl-tRNA binding activity. Alkylation of Cys-137 inhibits the ability of EF-Tu to bind guanine nucleotides and elongation factor EF-Ts. Cys-255 can be modified only under denaturing conditions and probably is buried within the native protein (Laursen et al., 1981; Miller et al., 1971; Arai et al., 1974). EF-Tu from the extreme thermophile, *Thermus thermophilus*, contains two cysteine residues that form a disulfide bridge in the native enzyme (Nakamura et al., 1978). This disulfide bridge can be reduced and the cysteines alkylated at 37 °C with retention of activity of the factor, in contrast to the *E. coli* factor. *T. thermophilus* EF-Tu is resistant to heat denaturation and has relatively high activity with ribosomes and other components from *E. coli*. The molecular weights and amino acid composition of the factors derived from *E. coli* and *T. thermophilus* are similar (Arai et al., 1978a,b).

Here we describe the results of nonradiative energy-transfer experiments using *T. thermophilus* EF-Tu labeled with a maleimidylcoumarin derivative (CPM) as the energy donor and reconstituted *E. coli* ribosomes labeled at specific sites with fluorescein. We calculate equilibrium constants for binding of EF-Tu to the ribosomes and distances between this factor and four points on the ribosome.

## Experimental Procedures

### *Materials*

Fluorescein-5-maleimide, fluorescein thiosemicarbazide, and CPM were purchased from Molecular Probes, Inc. (Junction City, OR), [8-$^3$H]guanosine 5′-diphosphate, ammonium salt (specific activity 12.7 Ci/mmol), and L-[$^{14}$C]phenylalanine (specific activity 450 Ci/mol) were purchased from Amersham (Arlington Heights, IL), casein hydrolysate was from Sigma

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[1] Abbreviations: CPM, 3-(4-maleimidylphenyl)-4-methyl-7-(diethylamino)coumarin; EF-G and EF-Tu, elongation factors G and Tu; GDP-NP, guanosine 5′-($\beta,\gamma$-imidotriphosphate); Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; poly(U), poly(uridylic acid); Tris, tris(hydroxymethyl)aminomethane; cps, counts per second.

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(St. Louis, MO), and autolyzed yeast extract powder was from Yeast Products Inc. (Clifton, NJ). All other chemicals were of reagent grade.

*Solutions.* The following solutions were used: solution A, 20 mM Tris-HCl (pH 7.9), 10 mM magnesium acetate, and 5 mM β-mercaptoethanol; solution B, 10 mM Tris-HCl (pH 7.5), 10 mM magnesium acetate, 100 mM $NH_4Cl$, and 5 mM β-mercaptoethanol; solution C, 50 mM Tris-HCl (pH 7.9), 10 mM magnesium acetate, 100 mM $NH_4Cl$, and 5 mM β-mercaptoethanol; solution D, 50 mM Tris-HCl (pH 7.5), 10 mM magnesium acetate, 100 mM $NH_4Cl$, 100 mM KCl, 5 mM dithioerythritol, 280 μM GDP-NP, 65 μg/mL poly(U), 10 μM Phe-tRNA, and 0.07% bovine serum albumin.

## Methods

*Growth of Thermus thermophilus.* Cells were grown for about 4–5 h at 70 °C in a broth consisting of 0.5% yeast extract, 0.5% casein hydrolysate, 0.15% dextrose, 0.2% NaCl, and 0.1% $KH_2PO_4$ (pH 7.2, adjusted with sodium hydroxide) with stirring and aeration. Bacteria were harvested by centrifugation at the mid-log phase and stored frozen at −80 °C. The yield was 500–600 g of cells/200 L of the medium.

*Preparation of RNAs.* Ribosomal RNAs were extracted from *E. coli* ribosomes as described by Nierhaus & Dohme (1979) and Odom et al. (1980). Unfractionated tRNA and $tRNA^{Phe}$ were isolated from *E. coli* as described by Robbins et al. (1981).

$tRNA^{Phe}$ was aminoacylated with [$^{14}$C]phenylalanine by using a partially purified phenylalanyl-tRNA synthetase fraction from *E. coli* as described (Robbins et al., 1981). It was assumed that one $A_{260}$ unit of $tRNA^{Phe}$ is equal to 1860 pmol.

*Preparation of 70S Ribosomes and Ribosomal Subunits.* Ribosomal subunits were prepared from *E. coli* K12 (strain A-19) as described previously (Odom et al., 1980). Ribosomes (70S) free of 30S and 50S subunits were prepared by zonal centrifugation of salt-washed ribosomes through a linear 10–30% sucrose (w/v) density gradient in a solution containing 20 mM Tris-HCl (pH 7.5), 50 mM ammonium chloride, 10 mM magnesium chloride, and 1 mM dithioerythritol (Dottavio-Martin et al., 1979).

*Labeling of Ribosomal RNAs.* Ribosomal RNAs were oxidized at their 3′ ends by incubation with 0.09 M sodium periodate in the dark, as described by Odom et al. (1980). Oxidized RNAs were incubated for 2 h in the dark with 2 mM fluorescein thiosemicarbazide in 0.1 M sodium acetate (pH 5.0), at 20 °C for 5S RNA, in 50 mM sodium phosphate (pH 7.0), at 25 °C for 16S RNA, and in 50 mM sodium phosphate (pH 7.0), at 0 °C for 23S RNA. Unbound label was removed by repeated phenol extractions and ethanol precipitations (Odom et al., 1980).

*Reconstitution of 30S Ribosomal Subunits.* The procedures of Traub & Nomura (1968) as modified by Hardy et al. (1969) were followed for isolation of a fraction containing the total protein from the 30S subunit, TP30, and for reconstitution of 30S particles containing labeled 16S RNA. Proteins from the 30S subunits were extracted with acetic acid. TP30 was incubated with labeled 16S RNA for 1 h at 42 °C in 30 mM Tris-HCl (pH 7.4), 330 mM KCl, 20 mM magnesium acetate, and 1 mM dithioerythritol.

*Reconstitution of 50S Subunits.* The methods described by Nierhaus & Dohme (1979) were followed for isolation of unfractionated proteins from the 50S subunit, TP50, and for reconstitution of 50S subunits containing labeled 5S RNA or 23S RNA; 50S proteins were extracted with acetic acid. The reconstitution involves incubation of 23S RNA, 5S RNA, and TP50, first for 20 min at 44 °C in 4 mM magnesium acetate and then for 90 min at 50 °C in 20 mM magnesium acetate in the reconstitution mixture described by Nierhaus & Dohme (1979). Activity of reconstituted ribosomes was measured by poly(U)-dependent polyphenylalanine synthesis.

*Purification of S1.* Ribosomal protein S1 was isolated by the method of A. R. Subramanian (personal communication). About 190 nmol of 30S subunits in 1 M $NH_4Cl$, 20 mM magnesium acetate, 6 mM β-mercaptoethanol, and 10 mM Tris-HCl (pH 7.5) was applied to a 30-mL (1.5 × 17 cm) poly(U)–Sepharose 6B column [prepared from cyanogen bromide activated Sepharose 6B by the method of Lindberg & Persson (1972)], equilibrated with the same solution. Most of the components of the 30S particle except protein S1 pass through the column with little, if any, retardation. This eluate is used as a source of 30S subunits that lack S1. Protein S1 binds to the poly(U) and thus is retained on the column. The column was washed with the buffer indicated above until the optical density at 260 nm of the eluate was below 1.0. Then most of the remaining material was eluted with 10 mM Tris-HCl (pH 7.5) and 6 mM β-mercaptoethanol. Finally S1 was eluted with a solution containing 10 mM Tris-HCl (pH 7.5) and 7 M urea. S1 eluted from the poly(U) column, although essentially free of other proteins, contains a significant amount of material that absorbs light at 260 nm. For removal of this material, S1 is chromatographed on DE 52 cellulose as described by Labischinski & Subramanian (1979). The S1 eluted from the poly(U) column was dialyzed against 10 mM sodium acetate (pH 5.5) in 6 M urea and then was applied to a 2.5-mL (0.7 × 6.5 cm) DE 52 column equilibrated with the same buffer. S1 was eluted from the DE 52 column with a 40-mL linear KCl gradient (0–200 mM KCl in 10 mM sodium acetate, pH 5.5, and 6 M urea). The $A_{280}/A_{260}$ ratio of the preparation was about 1.2. Finally, the S1 solution was dialyzed against 10 mM Hepes (pH 7.5) and stored at −80 °C.

*Purification of EF-Tu.* EF-Tu was purified from *Thermus thermophilus* by the method of Arai et al. (1978a), except that the crystallization step was omitted. The final EF-Tu preparation was extensively dialyzed against 50 mM $NH_4HCO_3$ and lyophilized. About 180 mg of the factor was purified from 550 g of wet cells.

*Labeling of EF-Tu with Fluorescent Dyes.* The disulfide bond of EF-Tu was reduced by sodium cyanoborohydride, and the resulting sulfhydryl groups were reacted with CPM or fluoresceinmaleimide by using the following procedure: 1 mg of lyophilized EF-Tu was dissolved in 100 μL of a solution containing 10 mM Tris-HCl (pH 8.5), 10 mM magnesium acetate, and 0.1 M sodium cyanoborohydride and then incubated for 60 min at 37 °C. After reduction 2 μL of 1 M Hepes–KOH buffer (pH 7.0) and 1 μL of either 100 mM CPM or 100 mM fluoresceinmaleimide in formamide were added, and the mixture was incubated at 37 °C. After 30 min 1 μL of the labeling reagent solution was added, and the incubation was continued for an additional 30 min, at which time unreacted labeling reagent was inactivated by the addition of 2 μmol of cysteine. Labeled EF-Tu was isolated by chromatography on a 0.7 × 8.5 cm column of Sephadex G-25 equilibrated with solution A. The molar extinction coefficient, ε, of fluorescent EF-Tu at maximum absorption of the dye was determined by comparison of the spectra before and after proteinase K treatment (cf. legend to Figure 2) to the spectrum of CPM–cysteine. ε of CPM–Cys was calculated in the following way. A given amount of CPM was dissolved in formamide to obtain a concentration of the dye of 10 mM and then

diluted by addition of 500 volumes of 40 μM cysteine in solution A. The spectrum was taken about 5 min after this addition. ε of CPM–Cys at 397 nm was 29 800. The labeling ratio of the dye to the EF-Tu approached 1:1. If desired, CPM–EF-Tu was concentrated by ammonium sulfate precipitation (70% saturation).

EF-Tu was also labeled under denaturing conditions to reach two fluorophores per protein molecule. One milligram of EF-Tu was treated with sodium cyanoborohydride as above, and then 2 μL of 1 M Hepes–NaOH (pH 7.8) and 230 mg of solid guanidine hydrochloride were added to 100-μL solution of the reduced factor. The mixture was incubated at 37 °C for 10 min, and then 2 μL of 100 mM CPM, dissolved in formamide, was added. After 15, 30, and 45 min, additional 1-μL aliquots of CPM were added to the mixture. Unreacted CPM was inactivated by addition of 2 μmol of cysteine, and then the mixture was passed through a Sephadex G-25 column (0.7 × 8.5 cm) equilibrated with 2 M urea in solution A. The labeled factor may be dialyzed against solution A without visible precipitation.

*$^{125}$I-Labeled EF-Tu.* The lactoperoxidase method as described by Morrison (1980) was used to label EF-Tu with radioactive iodine. One millicurie of carrier-free Na$^{125}$I solution and 4 μg of lactoperoxidase were added to 40 μL of a solution containing EF-Tu (1 mg/mL) that had been previously labeled with CPM or fluoresceinmaleimide and then dialyzed against 50 mM sodium phosphate (pH 7.4). The iodination reaction was initiated by addition of 7 μL of 1 mM hydrogen peroxide. This addition was repeated 4 times at 1-min intervals. Iodinated protein was separated from free iodide by chromatography twice on Sephadex G-25 columns (20 × 0.6 cm) previously equilibrated with solution A. The amount of free iodide was less than 10% of the total radioactive material in the preparations of the labeled factor, as judged by the trichloroacetic acid precipitation method (Mans & Novelli, 1961). Specific activity was approximately 35 000 cpm/pmol of EF-Tu.

*Labeling of Ribosomal Protein S1 and Reconstitution of 30S Subunits with Labeled Protein S1.* Solid guanidine hydrochloride was added to a solution of S1 (about 3 mg of protein/mL in 10 mM Hepes–KOH, pH 7.5) to give a final guanidine concentration of about 6 M. Then solid dithioerythritol was added to give a final concentration of 20 mM, and the pH was adjusted to 8.3 by the addition of solid Tris base. The mixture was incubated for 30 min at 37 °C. Dithioerythritol was removed by passing the mixture through a Sephadex G-25 column (1.5 × 23 cm) equilibrated with a solution containing 7 M guanidine hydrochloride and 10 mM 1,4-piperazinediethanesulfonic acid–KOH (pH 6.8). Labeling of reduced S1 was accomplished by incubation for 2 h at room temperature with 0.5 mM final concentration of fluoresceinmaleimide. The sample was passed through a Sephadex G-75 column (1.5 × 23 cm) equilibrated with 7 M urea and 10 mM Hepes–KOH (pH 7.5), and the eluate was dialyzed overnight against two 2 L volumes of 10 mM Hepes (pH 7.5), and then it was concentrated to about 1.5 mg/mL by lyophilization. Two molecules of fluorescein were bound per molecule of S1. The extent of labeling was determined from the absorption at 497 and 280 nm, taking 49 000 as the molar extinction coefficient of unlabeled S1 at 280 nm (Linde et al., 1979). The molar extinction coefficient of S1 labeled with fluoresceinmaleimide was determined by comparison of the absorption of the labeled protein before and after hydrolysis by proteinase K, carried out as described for EF-Tu in the legend to Figure 2. The absorption maximum shifted from 497 to 493 nm after hydrolysis, and the maximum absorption increased by 0.18, corresponding to a molar extinction coefficient of 58 000 at 497 nm for each fluorescein residue on the undigested protein. The change in absorption upon hydrolysis was not detectably different for S1 labeled with one or two fluorescein residues. A molar extinction coefficient of 69 000 at 490 nm was determined directly for fluoresceinmaleimide. The absorption spectrum between 260 and 550 nm of fluoresceinmaleimide did not change detectably upon reaction with β-mercaptoethanol, reduced glutathione, or cysteine under the conditions used. For calculation of the concentration of fluorescein-labeled protein, absorption at 280 nm was reduced by a factor of 0.25 of the absorption at 497 nm.

For binding of labeled S1 to deficient ribosomes, a solution of 8 μM 30S subunits free of protein S1 obtained during S1 purification was incubated with 9.5 μM fluorescein–S1 in solution B for 10 min at 37 °C, and then reconstituted 30S subunits were centrifuged for 3 h at 150000*g*. The pellet was suspended in solution B. The ratio of the fluorescein–S1 to 30S subunits approached 1:1.

*Translation of Poly(U).* This procedure has been described by Odom et al. (1980).

*Formation of EF-Tu·GDP Complex.* Binding of GDP to EF-Tu was performed essentially as described by Arai et al. (1972). [$^3$H]GDP (250 pmol) (0.5 Ci/mmol) in 50 μL of solution C was incubated with variable amounts of EF-Tu for 20 min at 37 °C, then 2 mL of cold solution C was added, and mixtures were passed through Millipore filters (0.25 μm) which were then washed twice with 3.0 mL each of cold solution C. The filters were dried, and their radioactivity was determined by liquid scintillation counting.

*Binding of Phe-tRNA to Ribosomes.* Binding of [$^{14}$C]-Phe-tRNA to ribosomes was carried out as described by Wagner & Sprinzl (1979) with some modifications. Reaction mixtures of 35 μL containing 50 mM Tris-HCl (pH 7.5), 15 mM magnesium acetate, 100 mM $NH_4Cl$, 100 mM KCl, 5 mM dithioerythritol, 5 μg of poly(U), 250 μM GTP, 2 nmol of unfractionated *E. coli* tRNA, and 28 pmol of ribosomes were preincubated for 10 min at 37 °C to decrease EF-Tu-independent binding of Phe-tRNA to the ribosomes. After preincubation, 40 μL of a solution containing 50 mM Tris-HCl (pH 7.5), 100 mM $NH_4Cl$, 100 mM KCl, 5 mM dithioerythritol, 50 pmol of [$^{14}$C]Phe-tRNA (50 Ci/mol), and variable amounts of EF-Tu was added, and the incubation was continued for 20 min at 37 °C. After incubation the reaction mixtures were diluted by addition of about 20 volumes of cold solution C and then passed through nitrocellulose filters which were then washed twice with 3 mL each of cold solution C. Radioactivity of the filters was determined by liquid scintillation counting.

*Fluorescence Measurements.* Emission and excitation spectra were taken with a photon counting spectrofluorometer, SLM Instruments Inc., Model 8000. When spectra were taken, data were accumulated at 1-nm intervals with a scanning rate of 1 s per wavelength increment. All fluorescence measurements were made at an absorption of less than 0.1 at the excitation maxima.

Titration of CPM–EF-Tu with ribosomes was performed in a 2 × 10 mm quartz cuvette at 25 ± 1 °C, in solution D. A solution of CPM–EF-Tu was titrated with a solution containing equimolar amounts of 30S and 50S subunits. It was assumed that one $A_{260}$ unit of 30S, 50S, and 70S ribosomes corresponds to 75, 44, and 28 pmol, respectively (Hill et al., 1969). The excitation beam (397 nm) passed through a 10-nm band-pass filter centered at 400 nm and then through the

10-mm path length of the cuvette. Emission was recorded at 470 nm for 100 s for each ribosome concentration, 20 s after gentle stirring of the reaction mixture with a 1.5 mm thick glass rod. The fluorescence intensity was about 2000 cps. The fluorescence of the blanks, i.e., solution D as well as fluorescein-labeled ribosomes in solution D, was 390 cps, and this value was subtracted from the data. Under these conditions the standard deviation for a specific determination usually did not exceed 0.3%.

Quantum yield was calculated by comparison of the fluorescence of the probe to the fluorescence of a standard whose quantum yield is known (Odom et al., 1980). Quinine sulfate in 0.1 N sulfuric acid, $Q = 0.7$ (Scott et al., 1970), and disodium fluorescein in 0.1 M NaOH, $Q = 0.92$ (Weber & Teale, 1957), were used as standards for CPM–EF-Tu and fluorescein-labeled proteins, respectively. The shape of the emission fluorescence curve of the CPM–EF-Tu did not change during titration with ribosomes; therefore, the quantum yield of the ribosome-bound CPM–EF-Tu was estimated according to the equation

$$Q_b = Q_f(F_b/F_f)$$

where $Q_b$ and $Q_f$ are quantum yields of the bound and free factor, respectively, and $F_b$ and $F_f$ are the relative fluorescence intensities at 470 nm of the bound and free factor, respectively. Fluorescence polarization was determined according to the equation

$$P = \frac{I_\parallel - I_\perp}{I_\parallel + I_\perp}$$

where $I_\parallel$ is the intensity of fluorescence polarized parallel to the excitation light and $I_\perp$ is the intensity polarized perpendicular to the excitation beam (Kaye & West, 1967). The polarization of the donor (CPM–EF-Tu) was measured at λ emission of 470 nm and λ excitation of 397 nm. For acceptors (fluorescein-labeled ribosomes) excitation and emission were 470 and 520 nm, respectively. $R_0$, the distance at which the energy transfer proceeds at half of its maximum rate, was calculated according to the equation

$$R_0 = 9.79 \times 10^3(Q_0 n^{-4} \kappa^2 J)^{1/6}$$

where $Q_0$ is the quantum yield of the energy donor under the experimental conditions in the absence of an acceptor, $n$ is the refractive index of the medium, $\kappa^2$ is an orientation factor for dipole–dipole transfer, and $J$ is the spectral overlap integral (Förster, 1959; cf. Odom et al., 1980; Robbins et al., 1981). The distance, $r$, between probes was calculated by

$$r = R_0(E^{-1} - 1)^{1/6}$$

where $E$ is the efficiency of energy transfer between the probes. CPM–EF-Tu was titrated with nonfluorescent or fluorescein-labeled ribosomes, and the fluorescence was recorded. Reciprocal values for decrease of fluorescence vs. reciprocal ribosome concentrations were extrapolated to infinite concentration of ribosomes, as shown in Figure 7. $E$ was calculated as

$$E = \frac{F_0'}{F_0} - \frac{F_i'}{F_i}$$

where $F_0'$ and $F_i'$ are the fluorescence values of the CPM–EF-Tu in the presence of infinite concentrations of unlabeled and labeled ribosomes, respectively, and $F_0$ and $F_i$ are the fluorescence values of unquenched CPM–EF-Tu before titration with unlabeled and labeled ribosomes, respectively.

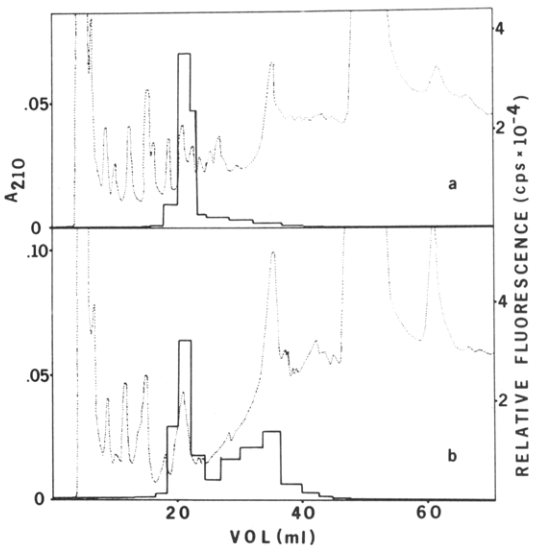


FIGURE 1: Analysis of fluorescent peptides by high-pressure liquid chromatography. After reduction of the disulfide bridge of EF-Tu with sodium cyanoborohydride, the factor was incubated with CPM: (a) under nondenaturing conditions; (b) in 8 M guanidine hydrochloride as described under Methods. Trypsin digestion of both preparations of labeled EF-Tu was performed in 195 mM ammonium bicarbonate (pH 8.0) for 6 h at 37 °C; trypsin:EF-Tu ratio was 1:20, by weight. The samples were lyophilized to dryness and then dissolved in water. About 40 μg of digested EF-Tu was applied to a μBondapak $C_{18}$ column (3.9 × 300 mm). The column was developed with 40 mL of a 20–50% linear gradient of $CH_3CN$ in a 8–5 mM linear gradient of phosphate buffer (pH 3.0), followed by an additional 30 mL volume at the highest concentration of $CH_3CN$. The flow rate was 2 mL/min. At least four nonfluorescent peptides were eluted with 75% $CH_3CN$ (not shown); all fluorescence loaded on the column was eluted quantitatively; optical density at 210 nm (···); fluorescence at 480 nm (—) with excitation at 397 nm.

Because of pipetting errors, values of $F_0$ and $F_i$ may be slightly different.

## Results

*Labeling of EF-Tu with CPM and Spectral Properties of CPM–EF-Tu.* Most maleimidyl derivatives react preferentially with the sulfhydryl groups of proteins at neutral pH (Liu, 1977). The specificity of labeling with the fluoresceinmaleimide and CPM obtained under the conditions used here was analyzed by digesting the labeled proteins with trypsin followed by high-pressure liquid chromatography of the resulting tryptic peptides. Only one peak of fluorescence was observed with the digest of EF-Tu that had been labeled with CPM under nondenaturing conditions (Figure 1a). Only one fluorescent peptide was visible after two-dimensional analysis by thin-layer chromatography and subsequent electrophoresis of peptides obtained from exhaustive tryptic digestion (results not shown). Fluorescence in other fractions was near background level, indicating very low reaction with amino groups under the conditions used. The second sulfhydryl group also was labeled when EF-Tu was reacted with CPM in the presence of 8 M guanidine hydrochloride. Two fluorescent peaks were in digests of EF-Tu labeled under these conditions (Figure 1b). Only EF-Tu labeled under nondenaturing conditions was used for the experiments described below.

The excitation and emission spectra of CPM–EF-Tu before and after proteolytic digestion with proteinase K are shown in Figure 2. The relative fluorescence intensity of CPM–EF-Tu decreased during digestion, and a shift in the emission maximum from 470 to 485 was observed. There was no detectable change in the excitation spectrum of CPM–EF-Tu during digestion. The shape of the spectra after protease

Table I: Spectral Properties of Fluorescent EF-Tu Derivatives and Fluorescein-S1

| protein | fluorophore | $A_{max}$ (nm) | $\epsilon(A_{max}) \times 10^{-3}$ | max emission (nm) | $Q$ of labeled protein[a] | |
|---|---|---|---|---|---|---|
| | | | | | free | bound to ribosome |
| EF-Tu | CPM | 397 | 25.0 | 470 | 0.70 | 0.65 |
| EF-Tu | fluorescein | 496 | 60.6 | 520 | 0.38 | 0.37 |
| S1 | fluorescein | 497 | 58.5 | 523 | 0.27 | 0.25 |

[a] Spectral properties of the free proteins were measured in solution A for fluorescent EF-Tu derivatives and in solution B for fluorescein-S1. The quantum yield, $Q$, of CPM-EF-Tu bound to ribosomes was estimated by extrapolation of the values of the fluorescence to the infinite concentration of the ribosomes in the titration experiments (for details see text related to Figure 7). $Q$ of free fluorescein-S1 or reconstituted in ribosomes was measured in solution B. $A$, absorption; $Q$, quantum yield; $\epsilon$, molar extinction coefficient.

Table II: Activity of Unlabeled and Fluorescein-Labeled Protein S1 in Enhancing Poly(U)-Dependent Polyphenylalanine Synthesis[a]

| S1 added | polyphenylalanine synthesized (pmol) |
|---|---|
| none | 234 |
| unlabeled | 620 |
| fluorescein | 543 |

[a] The assay was as described by Odom et al. (1980), except that 30S ribosomal subunits were replaced by 30S subunits deficient in protein S1. The specific activity of the [$^{14}$C]Phe was 5 Ci/mol, corresponding to about 10 cpm/pmol.

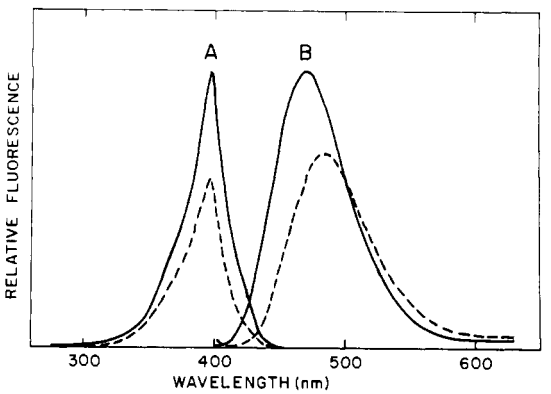


FIGURE 2: Fluorescence excitation and emission spectra of CPM-EF-Tu. The excitation and emission spectra of CPM-EF-Tu were taken before (—) and after (---) digestion. For digestion, 0.33 mg of CPM-EF-Tu in 1.0 mL of solution A was incubated with 24 $\mu$g of proteinase K in a sealed cuvette for 2 h at 25 °C. (A) Excitation spectra ($\lambda_{em}$ = 470 nm); (B) emission spectra ($\lambda_{ex}$ = 397 nm).

digestion were indistinguishable from those of the CPM-Cys adduct. The spectral properties of CPM-EF-Tu are given in Table I.

*Properties of Ribosomal Protein S1 Labeled with Fluoresceinmaleimide Free and Reconstituted into 30S Subunits.* Protein S1 ($M_r$ 61 159; Schnier et al., 1982) contains two cysteine residues which can be labeled with maleimide derivatives under denaturing conditions as described under Methods. The labeling ratio approached two fluorophore residues per S1 molecule, indicating reaction of both sulfhydryl groups. The spectral properties of S1 labeled under denaturing conditions with fluoresceinmaleimide are summarized in Table I. The activity of labeled S1 in enhancing polyphenylalanine synthesis was about 80% that of the unlabeled protein, as shown in Table II. It has, however, been reported (Kolb et al., 1977) that blocking of the sulfhydryl groups of S1 destroys its activity to promote natural messenger RNA binding to 30S subunits.

*Activity of Labeled EF-Tu.* EF-Tu can carry out a number of functions: bind GDP or GTP, form a ternary complex with GTP and aminoacyl-tRNA, promote binding of aminoacyl-tRNA to ribosomes, and catalyze GTP hydrolysis (Kaziro,

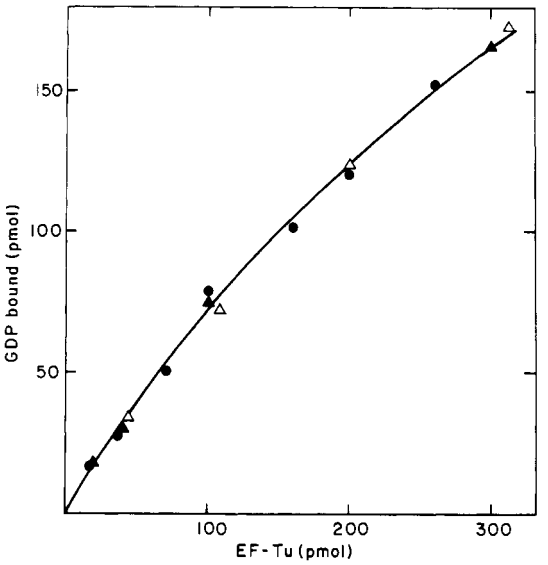


FIGURE 3: Formation of the EF-Tu-GDP complex. Reaction mixtures contained [$^3$H]GDP and indicated amounts of EF-Tu. Unlabeled EF-Tu (●); EF-Tu labeled with CPM (▲) and fluoresceinmaleimide (△). A background value (no EF-Tu), equivalent to 4 pmol of GDP/sample, was subtracted.

1978). Each of these functions can be determined separately as a function of EF-Tu activity. We have used binding of GDP to EF-Tu and EF-Tu-dependent binding of Phe-tRNA to ribosomes to test the activity of EF-Tu used for the studies described below. The dissociation constant of the GDP·EF-Tu complex with the *T. thermophilus* factor is $1.1 \times 10^{-9}$ M (Arai et al., 1978b). Thus, GDP binding may be used as a convenient measure of the activity of a factor preparation. Results for GDP binding to native and labeled EF-Tu are shown in Figure 3.

Activity of the purified factor was about 18 pmol of GDP bound per $\mu$g of protein. Assuming a molecular weight of 50 000 for EF-Tu (Arai et al., 1978a), about 90% of the protein in the EF-Tu preparation is capable of binding GDP. This is in good agreement with previously published data (Arai et al., 1978a). GDP binding activity with EF-Tu labeled with CPM or fluoresceinmaleimide was indistinguishable from that of the unmodified factor. EF-Tu labeled with these probes promoted binding of Phe-tRNA to ribosomes to the same extent as unlabeled factor, as shown in Figure 4. *T. thermophilus* EF-Tu gave about 30% higher activity in this assay system than our preparation of *E. coli* EF-Tu (data not shown).

*Binding of EF-Tu to Ribosomes.* To determine energy transfer from fluorescence intensity measurements, it is desirable to have a high proportion of the energy donor fluorophore paired with probes that serve as the energy acceptor. A correction factor is required to account for unpaired donor. This factor may be difficult to determine reliably and can be a major source of error for distance calculations by this me-

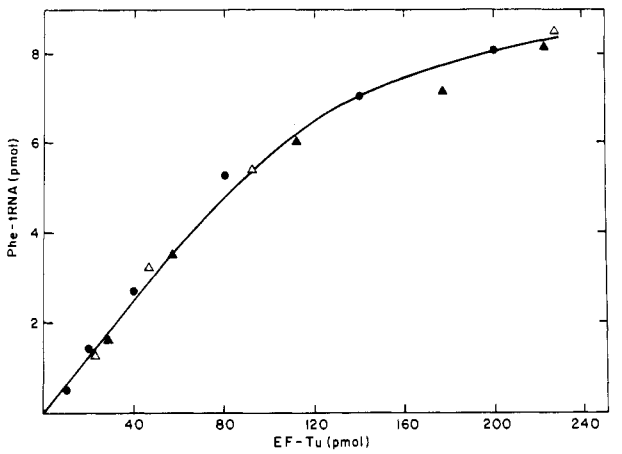


FIGURE 4: EF-Tu-dependent binding of Phe-tRNA to ribosomes. Reaction mixtures containing poly(U), unfractionated *E. coli* tRNA and 70S ribosomes were preincubated to block the ribosomal P site with deacylated tRNA, then a mixture containing [$^{14}$C]Phe-tRNA and various amounts of EF-Tu was added, and the incubation was continued for 20 min as described under Methods. *T. thermophilus* EF-Tu, not labeled (●), labeled with CPM (▲), or labeled with fluoresceinmaleimide (△). A background value equivalent to 1 pmol of Phe-tRNA/sample containing no EF-Tu was subtracted.

thod. The experiments described below, in which CPM–EF-Tu serves as the energy donor for energy transfer to probes on ribosomal components, involve relatively weak binding and rapid dissociation of the EF-Tu·ribosome complexes formed under a variety of conditions. It is not posible to form the EF-Tu·ribosome complex, collect the ribosomes by centrifugation, and then resuspend the sample for measurement of fluorescence, as has been done for tRNA·ribosome or S1·ribosome complexes, without dissociation of EF-Tu. Chemical or photochemical cross-linking of EF-Tu to the ribosomes was not attempted because of the artifacts that might be generated. Two approaches were used to estimate the number of EF-Tu binding sites per ribosome. One method involved measuring binding of labeled EF-Tu to ribosomes at a number of different EF-Tu concentrations and then extrapolating the plotted data to infinite EF-Tu concentration. For these experiments $^{125}$I-labeled CPM–EF-Tu was bound to 70S ribosomes or ribosomal subunits at various concentrations, then the ribosomes were collected by centrifugation, and the radioactivity of the pelleted ribosomes was measured. The results, shown as a Scatchard-type plot in Figure 5, indicate that native 70S ribosomes or a 1 to 1 ratio of ribosomal subunits gave limiting binding of about 0.9 molecule of EF-Tu per ribosome. Similar results were obtained from calculations based on changes in the intensity of fluorescence from CPM–EF-Tu that occurs upon binding of the labeled EF-Tu to ribosomes. Concentration-dependent binding was not observed with either 30S or 50S subunits, although fluorescence quenching was observed with the latter, as described below. Apparent binding similar in magnitude to that observed with 30S subunits was seen if GDP was substituted for GDP-NP or if Phe-tRNA or poly(U) was omitted from the reaction mixture (data not shown). This appears to be nonspecific binding or coprecipitation that is detected by the centrifugation method. The centrifugation method is not useful for fluorescence energy transfer studies because of dissociation of the EF-Tu·ribosome complex after resuspension of the ribosomal pellet. Therefore, another approach was used to determine the optimal GDP-NP or Phe-tRNA concentrations for maximum binding of EF-Tu to ribosomes and for energy transfer between fluorophores. In those experiments the solution of CPM–EF-Tu and ribosomes was titrated with GDP-NP or Phe-tRNA, or CPM–EF-Tu

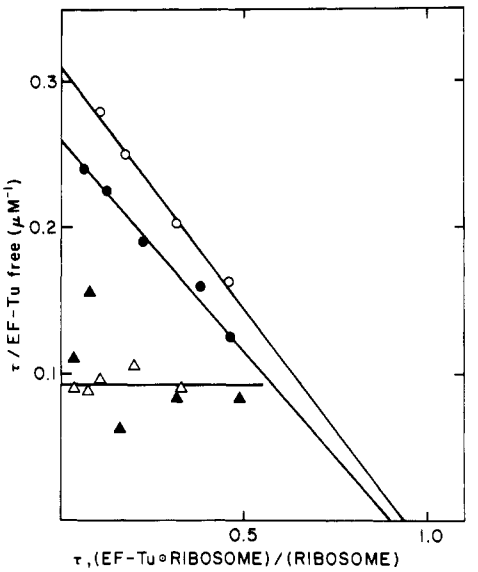


FIGURE 5: Binding of $^{125}$I-labeled CPM–EF-Tu to ribosomes. 70S ribosomes (560 pmol), 30S subunits (1500 pmol), 50S subunits (880 pmol), or an equimolar mixture of 30S and 50S subunits (560 pmol) and a variable amount of $^{125}$I-labeled CPM–EF-Tu (1000 cpm/pmol) were suspended in 50 μL of solution D and then incubated for 20 min at 37 °C. After centrifugation for 2 h at 190000*g*, the supernatant was removed, and the pellet was suspended in solution C. Radioactivity and optical density at 260 nm were measured. 70S (●), mixture of 30S and 50S (○), 30S (△), and 50S (▲). The following amounts of CPM–EF-Tu were found in the pellets at the highest concentration of CPM–EF-Tu (4.3 μM) used: 70S, 71 pmol; 30S plus 50S subunits, 67 pmol; 30S subunits, 135 pmol; 50S subunits, 84 pmol. A blank value equivalent to 2 pmol of $^{125}$I-labeled CPM–EF-Tu found for a sample without ribosomes was subtracted; $\tau$ is the molar ratio of EF-Tu bound to ribosomes (ribosomal subunits) to free ribosomes (ribosomal subunits).

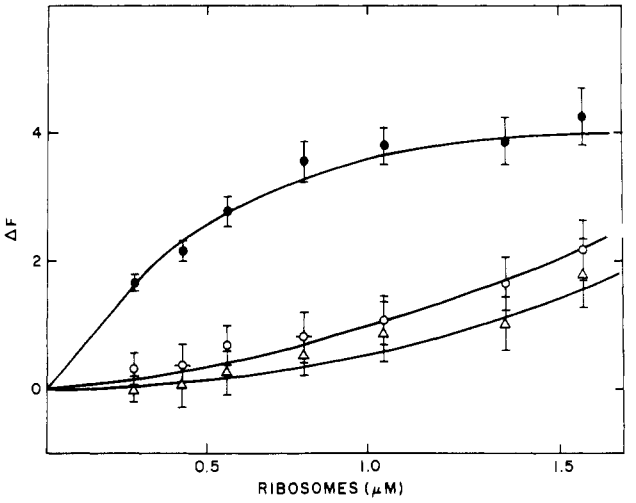


FIGURE 6: Titration of CPM–EF-Tu with unlabeled ribosomes. The difference in fluorescence between samples before and after addition of ribosomes was measured and expressed as percentage of the fluorescence of the unquenched sample ($\Delta F$) vs. concentration of ribosomes. Solution D (140 μL) containing 270 nM CPM–EF-Tu was titrated with 11 μM of ribosomes. The data were corrected for dilution. Complete system (●), Phe-tRNA omitted (○), or GDP-NP omitted (△). Points and error bars represent the average and standard error of four different determinations.

was titrated with ribosomes, and changes of fluorescence were recorded.

EF-Tu binds to the 70S ribosomes with a binding constant of $(3 \pm 1.2) \times 10^6$ M$^{-1}$ in the presence of the complete system as listed in the legend to Figure 6. Binding was greatly reduced if GDP-NP was replaced by GDP or if Phe-tRNA

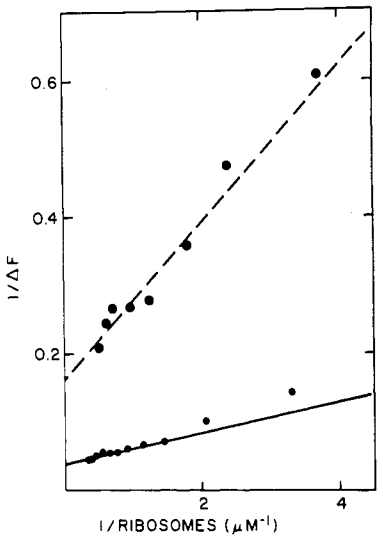


FIGURE 7: Double-reciprocal plot of CPM–EF-Tu titrated with 11 μM unlabeled 70S ribosomes (---) or with equimolar mixture of unlabeled 50S subunits and 30S subunits (8 μM) containing fluorescein-labeled protein S1 and 3.2 μM free fluorescein–S1 (—) in solution D. Points represent the average of four different determinations at each ribosome concentration used. The slope of the two lines, their $y$ intercept corresponding to infinite ribosome concentration, and the standard deviation, $\sigma$, on this intercept were calculated by linear regression analysis of the original data points. Nonradiative energy transfer, $E$, between the two probes is the difference of the reciprocals of the two $y$ intercept values. $E \pm \sigma$ is 0.24 ■ 0.02 for these data.

was omitted from the reaction mixture (Figure 6). Binding of CPM–EF-Tu to 50S subunits was measurable with this system; however, the binding constant was at least 1 order of magnitude lower than with 70S ribosomes ($K_b \simeq 10^5$). Binding to 30S subunits was not detected.

*Energy-Transfer Experiments.* In all experiments CPM–EF-Tu was used as the energy donor. Fluorescein covalently linked at different specific points on the ribosome was the energy acceptor. Since the interaction between ribosomes and EF-Tu is relatively weak, energy transfer was determined by titration of CPM–EF-Tu with ribosomes and then extrapolation of the fluorescence data to infinite ribosome concentration, at which point all the EF-Tu present in the system should exist as a 70S·EF-Tu complex. Two processes contribute to quenching of fluorescence from CPM–EF-Tu during titration with fluorescein-labeled ribosomes. One is caused by changes in the local environment of the CPM fluorophore and does not involve energy transfer to fluorescein which constitutes the second process. The decrease in fluorescence intensity caused by changes in the local environment was determined by titration of CPM–EF-Tu with unlabeled ribosomes. Data from such titrations in the presence of Phe-tRNA and the GTP analogue, GDP-NP, and in the absence of one of the two components are shown in Figure 6. The results indicate that both Phe-tRNA and GDP-NP are required for efficient binding of EF-Tu to ribosomes, as anticipated. Extrapolation of the data indicates about 6% quenching at infinite ribosome concentration.

For determination of energy transfer, the type of experiment represented by Figure 6 was repeated by using fluorescein-labeled ribosomal subunits, and a double-reciprocal plot was constructed of the resulting data. Results for energy transfer from CPM–EF-Tu to fluorescein–S1 are shown in Figure 7. A linear extrapolation to infinite ribosome concentration was made from the approximately linear portion of the titration curve that is generated at high ribosome concentrations.

Table III: Energy Transfer from CPM–EF-Tu to Fluorescein on S1 or Ribosomal RNAs in Ribosomes

| location of fluorescein[a] | polarization (acceptor)[b] | half-height limits of $Q(r'/r)$[c] | $E$ (% quenching) | $r$ (Å)[d] | limits of $r$[e] (Å) |
|---|---|---|---|---|---|
| S1 | 0.03 | 0.95–1.05 | 24 ± 3 | 62 to 68[f] | 59–65<br>65–71 |
| 16S | 0.05 | 0.94–1.06 | 5 ± 2 | 82 | 77–87 |
| 5S | 0.01 | 0.95–1.04 | 11 ± 2 | 70 | 67–74 |
| 23S | 0.09 | 0.93–1.08 | 9 ± 2 | 73 | 68–78 |

[a] S1 was labeled at its cysteine residues. Ribosomal RNAs were labeled at their 3′ ends. [b] Polarization of donor ($\lambda_{excitation}$ = 397 nm, $\lambda_{emission}$ = 470 nm) was 0.2; acceptor, $\lambda_{excitation}$ = 470 nm and $\lambda_{emission}$ = 520 nm. [c] As determined by the full width at half-maximum height of the function $(r'/r)$. $Q(r'/r)$ is the apparent distribution of distances between donor and acceptor pairs estimated from polarization data as described by Haas et al. (1978); $r'$ is the distance between the donor and acceptor pair calculated from $R_0$ for which $\kappa^2 = {}^2/_3$; $r$ is the actual distance between the donor and acceptor pair. [d] $R_0$, the distance for half-maximum energy transfer assuming an orientation factor for dipole–dipole transfer $\kappa^2 = {}^2/_3$, was equal to 50 Å for all pairs used. [e] Limits of $r$ were calculated as previously described (Odom et al., 1980) by dividing the values of $r$ by the half-height limits of $Q(r'/r)$. [f] Limits for the distance to the closest fluorescein acceptor calculated as described in the text.

Energy transfer is taken as the difference in the change in fluorescence at infinite ribosome concentration for labeled and unlabeled ribosomes. The standard error for energy transfer was calculated by a linear regression analysis of the two sets of data. A similar analysis was carried out for energy-transfer experiments involving ribosomes reconstituted with a ribosomal RNA labeled with fluorescein at its 3′ end. The results are presented in Table III. Limits of the distances between probes shown in Table III have been estimated from fluorescence polarization data by the method of Haas et al. (1978) and represent the uncertainty of the value of ${}^2/_3$ for the orientation factor $\kappa$, which assumes random orientation of the probes.

Interpretation of energy transfer to S1 is complicated by the presence of a fluorescein residue on each of its two cysteine residues. Energy transfer between probes attached to these two cysteine residues indicates that they are less than 25 Å apart (unpublished data). The $R_0$ value for the CPM–fluorescein pair is 50 Å. The efficiency of energy transfer to either of the two fluorescein acceptors will depend upon their position relative to the donor. One limiting situation will be given if the two acceptors are equally distant from the donor. The equation representing this situation can be derived as follows: Let $E_1$ be the efficiency of energy transfer which would occur in the absence of the other acceptor. $E_2$ will be the overall efficiency of energy transfer in the presence of both acceptors. Then in the presence of one acceptor

$$E_1 = k_t/(k_t + k_s)$$

or

$$k_s = (1 - E_1)k_t/E_1$$

where $k_t$ is the rate constant for transfer to one acceptor and $k_s$ is the sum of all of the other rate constants.

In the presence of both acceptors the rate constant for transfer would be twice the rate constant for transfer to one acceptor or $2k_t$; therefore

$$E_2 = \frac{2k_t}{2k_t + k_s} = \frac{2k_t}{2k_t + (1 - E_1)k_t/E_1} = \frac{2E_1}{E_1 + 1}$$

$$r = R_0(1/E_1 - 1)^{1/6}$$

thus

$$r = R_0(2/E_2 - 2)^{1/6}$$

and $r = 1.36R_0 = 68$ Å.

The other limiting situation will be generated when the two acceptors are at maximum and minimum distances from the donor; i.e., a straight line can be drawn through the positions of the three probes. In this situation the distance from the donor to each of the two probes differs by up to 25 Å, and the distance from the donor to the closest acceptor, $r$, can be calculated from

$$\frac{1}{E_2} = 1 + \frac{r^6(r + 25)^6}{R_0^6[r^6 + (r + 25)^6]}$$

In this case $r \geq 62$ Å, and the distance to the furthest fluorescein acceptor is $\leq 87$ Å. The limiting distances to the closest acceptor are given for S1 in Table III.

*Error due to S1 Dissociation.* Early studies of ribosomal proteins demonstrated that S1 was present in less than stoichiometric amounts on 30S ribosomal subunits prepared in several ways. Values as low as 0.1–0.5 copy per subunit were observed (Traut et al., 1969; Voynow & Kurland, 1971; Weber, 1972). One explanation of these findings was that S1 is not tightly bound and is partially lost during subunit isolation. This hypothesis was supported by the observation that S1 bound to 30S subunits was readily exchangeable with S1 free in solution (Laughrea & Moore, 1977; Subramanian & Van Duin, 1977; Robertson et al., 1977). Sillers & Moore (1981) found an apparent dissociation constant of 0.15 μM for the S1·30S complex and used dimethyl adipimidate to cross-link S1 to 30S subunits for neutron-scattering experiments.

Fluorescein–S1 serves as the energy acceptor in the experiments reported here, and the observed energy transfer will be decreased by a factor that is directly proportional to the ribosomes that lack S1. Dissociation of fluorescein–S1 from 30S subunits in the presence of 50S subunits and poly(U) was analyzed by the following experiment. An equimolar amount of 50S subunits was added to fluorescein–S1·30S subunits to obtain the final concentration of 2.2 μM in solution D, and then the solution was centrifuged for 30 min at 150000$g$ at room temperature in an air-driven ultracentrifuge (Airfuge, Beckman). In the presence of ribosomes 13% of the labeled S1 was found in the supernatant, as measured by comparing fluorescence of the solution before and after centrifugation. In a parallel control lacking ribosomes, more than 95% of S1 was found in the supernatant. The value of 13% corresponds to an apparent dissociation constant of less than 40 nM. This value is about one-fourth of that observed for S1 dissociation from 30S subunits in the absence of 50S and poly(U) (Sillers & Moore, 1981).

The results obtained in the experiment described above may be subject to considerable error, but it appears safe to assume that the dissociation constant in the presence of poly(U) and 50S subunits is below that of the S1·30S complex, 0.15 μM, which we take as an upper limit to estimate the decimal fraction of ribosomes that lack S1 under the conditions used for energy transfer from the experiments represented by Figure 7.

When this dissociation constant is assumed, about 20% of the ribosomes would lack S1 at a ribosome concentration of 1 μM, corresponding to a corrected energy transfer of 30% rather than the 24% as given in Table III. Note that the experiments were carried out with a 40% molar excess of S1 to ribosomes. Energy transfer of 30% rather than 24% would correspond to a decrease in the calculated distances for S1 given in Table III of about 3 Å. However, most of the data points of Figure 7 were taken at ribosome concentrations greater than 1 μM, and the energy-transfer value used for the distance calculation was obtained by extrapolation of these data to infinite ribosome concentration. At this point there should be no ribosomes that lack S1. On this basis we conclude that dissociation of S1 from the ribosome complex does not generate a significant error in the calculated distances involving S1.

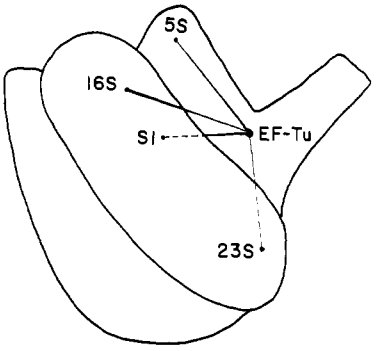


FIGURE 8: Schematic model of the *E. coli* ribosome showing the distances between CPM on EF-Tu and fluorescein on S1 or the 3′ ends of the ribosomal RNAs.

## Discussion

The remarkable thermal stability of EF-Tu from *Thermus thermophilus* appears to be an important factor in recommending its use rather than EF-Tu from *E. coli* for many types of studies. We have not detected any indication that the factors from the two organisms do not function in an equivalent manner with *E. coli* ribosomes. Indeed, in our hands the heat-stable factor consistently has given equal or higher enzymatic activity than the *E. coli* factor. We attribute this difference to inactive factor in preparations from *E. coli*. The data of Figure 3 indicate that about 90% of the protein in our EF-Tu preparations from the thermophile is active as judged by GDP binding. However, the errors inherent in determining protein concentration and the specific activity of GDP dictate caution in making a rigorous interpretation of this value.

The binding constant for association of EF-Tu from *T. thermophilus* with *E. coli* ribosomes, determined by changes in fluorescence under the conditions described above, was determined to be $3 \times 10^6$ $M^{-1}$. This appears to be in reasonable agreement with the corresponding value, $6.6 \times 10^6$ $M^{-1}$, for *E. coli* EF-Tu that was determined by Holschuh et al. (1981) by using a velocity sedimentation technique and different reaction conditions. The binding constant for 50S subunits only was about an order of magnitude lower than for 70S ribosomes. Binding to 30S subunits could not be detected.

Evidence has been presented that indicates that EF-G and EF-Tu binding sites on ribosomes overlap each other [for review see Matheson et al. (1980)]. EF-G binds to the 50S subunit close to the L7/L12 stalk, as determined by immunoelectron microscopy (Girshovich et al., 1981). EF-Tu can be cross-linked to proteins most of which are located close to the L7/L12 stalk, i.e., L5, L15, L20, L23, and L33 (San José et al., 1976; Fabian, 1976). No specific cross-linking to 30S proteins was observed (San José et al., 1976); however, the difference in the binding constants for 50S subunits and 70S ribosomes indicates that the 30S subunit must have some effect on the EF-Tu binding site in the intact ribosome. This conclusion appears to be consistent with the observations that EF-Tu bound to 70S ribosomes increases the accessibility of S7 and S10 for iodination and decreases that of S21 (Martinez & Modolel, 1979).

A schematic model (Figure 8) showing the positions of the probes attached to EF-Tu, S1, and the 3′ ends of ribosomal RNAs was generated from the energy-transfer data presented above. The approximate positions of the 3′ ends of the ribosomal RNAs are as located by immunoelectron microscopy (Stöffler-Meilicke et al., 1981).

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