Kinetic Studies on the Role of Elongation Factors 1β and 1γ in Protein Synthesis*

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An equilibrium isotope exchange technique was used to measure in an Artemia system the catalytic influence of elongation factor (EF) 1βγ on the dissociation of GDP from the complex of elongation factor 1α-[3H]GDP in the presence of an excess of free GDP. The kinetic data demonstrate that, in analogy to prokaryotes, dissociation of GDP occurs via the formation of a transient ternary complex of EF-1α-GDP-EF-1βγ. The rate constants for the dissociation of GDP from EF-1α-GDP and from the ternary complex EF-1α-GDP-EF-1βγ were found to be 0.7 × 10⁻⁵ and ≥0.7 s⁻¹, respectively. The equilibrium association constants of GDP to EF-1α-EF-1βγ and of EF-1βγ to EF-1α-GDP were found to be 2.3 × 10⁸ and 4.2 × 10⁶ M⁻¹, respectively. Judged from the known elongation rate in vivo and kinetic constants of nucleotide exchange, it was estimated that the recycling of EF-1α may be a rate-controlling step in eucaryotic translation.

As a model for GTP exchange, the formation of the ternary EF-1α-guanylyl (βγ-methylene)diphosphonate-EF-1βγ complex was also studied. It was observed that both an increase of the level of aminoacyl-tRNA and of temperature favored the dissociation of this complex, thereby enabling EF-1βγ to recycle as a catalyst. This behavior would explain the frequent occurrence of a heavy form of elongation factor 1 in extracts of the eucaryotic cell.

The eucaryotic elongation factor (EF) 1α and its complement EF-1βγ participate in the polypeptide elongation cycle of protein synthesis. The two factors have been purified from various eucaryotic sources and are considered to be functionally analogous to bacterial EF-Tu and EF-Ts, respectively (1-14). In analogy to bacterial EF-Tu, eucaryotic EF-1α promotes the binding of aminoacyl-tRNA (aa-tRNA) to ribosomes under hydrolysis of GTP. The sequence of reactions involved in this cycle can be schematically represented as follows (15).

\[
\text{EF-1α.GTP + aa-tRNA} \rightleftharpoons \text{EF-1α.GTP.aa-tRNA} \quad (1)
\]

\[
\text{EF-1α.GTP.aa-tRNA + ribosome} \rightleftharpoons \text{ribosome.aa-tRNA + EF-1α.GDP + P}_1 \quad (2)
\]

In the past, several models have been proposed to explain the stimulatory activity of EF-1βγ on the binding of aminoacyl-tRNA to ribosomes (1-6, 16, 17). At present, it is generally accepted that the stimulatory activity of EF-1βγ is due to an accelerated exchange of GDP bound to EF-1α with exogenous GTP (1-14, 18-20).

\[
\text{EF-1α.GDP + GTP} \rightleftharpoons \text{EF-1α.GTP + GDP} \quad (3)
\]

The guanine nucleotide exchange factor EF-1βγ and its constituent polypeptides, EF-1β and EF-1γ, have been purified from pig liver (3), silk gland (5), Artemia (7, 14), Krebs II mouse ascites tumor cells (8), chick brain (10), wheat germ (11), rabbit reticulocytes (12, 19), and calf brain (13). Earlier attempts to isolate a stimulatory exchange factor from yeast extracts were unsuccessful (21); but recently, the isolation of EF-1βγ from yeast has been achieved (22). Therefore, the conclusion can be drawn that this factor is universally present in eucaryotes. Although the stimulatory effect of EF-1βγ on the recycling of EF-1α had been detected first more than 10 years ago (1-6), little is known about the details of the reaction. In prokaryotes, it has been found that the exchange of GDP occurs via a ternary EF-Tu.GDP.EF-Ts complex and that the dissociation of GDP from the ternary complex seems not the rate-limiting process in procaryotic protein synthesis (23, 24). In order to determine what the situation is in eucaryotes, we initiated a similar type of study in Artemia using an equilibrium isotope exchange technique as described by Chau et al. (23) for Escherichia coli. Although we had EF-1βγ at our disposal, we mostly used EF-1βγ for the studies presented here. The reason is that, in many cellular extracts (1-14), the two subunits are tightly associated to each other so that there are reasons to believe that, in the cell, the factor occurs as a functional complex of EF-1βγ. For an up-to-date survey of the preparation and general properties of EF-1βγ of Artemia and especially the affinity of EF-1γ toward membrane and cytoskeleton structures of the cell, see Ref. 14.

EXPERIMENTAL PROCEDURES

Chemicals—The radiolabeled compound [8-³²P]GDP (10 Ci/mmol) was purchased from the Radiochemical Centre (Amersham, England). GDP, GTP, guanylyl (βγ-methylene)diphosphonate, and pure phenylalanine-specific transfer ribonucleic acid (tRNA₆₅₅₅) from yeast were obtained from Boehringer-Mannheim.

Elongation Factors—EF-1α from Artemia cysts was prepared as described (4). EF-1βγ was isolated from Artemia cysts by a simplified method (14). Briefly, postribosomal supernatant prepared as described (4) was fractionated with ammonium sulfate. EF-1βγ was isolated from Artemia cysts by a simplified method (14). Briefly, postribosomal supernatant prepared as described (4) was fractionated with ammonium sulfate. EF-1βγ was isolated from Artemia cysts by a simplified method (14). Briefly, postribosomal supernatant prepared as described (4) was fractionated with ammonium sulfate. EF-1βγ was isolated from Artemia cysts by a simplified method (14). Briefly, postribosomal supernatant prepared as described (4) was fractionated with ammonium sulfate. EF-1βγ was isolated from Artemia cysts by a simplified method (14). Briefly, postribosomal supernatant prepared as described (4) was fractionated with ammonium sulfate.

The amount and nature of nucleotide bound to EF-1α were determined by HPLC (25). 150 µg of EF-1α in 1 ml of sodium phosphate.
buffer (pH 2.8) were incubated for 10 min at 20 °C and analyzed on a polyethyleneimine column (4.6 × 250 mm; J. T. Baker Chemical Co.). Besides some background, a single peak eluted from the column. The elution time was 20 min, corresponding to that of GDP, GTP, GMP, and GMP-PCP eluted at 29, 15, and 25 min, respectively. The absorption spectrum of the material eluting in this peak was measured with the aid of a Beckman Acta MVI spectrophotometer and showed a shoulder at about 270 nm, characteristic for guanine nucleotides. EF-la isolated from Artemia salina cysts contained usually a GDP mole fraction of 0.9 ± 0.1 and is designated for this reason as GDP complex.

Equilibrium Isootope Exchange—EF-la. [3H]GDP complex was prepared as described elsewhere (14). Briefly, EF-la. GDP (16 μg) was incubated for 5 min at 37 °C with [3H]GDP (4 μM, 500 Ci/mol) in a 96-μl reaction mixture containing 40 mM Tris-HCl (pH 7.5), 0.2 mM dithiothreitol, 10 mM magnesium acetate, 100 mM NH₄Cl, 1 mg/ml bovine serum albumin, and 25% (v/v) glycerol. 80 μl of this complex were diluted with 840 μl of exchange buffer (20 mM Tris-HCl (pH 7.5), 10 mM magnesium acetate, 50 mM NH₄Cl, and 10% (v/v) glycerol). The reaction was initiated by addition of 160 μl of exchange buffer containing nucleotide and EF-la. GTP. At specified times, 110-μl aliquots were withdrawn from the reaction mixture. The reaction was immediately stopped by rapid filtration onto 0.45-pm nitrocellulose filters (Schleicher & Schüll). The filters were washed and counted as described previously (14). All reactions were performed in triplicate and at 0 °C (unless otherwise noted). The reaction mixture contained 300 nM EF-la. [3H]GDP and 140 μM GDP unless otherwise indicated.

The incorporation of [3H]GDP into EF-la. GDP was measured as described above, except that the complex formation reaction was omitted and that the exchange reaction was performed with 5 nM [3H]GDP (8.5 Ci/nmol). The data obtained from the equilibrium isotope exchange experiments were fit to a single exponential function using a least-squares fitting procedure as described for E. coli (23).

Miscellaneous Methods—Determination of protein concentration was performed as described (26).

The purity of nucleotides was examined by HPLC (25). The following mole fractions of contaminating nucleotide were detected: 0.06 GDP in GTP and 0.01 GTP in GDP. [3H]GDP was free of [3H]GTP, and GMP-PCP was free of GDP and GTP (detection limit was 0.001).

Yeast tRNA<sup>Met</sup> was aminoacylated with phenylalanine using E. coli charging enzymes (27). The degree of aminoacylation was monitored by amino acid analysis after hydrolysis for 10 min at 20 °C in 0.5 M NaOH.

RESULTS

In this paper, we present a kinetic study on the interactions among EF-la, EF-la. GMP, and nucleotides using two different approaches. First, we used the equilibrium isotope exchange approach to demonstrate the occurrence of the ternary EF-la-GDP-EF-la. GMP complex. The catalytic exchange of GDP was performed at 0 °C in the presence of GDP once a certain percentage of EF-la had lost its bound GDP. It looked as if EF-la. GMP-PCP-EF-la. GMP was free of GDP and GTP (detection limit was 0.001). This "end-product inhibition" is observable because EF-la. GMP is present in relatively small amounts at the beginning of the reaction.

As mentioned under "Experimental Procedures," EF-la isolated from Artemia contains a singly bound GDP. Therefore, EF-la. [3H]GDP was prepared by incubation of EF-la. GDP with an equivalent amount of [3H]GDP, the amount of label incorporated in the protein, as expected for complete exchange of the nucleotide. Thus, the amount of cold GDP bound and its full exchange capacity for [3H]GDP argue for a high percentage of active EF-la. In nearly all experiments, this complex was used as initial material.

Fig. 1 shows the time course of the exchange of [3H]GDP bound to EF-la with GDP free in solution and the influence of catalytic amounts of EF-la in the rate of dissociation. It is clear that the exchange rate increases markedly in the presence of EF-la. GTP. From Fig. 2 it can be seen that semilogarithmic representation of the data results in a straight line. Thus, the exchange reaction can be described by a single exponential function, indicating that the reaction follows first-order kinetics. Comparison of the two figures (0 versus 21 °C) reveals the temperature dependence of the exchange reaction because all other conditions were the same. In the presence of EF-la, the reaction was also temperature-dependent and followed first-order kinetics, too.

In order to obtain more information on the kinetics of the EF-la-mediated exchange, the reaction was carried out with various concentrations of EF-la and EF-la-GMP. As seen from Fig. 3, the observed first-order rate constant (k<sub>obs</sub>) is linearly dependent on the total concentration of EF-la. GTP. This catalytic behavior of EF-la can best be explained by assuming that EF-la interacts with EF-la-GTP to form the ternary EF-la-GTP-EF-la complex and that the dissociation of [3H]GDP from the ternary EF-la complex is much faster than the dissociation of [H]GDP from the binary EF-la-GMP complex.

The data obtained from the equilibrium isotope exchange experiments were fit to a single exponential function using a least-squares fitting procedure as described for E. coli (23).

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RESULTS

In this paper, we present a kinetic study on the interactions among EF-la, EF-la. GMP, and nucleotides using two different approaches. First, we used the equilibrium isotope exchange approach to demonstrate the occurrence of the ternary EF-la-GDP-EF-la. GMP complex. The catalytic effect of EF-la in the release of [3H]GDP from EF-la. [3H]GDP in the presence of a large excess of unlabeled GDP, and the calculation of the kinetic parameters describing this EF-la-mediated exchange reaction. These experiments were performed as described for the bacterial system (23). Second, we observed a drastic change in the rate of EF-la-dependent release of GDP from EF-la. GDP in the presence of GMP-PCP once a certain percentage of EF-la had lost its bound GDP. It looked as if EF-la became inactivated due to the formation of a stable complex of EF-la-GMP-PCP-EF-la. This "end-product inhibition" is observable because EF-la is present in relatively small amounts at the beginning of the reaction.

As mentioned under "Experimental Procedures," EF-la isolated from Artemia contains a singly bound GDP. Therefore, EF-la. [3H]GDP was prepared by incubation of EF-la. GDP with an equivalent amount of [3H]GDP, the amount of label incorporated in the protein, as expected for complete exchange of the nucleotide. Thus, the amount of cold GDP bound and its full exchange capacity for [3H]GDP argue for
1α-[3H]GDP complex. The simplest model for the mechanism of the exchange reaction is that first described by Chau et al. (23) in the case of bacterial elongation factor Tu:

\[
EF-1α·[3H]GDP \overset{k_1}{\underset{k_3}{\rightleftharpoons}} EF-1α + [3H]GDP
\]

in the absence of EF-1βγ, and:

\[
EF-1α·[3H]GDP + EF-1βγ \overset{k_2}{\underset{k_3}{\rightleftharpoons}} EF-1α·[3H]GDP·EF-1βγ \quad K'_1
\]

\[
EF-1α·[3H]GDP·EF-1βγ \overset{k_4}{\underset{k_5}{\rightleftharpoons}} EF-1α·EF-1βγ + [3H]GDP \quad K'_2
\]

\[
EF-1α·EF-1βγ + GDP \overset{k_6}{\underset{k_1}{\rightleftharpoons}} EF-1α·GDP·EF-1βγ \quad (6a)
\]

\[
EF-1α·GDP·EF-1βγ \overset{k_7}{\underset{k_8}{\rightleftharpoons}} EF-1α·GDP + EF-1βγ \quad (5a)
\]

in the presence of EF-1βγ. K'_1 and K'_2 refer to the association constants of the respective equilibrium Equations 5 and 6. Since the amount of free GDP is in large excess with respect to bound [3H]GDP, reincorporation of released [3H]GDP is negligible. Therefore, k_1, the first-order rate constant in the absence of EF-1βγ, is given by:

\[
\log \frac{[EF-1α·[3H]GDP]}{[EF-1α·[3H]GDP]_t} = k_1 (t_2 - t_1)
\]

where \([EF-1α·[3H]GDP]\), and \([EF-1α·[3H]GDP]_t\), represent the concentrations of EF-1α·[3H]GDP at any time t_1 and t_2, respectively. The observed first-order rate constant (k_{obs}) of exchange in the presence of EF-1βγ is dependent on the total concentrations of EF-1α·GDP, EF-1βγ, and GDP and is given by (see Footnote 3):

For the derivation of Equation 8, the original publication of Chau et al. (23) should be consulted; the same applies for the use of Equations 9-11, which were also derived earlier for the case of procaryotic EF-Tu and EF-Ts.
very abrupt decline of the rate of nucleotide exchange reflects a fast formation of an aggregate composed of EF-la-GMP-PCP. EF-1P-y molecules once the product of the concentration change reactions in the presence and absence of EF-1P-y. It is by EF-1B-y in the GMP-PCP system. Two aspects of the reaction the rate was comparable to that obtained in the absence of exogenous nucleotide. To our surprise, difficulties arose in using GTP, we initiated a series of experiments with GTP as the y-subunit has a positive effect on the exchange rate, EF-1P-y having by itself no measurable exchange activity.

Since during protein synthesis EF-1P-y exchanges GDP for GTP, we initiated a series of experiments with GTP as exogenous nucleotide. To our surprise, difficulties arose in obtaining reproducible time courses for this system. About one-third of the time courses showed an abrupt change in the exponential decay. Another one-third showed, after an abrupt decrease, a distinct hill, something unexplained so far. Finally, the remaining curves showed no change of the apparent reaction rate at all (results not shown). In all cases, the initial exchange rate was close to that of the GDP system. Under the same conditions used in this system, \( k_{on} \) was found to be \( 5.6 \times 10^{-3} \) s\(^{-1} \) in the presence of EF-1P or EF-1P-y.

To avoid the problems encountered with GTP, we preferred to use the nonhydrolyzable GTP analogue GMP-PCP. Fig. 7 shows a semilogarithmic plot of the data obtained from exchange reactions in the presence and absence of EF-1P-y. It is seen that almost 90 s an abrupt change in exchange rate occurs, revealing that the overall reaction does not follow simple first-order kinetics. On closer inspection, however, the initial rate follows first-order kinetics and is comparable to those measured without EF-1P-y. The rate constants of the reaction rate at all (results not shown). In all cases, the initial exchange rate was close to that of the GDP system. Under the same conditions used in this system, \( k_{on} \) was found to be \( 5.6 \times 10^{-3} \) s\(^{-1} \) in the presence of EF-1P or EF-1P-y.

For pig liver EF-1H (a heavy form of EF-1), it has been reported (28) that in the presence of GTP, dissociation of EF-1H into EF-1α and EF-1βγ is stimulated by higher temperatures. If the formation of a stable complex of the type EF-1H as seen in pig liver is responsible for the elimination of EF-1βγ from the reaction mixture, then one would expect, given the functional analogy of GTP with GMP-PCP, a temperature dependence of the bend of Fig. 7. As seen from Table 1, an increase of temperature shifts the bend toward a greater percentage of \([\text{H}]\text{GDP} \) released from EF-1H. The rate constants in the second part of the [\( \text{H} \)]GDP release were again comparable with those measured without EF-1βγ. Thus, the resulf suggests that formation of EF-1α-GMP-PCP-EF-1βγ occurs and that, in analogy with pig liver EF-1H, low temperatures favor stabilization of the complex. Using the data of Table 1, extrapolation from 0 to 27 °C gives GTP dissociation rate constants of \( k_1 = 14 \) s\(^{-1} \) and \( k_2 = 14 \times 10^{-3} \) s\(^{-1} \) at the optimal living temperature of the shrimp.

In the cell, aminoacyl-tRNA forms with EF-1α-GTP the EF-1α-GTP-aminoacyl-tRNA complex (15), which is ultimately the product of the concentration

\[ \text{EF-1α-GMP-PCP} + \text{EF-1βγ} \rightarrow \text{EF-1α-GMP-PCP-EF-1βγ} \]

(12)
mately the substrate for polypeptide elongation. As a consequence, EF-1α-GMP-PCP-EF-1βγ should be prevented from being formed when aminoacyl-tRNA is included in the reaction mixture. In the presence of Phe-tRNA (90 μM), the bend in the curve of Fig. 7 disappeared and the reaction followed again first-order kinetics (results not shown). In the presence of Phe-tRNA, $k_{\text{obs}}$ was found to be $5.1 \times 10^{-3}$ s$^{-1}$ (21°C). Presumably, the concentration used, Phe-tRNA drives the reactions toward formation of the ternary EF-1α-GMP-PCP-Phe-tRNA complex, thus preventing depletion of free EF-1βγ as a catalyst. This experiment therefore also offers a good explanation for the occurrence of EF-1H complexes in the cell under conditions where low concentrations of free aminoacyl-tRNA are present.

**DISCUSSION**

Our results demonstrate the strong coupling between the nucleotide binding part (EF-1α) and the nucleotide exchange part (EF-1βγ) of the polypeptide elongation factor 1 (EF-1αβγ) of *Artemia*. Especially the association between EF-1α-GMP-PCP and EF-1βγ seems strong when compared to the association of EF-1α-GDP and EF-1βγ. Given the functional analogy of GMP-PCP with GTP, formation of EF-1α-GMP-PCP-EF-1βγ may relate to the frequent occurrence of EF-1H (EF-1αβγ) complexes in animal cells (29). The most important feature of the GDP dissociation reaction is that EF-1βγ catalyzes the exchange of $[^{3}H]GDP$ bound to EF-1α with free GDP via the formation of a transient ternary complex of EF-1α-[^3H]GDP-EF-1βγ. This reaction follows first-order kinetics and reveals that the GDP dissociation is consistent with the proposed model as expressed in Equations 5 and 6. Although this type of catalysis has been shown to be valid for bacterial EF-Tu-GDP and EF-Tu (23, 24), our report is, to the best of our knowledge, the first one to describe the kinetics for a eucaryotic elongation factor in detail. Most important is that the ternary complex EF-1α.GDP-EF-1βγ releases GDP with a catalytic rate constant of at least $k_1 = 14 \times 10^{-3}$ s$^{-1}$ at 27°C; whereas in the absence of EF-1βγ, the loss of GDP from EF-1α.GDP occurs with a rate constant of only $k_1 = 14 \times 10^{-3}$ s$^{-1}$ at 27°C.

For an apprehension of the possible physiological significance of EF-1βγ as a necessary regulatory element of the elongation step in *vivo*, it is useful to estimate whether in the cell the rate of conversion of EF-1α.GDP into EF-1α.GTP represents actually a rate-limiting step in elongation. For this purpose, we compare the rate of elongation of eucaryotic protein synthesis with the recycling rate of EF-1α as determined in vitro with and without EF-1βγ. If comparable, changes in exchange rates may cause changes in rates of elongation.

For rabbit reticulocytes, an elongation rate of 2.5 amino acids/s/ribosome has been reported in *vivo* (30). The molar ratio of EF-1α to ribosomes has been found to be on average 25, at least in a reticulocyte system (31). At a 25-fold excess of EF-1α compared to ribosomes, the in *vivo* rate of formation of the EF-1α-GTP-EF-1βγ-aminacyl-tRNA from EF-1α-GDP should be at least $5%$ as fast as the elongation rate (cf. Ref. 23). This means that EF-1α-GDP should lose its GDP with a rate of at least $2.5/25 = 0.1$ s$^{-1}$ to keep up with elongation. With regard to how much of EF-1α in the cell is subject to recycling via EF-1βγ at a particular time, it is estimated that EF-1βγ is present in a molar ratio to EF-1α of roughly only 0.1 (31). For this reason, only one-tenth of the total amount of EF-1α can, at the most, complex to EF-1βγ. Furthermore, there are two types of complexes, EF-1α-GDP-EF-1βγ and EF-1α-GTP-EF-1βγ, the ratio being determined by their respective equilibrium association constants and the internal cellular ratio of GDP and GTP. We have strong indications that EF-1α-GMP-PCP has a higher affinity to EF-1βγ than EF-1α-GDP (Fig. 7). A similar trend is reported for the procaryote *E. coli* (24). On the assumption that only 1% of the total cellular EF-1α is involved in a ternary complex of EF-1α-GDP-EF-1βγ, a rate of 100 $×$ 0.1 s$^{-1}$ = 10 s$^{-1}$ would still be consistent to accelerate the GDP dissociation from EF-1α.GDP-EF-1βγ to within the range of EF-1H elongation. As it is, on the other hand, a requirement for EF-1βγ in *vivo* is strongly indicated because otherwise it is hard to see how the nucleotide exchange rate would even approximate that of the elongation.

We have obtained evidence that EF-1βγ also accelerates the release of GDP from EF-1α.GDP in the presence of exogenous GTP. For unknown reasons, this exchange was easier to study in the presence of the nonhydrolyzable GTP analogue GMP-PCP. Especially at low temperatures, it appeared that EF-1α-GMP-PCP forms a tight complex with EF-1βγ, thus slowing down further exchange of GDP from the residual complex of EF-1α-GDP. Formation of this complex was confirmed by the observation that aminoacyl-tRNA could prevent EF-1α-GMP-PCP from forming such a putative complex with EF-1βγ. A similar effect of aminoacyl-tRNA was first observed by Romero et al. (24) studying bacterial EF-Tu. The shift with temperature of the sharp bend in the curve of the exchange reaction of EF-1α-[^3H]GDP against GMP-PCP toward larger degrees of [^3H]GDP exchange and the effect of Phe-tRNA agrees with other studies in which one has observed that the dissociation of EF-1αβγ into EF-1α and EF-1βγ in the presence of GTP increased with increase of temperature (18, 28) or addition of aminoacyl-tRNA (28).

Notwithstanding the limitations of the calculation of the in *vivo* GDP dissociation rate, it appears that EF-1βγ brings the dissociation rate of GDP from EF-1α.GDP to a level of the in *vivo* rate required for elongation. In our opinion, this makes EF-1βγ an interesting candidate for modulating elongation rates in *vivo* and possibly a factor which influences error rates because it is involved in aminoacyl-tRNA binding. In this context, it is also interesting that, although EF-1γ is inert by itself, it does in combination with EF-1β enhance the rate of nucleotide exchange compared to EF-1β alone (Fig. 6), something expected if EF-1γ somehow regulates the function of EF-1β in the cell. Moreover, we have found that purified EF-1βγ behaves as a protein kinase (cf. Ref. 32) of the type casein kinase II. In fact, the β-chain is always phosphorylated at a specific serine residue, and the propensity to phosphorylate EF-1βγ resides clearly in our EF-1γ preparations and not in the EF-1β chain alone. The role and precise origin of the β-phosphorylation are presently under study.

In contrast to bacterial EF-Ts, a need for EF-1βγ in eucaryotic protein synthesis has been questioned seriously (33). It
is true that in the bacterial system, GDP is much more tightly bound to EF-Tu than GTP so that a mechanism must ensure that EF-Tu-GTP is regenerated once the GTP has been hydrolyzed on the ribosome. In the case of the eucaryotic system, however, the protein possesses a comparable affinity to both nucleotides, namely $K_d$ (EF-1a·GTP) = $8.5 \times 10^{-7}$ M and $K_d$ (EF-1a·GDP) = $5.3 \times 10^{-7}$ M (34), whereas $K_d$ (EF-Tu·GTP) = $3 \times 10^{-7}$ M and $K_d$ (EF-Tu·GDP) = $8 \times 10^{-8}$ M (35). Provided the ratio of GTP to GDP is high in the cell, the equilibrium would be driven in the direction of EF-1a·GTP even without EF-1P-y. However, this fact tells nothing about a requirement of EF-1P-y to accelerate protein synthesis when they want to understand mRNA-specific effects which are not accounted for by simple modulations of the rate of transcription. The broad resemblance in function between EF-1a and eucaryotic initiation factor 2 on one hand and between EF-1β and the guanine nucleotide exchange factor on the other (39) makes it unlikely that regulatory processes would be confined only to steps in initiation. The most ancient form of regulation of translation of biopolymers may have been started at the level of elongation, the principle of nucleotide exchange as a regulator of catalytic action being preserved later on in other systems like initiation factors and G-proteins (39).

REFERENCES