Mg\(^{2+}\) and a Key Lysine Modulate Exchange Activity of Eukaryotic Translation Elongation Factor 1β*

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To sustain efficient translation, eukaryotic elongation factor 1β (eEF1β) functions as the guanine nucleotide exchange factor for eEF1A. Stopped-flow kinetics using 2'-O-methylanthraniloyl (mant)-GDP showed spontaneous release of nucleotide from eEF1A is extremely slow and accelerated 700-fold by eEF1β. The eEF1β-stimulated reaction was inhibited by Mg\(^{2+}\) with a K\(_{\text{M}}\) of 3.8 mM. Previous structural studies predicted the Lys-205 residue of eEF1β plays an important role in promoting nucleotide exchange by disrupting the Mg\(^{2+}\) binding site. Co-crystal structures of the lethal K205A mutant in the catalytic C terminus of eEF1β with eEF1A and eEF1A-GDP established that the lethality was not due to a structural defect. Instead, the K205A mutant drastically reduced the nucleotide exchange activity even at very low concentrations of Mg\(^{2+}\). A K205R eEF1β mutant on the other hand was functional in vivo and showed nearly wild-type nucleotide dissociation rates but almost no sensitivity to Mg\(^{2+}\). These results indicate the significant role of Mg\(^{2+}\) in the nucleotide exchange reaction by eEF1β and establish the catalytic function of Lys-205 in displacing Mg\(^{2+}\) from its binding site.

Successive rounds of translation elongation are required for the stepwise addition of amino acids onto the growing polypeptide chain (reviewed in Ref. 1). Several elongation factors are required to facilitate efficient protein synthesis. The eukaryotic translation elongation factor 1 (eEF1) complex consists of three or four subunits: eEF1A, eEF1β, eEF1βy, and eEF1ββ. The eEF1A subunit, which is homologous to prokaryotic EF-Tu, facilitates the transport of aminoacyl-tRNA (aa-tRNA) to the A-site of the elongating ribosome. Following formation of the proper codon-anticodon pair and the peptidyl transfer reaction, eEF2 catalyzes translocation. This movement of the peptidyl tRNA to the P-site, the deacylated tRNA to the E-site, and the mRNA by three nucleotides allows for another elongation cycle. The fungal-specific eEF3 protein promotes release of the deacylated tRNA from the E-site (2, 3). All three of these elongation factors are essential nucleotide-binding proteins.

After the delivery of aa-tRNA to the ribosome, GTP bound to eEF1A is hydrolyzed to GDP. The guanine nucleotide exchange factor (GEF) complex eEF1B recycles eEF1A back to the active GTP form by stimulating GDP release, allowing rebinding of GTP and subsequently aa-tRNA for further rounds of peptide chain elongation (4). The yeast S. cerevisiae eEF1B complex contains the eEF1β and eEF1βy subunits, whereas mammalian systems have an additional eEF1ββ subunit (reviewed in Ref. 1). The eEF1β subunit is catalytic (5), whereas eEF1βy binds to and stimulates the nucleotide exchange activity of eEF1β in vitro (6). Co-crystal structure of the eEF1A-C terminus of eEF1β revealed that eEF1A has three defined domains, and eEF1β spans domains I and II of eEF1A to create two interfaces. Due to the proposed overlap of the aa-tRNA recognition site with that of eEF1β in domain II of eEF1A, the eEF1A-GTP-eEF1β intermediate complex is also proposed to interact with aa-tRNA before the dissociation of eEF1β (7). eEF1A is also an actin-binding and -bundling protein (8). Actin and aa-tRNA binding, however, are mutually exclusive (9). Thus, eEF1β may modulate access of these two substrates to eEF1A. The third eEF1ββ subunit of the mammalian system has sequence similarity to eEF1β and can function as a GEF. eEF1ββ may play a role in the organization of the eEF1B complex with aa-tRNA synthetases, particularly valyl tRNA synthetase (10). eEF1β is essential in vivo (11). However, its requirement for viability can be bypassed by an extra copy of the TEF1 or TEF2 genes encoding eEF1A (12), which is referred to as an eEF1β-Deficient strain. Two eEF1β isoforms encoded by the TEF3 and TEF4 genes are expressed in the cell, but both genes can be deleted with no adverse effect on cell growth (13). The loss of either eEF1B subunit promotes a high level of resistance to oxidative stress, indicating a potential role in a post-transcriptional control mechanism under stress conditions (14).

Recently, a structural model of the mechanism of eEF1β-
catalyzed guanine nucleotide exchange was proposed (7, 15). The structure predicts that Lys-205 of eEF1Bα is critical for the nucleotide exchange mechanism by displacing the Mg^{2+} ion associated with the bound GDP, consequently releasing GDP and stabilizing the nucleotide free state of eEF1A. The essential function of this residue is also supported by the lethality of the K205A mutant (15). Prior kinetic analysis demonstrated that the function of a GEF protein is to overcome the possible inhibition of nucleotide exchange caused by the physiological levels of Mg^{2+} (reviewed in Ref. 17). The mechanism of nucleotide dissociation by the eukaryotic elongation GEF has not yet been determined. Prior kinetic analysis demonstrated that EF-Ts, the GEF for EF-Tu, accelerates the dissociation of GDP by 6 × 10^4 (18). It was also shown that the removal of Mg^{2+} from the EF-Tu-GDP complex accelerates nucleotide dissociation by a factor of 150–300 in the absence of EF-Ts (18).

In this study, the interaction between GDP and wild-type and mutants of Lys-205 of the eukaryotic GEF eEF1Bα was determined at varying Mg^{2+} concentrations. The x-ray crystal structure of a complex between the C-terminal fragment of the K205A eEF1Bα mutant and eEF1A, with and without GDP, revealed that there are no large structural changes as compared with the wild-type structure. Using a fluorescent guanine nucleotide analog (2′-(or 3′)′-O-N-methylanthraniloyl-GDP (mant-GDP)) and stopped-flow kinetics methods, we show that the guanine nucleotide exchange of eEF1A is accelerated 700-fold by wild-type eEF1Bα. The GDP dissociation rate of the eEF1A-eEF1Bα complex is highly Mg^{2+}-dependent, which unlike EF-Tu is not a property of spontaneous GDP dissociation of eEF1A in the absence of its GEF. On the other hand, the K205A eEF1Bα mutant reduces the rate of GDP dissociation, and increasing the concentration of Mg^{2+} does not influence the GDP dissociation rates due to the inability of K205A to disrupt the Mg^{2+} binding pocket. Interestingly, K205A eEF1Bα also dissociates slowly from eEF1A compared with the wild-type protein. In vivo analysis of the lethal K205A mutation was performed using an eEF1Bα-deficient strain supported by excess eEF1A (12). The K205A mutant suppressed the growth and translational defects of a strain modified to survive without eEF1Bα, indicating that it is not a complete loss of function mutant. Substituting Lys-205 by arginine, however, results in a protein sufficient for viability in vivo with a slight growth defect compared with the wild-type strain. The K205R protein, however, displays a slightly reduced GDP dissociation rate constant that is independent of the Mg^{2+} concentration, likely due to the occlusion of the Mg^{2+} binding site. Taken together, these results demonstrate the kinetic effects of a eukaryotic guanine nucleotide exchange factor and the key role of Mg^{2+}.

### Experimental Procedures

#### Yeast Techniques and Mutant Preparation—S. cerevisiae

strains used are listed in Table 1. Standard yeast genetic methods were employed (19). Yeast cells were grown in either YEPD (1% Bacto yeast extract, 2% peptone, 2% dextrose) or defined synthetic complete media (C or C-Ura-Leu) supplemented with 2% dextrose as a carbon source. Yeast were transformed by the lithium acetate method (20). The K205A and K205R eEF1Bα mutants were prepared in pTKB500 (TEF5 LEU2) by PCR mutagenesis using the QuikChange method (Stratagene) with oligonucleotides K205A 5′-CCGATA TTTGCTGAT TACAAGC GTT TAAAAGGCC-3′ or K205R 5′-CCGATATTGCTGAT TACAAGC GTT TAAAAGGCC-3′. The resulting plasmids pTKB526 (tef5 K205A) and pTKB590 (tef5 K205R) as well as the wild-type eEF1Bα plasmid and an empty vector pRS315 (LEU2) were transformed separately into the eEF1Bα-deficient strain, TKY298. The plasmids in each strain were maintained by growth on C-Ura-Leu media. pTKB590 was also transformed into the strain TKY406 (tef5△TRP1 pTFS URA3), and the ability of the mutant to function in place of wild-type eEF1Bα was monitored by growth on 5-fluoroorotic acid, resulting in the production of strain TKY925 expressing only K205R eEF1Bα.

**Growth and Drug Assays**—Growth of strains at the permissive and non-permissive temperatures was assayed by spotting equal amounts of 1/10 serial dilutions of cells on YEPD or C-Ura-Leu media at 13, 24, 30, and 37 °C for 2–7days. The doubling times were determined by measuring the growth of cell cultures in liquid YEPD or C-Ura-Leu media. Strains grown for 1day at 30 °C were diluted to an A_{600} of ~0.07 in the appropriate media and incubated at 30 °C with vigorous shaking. The optical density (A_{600}) was assayed approximately every hour, and doubling times were determined by continually maintaining the cells in the log phase by dilution when the A_{600} reached mid-log phase (0.4–0.6 A_{600} units). Drug sensitivity assays

### Table 1

| Strain | Genotype                  | Source |
|--------|---------------------------|--------|
| TKY235 | MAT a ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 met2-1 his4-713 tef5::TRP1 pTEFS LEU2 | (27)   |
| TKY298 | MAT a ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 met2-1 his4-713 tef5::TRP1 pTEFS LEU2 | (27)   |
| TKY368 | MAT a ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 met2-1 his4-713 tef5::TRP1 pTEFS LEU2 | This work |
| TKY406 | MAT a ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his4-713 tef5::TRP1 pTEFS URA3 | This work |
| TKY925 | MAT a ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 met2-1 his4-713 tef5::TRP1 pTEFS LEU2 | This work |
| TKY915 | MAT a ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his4-713 tef5::TRP1 pTEFS URA3 | This work |
| TKY951 | MAT a ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his4-713 tef5::TRP1 pTEFS URA3 + pTEFS URA3 | This work |
| TKY952 | MAT a ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his4-713 tef5::TRP1 pTEFS URA3 + pTEFS URA2 + pTEFS K205R URA2 | This work |
| TKY953 | MAT a ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his4-713 tef5::TRP1 pTEFS URA3 + pTEFS K205A LEU2 | This work |
were performed on cultures of each strain grown at 30 °C in YEPD or C-Ura-Leu media to mid-log phase, 0.3 ml was then spread plated onto the corresponding solid media, and 10 μl of each drug was pipetted onto a sterile BBL 1/4-inch diameter paper disc. The concentrations of drugs used were 10 μmol cycloheximide, 25 mM hygromycin B, and 406 mM paromomycin. The plates were incubated for 2 days at 30 °C. Sensitivity to each drug was measured by the radius of the zone of growth inhibition in millimeters around each disc.

**Protein Purification and Crystallization**—eEF1A was purified as described (21) with the following modifications. TKY368 was grown in YEPD media and centrifuged, and the cell pellet was frozen and stored in liquid nitrogen. Two ml/g of lysis buffer (60 mM Tris, pH 7.5, 50 mM NH₄Cl, 5 mM MgCl₂, 0.1 mM EDTA, pH 8.0, 10% glycerol, 1 mM dithiothreitol (DTT), and 0.2 mM phenylmethylsulfonyl fluoride) was added to the thawed cell pellet, and lysed by sonication, and the recombinant lactopyranoside at 37 °C for 2–3 h. Cells were harvested by centrifugation and lysed by sonication, and the recombinant protein was purified in accordance with the QIAexpressionist protocol for His₆-tagged proteins under native conditions. Purified protein was dialyzed with 20 mM Tris, pH 8.0, 0.1 mM EDTA, pH 8.0, 25% glycerol, 1 mM DTT, and 0.2 mM phenylmethylsulfonyl fluoride and loaded onto a Source 15S PE 4.6/100 column (Amersham Biosciences). The column was washed with 150 to 300 mM KCl using an AKTA fast-protein liquid chromatography system (Amersham Biosciences). Cellular debris was removed by two rounds of centrifugation for 30 min at 8,000 rpm and 90 min at 50,000 rpm. The supernatant was passed through a 0.22-μm pore-size filter, glycerol was added to a final concentration of 25% and the protein solution applied to DE52 (Whatman) pre-equilibrated with buffer 1 (20 mM Tris, pH 7.5, 50 mM KCl, 10% glycerol, 1 mM DTT, and 0.2 mM phenylmethylsulfonyl fluoride) with 100 mM KCl. The material eluted with 500 mM KCl was dialyzed overnight with 20% glycerol.

**TABLE 2**

| Data collection | Apo | GDP |
|----------------|-----|-----|
| Space group    | P212121| P212121 |
| Cell parameters|     |     |
| A (Å)         | 65.75| 63.90 |
| B (Å)         | 93.53| 91.23 |
| C (Å)         | 93.30| 92.47 |
| Wavelength, λ (Å) | 0.9778 | 0.9778 |
| Resolution (Å) | 99-1.79 (1.86-1.79) | 20-2.6 (2.69-2.6) |
| Completeness (%) | 88.6 (69.9) | 97.4 (90.9) |
| Mean I/σ(I)   | 29.8 (3.4) | 14.0 (1.4) |
| Rmerge (%)    | 2.9 (23.1) | 8.0 (12.4) |
| Redundancy    | 5.1 (3.4) | 3.4 (2.0) |

Values in parentheses are for the outer resolution shell.

| Refinement | Apo | GDP |
|------------|-----|-----|
| Resolution (Å) | 20-1.8 | 20-2.6 |
| Reflections | Free | Free |
| Working | 45,484 | 15,698 |
| Free | 922 | 980 |
| Total atoms, protein/ligand/water | 4,066/0/539 | 4,088/28/268 |
| Rmerge (%) | 21.0/23.6 | 22.0/29.2 |
| Root mean square deviation | Bonds (Å) | 0.006 | 0.01 |
| Angles (°) | 1.31 | 1.36 |

*Rmerge* is identical to *R* on a subset of test reflections not used in refinement.

A pET11d vector expressing the N-terminally His₆-tagged eEF1Bα fusion protein (TKB448) was used to prepare the K205A and K205R mutants using the QuikChange method (Stratagene). The resulting plasmids were pTKB529 and pTKB897, respectively. One liter of *Escherichia coli* strain BL21 containing one of the expression plasmids was grown to an optical density of 0.6 in Luria broth with 100 μg/ml ampicillin medium. Protein expression was induced with 1 mM isopropyl-β-D-thiogalactopyranoside at 37 °C for 2–3 h. Cells were harvested by centrifugation and lysed by sonication, and the recombinant protein was purified in accordance with the QA expressionist protocol for His₆-tagged proteins under native conditions. Protein-containing fractions were dialyzed with 20 mM Tris, pH 7.5, 1 mM DTT, 0.1 mM EDTA, pH 8.0, 100 mM KC1, and 10% glycerol.

Expression and purification of the yeast eEF1Bα K205A mutant encoded in a pET11d expression vector for crystallography and subsequent complex formation with yeast eEF1A was essentially performed as for the native protein complex (22). Crystallization was based on the conditions used for the native complex but occurred at a slightly lower pH and polyethylene glycol 2000 monomethanol ether concentration.

**Data Collection and Structure Refinement**—The reservoir solution of the crystallization tray was substituted with cryobuffer 1 (12% polyethylene glycol 2k monomethanol ether, 20% glycerol, 100 mM Tris-HCl, pH 8, 100 mM KC1, and 3 mM DTT) 20 h prior to data collection. The crystal was then transferred to cryobuffer 1 for 10 min prior to flash freezing in the nitrogen stream and collecting data. A second crystal had its reservoir substituted with cryobuffer 2 (37.5% polyethylene glycol 750 monomethanol ether, 100 mM Tris-HCl, pH 7.6, 100 mM KC1, 3 mM DTT, 2 mM GDP, and 5 mM MgCl₂) 4 h before data collection. The crystal was transferred 10 min before data collection to cryobuffer 2 and frozen as the first crystal, and data were collected. Data were collected at the XRD1 beamline at Elettra in Trieste, Italy. A high and low resolution data set was collected for the apo complex and a single data set for the nucleotide soaked crystal. All data were processed with the HKL package (23) (Table 2). The native structure (PDB ID code 1F60) was used for solving both structures by rigid body refinement in CNS (24). The search model was divided into the three structural domains of eEF1A (2–240, 241–333, and 334–441) and the C-terminal domain of eEF1Bα (117–204). Manual model rebuilding was performed in O (25). The electron density for residue 205 in eEF1Bα corresponded to the expected alanine (Fig. 1A), confirming the mutation. The model for the eEF1A-eEF1Bα-GDP complex was initially refined without GDP. After two rounds of refinement of the search model against the data for the GDP- and Mg²⁺-soaked crystal, a GDP molecule was fitted into the density of a Fₘ – Fₘ map.
The Mg$^{2+}$ ion present in cryobuffer 2 could not be modeled. After iterative cycles of model refinement in O and CNS, and addition of water molecules, the quality of both structures was checked with PROCHECK (26) and Oop2.5 A few minor refinements were performed, and the structures were refined in CNS a final time (Table 2). For the nucleotide-free complex residues 5–441 of eEF1A, 117–206 of eEF1Bα, and 539 water molecules could be modeled. For the GDP-containing complex these numbers were 2–441, 117–206 and 268, respectively. The nucleotide-free and GDP-containing structures have been deposited at the Protein Data Bank with IDs codes 2B7C and 2B7B, respectively. Fig. 1 was made using PyMOL.6

**Mant-GDP Binding Assay**—mant-GDP was purchased from Molecular Probes. The 400-μl mant-GDP solution was diluted with 5 ml of dH$_2$O and loaded onto a 1-ml Hi-Trap DEAE-Sepharose Fast Flow column (Amersham Biosciences) pre-equilibrated with 20 mM triethylammonium bicarbonate, pH 7.8, using an AKTA fast-protein liquid chromatography system (Amersham Biosciences). The column was washed with 2 column volumes of 20 mM triethylammonium bicarbonate and eluted using a linear gradient from 0.02 to 1 M triethylammonium bicarbonate over 20 column volumes. All nucleotide-containing fractions were combined, lyophilized using a speed vacuum, resuspended in 100 μl of dH$_2$O, and stored at −20°C. The purity of the fluorescence-labeled nucleotide was analyzed by thin layer chromatography, and the concentration was determined by reading the absorbance at 356 nm and using the absorption coefficient of the N-methylanthraniloyl group ε$_{356}$ = 5800 M$^{-1}$ cm$^{-1}$.

The affinity for mant-GDP at 25°C was determined by a fluorometric titration assay as previously described with minor modifications (29). Using a FluoroMax-2 spectrophuorometer (Jobin Yvon, Spex Instruments S.A., Inc.), 1 μM eEF1A and 2.5 ml of binding buffer (10% glycerol, 50 mM Tris-Cl, pH 8.0, and 5 mM MgCl$_2$) was placed in a 10×10×40-mm quartz cuvette with a magnetic stirring bar. Aliquots of mant-GDP were added with continuous stirring for 3 min before measuring the fluorescence ($F_{obs}$) with fluorescence resonance energy transfer via excitation at 280 nm and emission of 440 nm for the mant moiety of the nucleotide with both slit widths of 10.05 nm. The protein and nucleotide complex-dependent fluorescence values ($F_{em}$) were obtained by correcting for titration volume and inner filter effect using Equation 1, plotted against mant-GDP concentration and fit to Equations 2 and 3,

$$F_{em} = F_{obs} \times (V_0/V) \times 10^{[0.5(A_{ex}(280) + A_{em}(440))]} \quad (Eq. \ 1)$$

$$F_{em} = C + f_{eb}E_b + f_{gb}M_i \quad (Eq. \ 2)$$

$$E_b = (K_d + M_i + E_i - ((K_d + M_i + E_i)^2 - 4M_iE_i))^{0.5}/2 \quad (Eq. \ 3)$$

where $V$ is the final volume, $V_0$ is the initial volume, $A_{ex}(280)$ is the excitation absorbance of mant-GDP, and $A_{em}(440)$ is the emission absorbance of mant-GDP, $C$ is background fluorescence, $f_M$ is the fluorescence coefficient of free mant-GDP, $f_E$ is the fluorescence coefficient of mant-GDP bound to eEF1A, $E_i$ is the total eEF1A protein concentration, $M_i$ is the total concentration of mant-GDP, the concentration of eEF1A bound to mant-GDP is $E_o$, and $K_d$ is the mant-GDP dissociation constant.

**Stopped-flow Kinetics Measurements**—mant-nucleotide fluorescence was monitored via fluorescence resonance energy transfer from endogenous tryptophans and tyrosines of eEF1A excited at 280 nm, and light was measured after passing through a KV399 filter of a stopped-flow fluorometer (KinTek). The maximum emission of mant-GDP is 440 nm. All experiments were performed in reaction buffer (10% glycerol, 50 mM Tris-Cl, pH 8.0, and 50 mM KCl) at 37°C. MgCl$_2$ was added to various concentrations, and reaction buffer without MgCl$_2$ contained 5 mM EDTA, pH 8.0. Reaction buffer containing 1 μM eEF1A and 1 μM mant-GDP was rapidly mixed with 45 μg GDP and eEF1Bα in reaction buffer. Dissociation of eEF1A-mant-GDP complexes were initiated by excess non-labeled GDP and catalyzed by eEF1Bα. No fluorescence change was observed when mant-GDP was mixed with the reaction buffer alone. At least seven fluorescence trace curves were averaged for each condition, and the resulting averaged curves were fitted to single or double exponential decay (Equation 4) to calculate the dissociation rate constants and amplitudes,

$$F = F_o + A_1e^{-k_1t} + A_2e^{-k_2t} \quad (Eq. \ 4)$$

where $A$ is the amplitude of each phase, $k$ is the dissociation rate constant of each phase, $t$ is time, $F_o$ is the final fluorescence value, and $F$ is fluorescence. All data fitting and residuals were performed using Sigma Plot version 9 (Systat Software, Inc.).

**RESULTS**

**Inhibition of eEF1Bα Function by the K205A Mutant Is Not Due to a Structural Defect**—Prior analysis demonstrated that a K205A mutation of eEF1Bα is not viable as the only form of the protein, although it maintains the ability to bind to eEF1A as determined by a pull-down assay (15). The apo structure of the wild-type eEF1A-eEF1Bα C-terminal catalytic fragment shows that Lys-205 inserts into the GDP/GTP-Mg$^{2+}$ binding pocket of eEF1A (7). Based on the ability to maintain substrate binding, co-crystal structures were resolved of the C-terminal fragment of amino acids 117–206 of eEF1Bα K205A with eEF1A and eEF1A-GDP at resolutions of 1.8 and 2.6 Å, respectively. Mg$^{2+}$ was included during crystallization for the GDP structure, but Mg$^{2+}$ ions were not detectable in the density. Comparison to the structure of the wild-type eEF1A-eEF1Bα C terminus complex indicates that there are no major changes in the K205A mutant structure except the lack of the Lys-205 side chain, supporting that this residue of eEF1Bα has a functional requirement for catalytic activity (Fig. 1A).

The electron density for residue 205 of eEF1Bα clearly corresponds to an alanine in both structures (Fig. 1A). In previous structures of nucleotides bound to the yeast eEF1A-eEF1Bα C terminus complex, the β- and γ-phosphates appeared mobile. The structure of the mutant complex with GDP revealed the position of the β-phosphate for the bound nucleotide, but the
Mg$^{2+}$ ion present in the cryo solution could not be modeled (Fig. 1B). The α-phosphate of GDP in the structure presented here interacts with the backbone amide group of T22 of eEF1A and two water molecules. The β-phosphate is positioned between the N terminus of helix A and the P-loop preceding it. The oxygen atoms of this phosphate interact with the backbone amide groups of Gly-19, Lys-20, and Ser-21 of eEF1A. The positive dipole of helix A contributes to the electrostatic binding of both the α- and β-phosphates. One of the β-phosphate oxygens also interacts with a water molecule 2.53 Å away. This water molecule lies approximately at the position of the Nζ atom from Lys-205 in the native nucleotide-containing complexes of eEF1A and the C-terminal fragment eEF1Bα. Asp-17 from the P-loop bends slightly toward the β-phosphate, but no other changes are seen in this region. Another minor difference between the six yeast complex structures is the orientation of Trp-194. This residue lines the GDP/GTP binding site in a region involved in binding the base of the nucleotide. In the native nucleotide-free and GDP-containing structures, as well as the two structures presented here, this side chain points in one direction, whereas in the GDPNP structure and the GDP-Mg$^{2+}$ structure it is flipped ~180° around the Cζ–Cγ bond and rotated slightly about the Cα–Cγ bond. However, the indole side chain lies in the same plane in all of the structures.

When comparing the phosphate-binding site of *Sulfolobus solfataricus* archael EF1A-GDP (30) with all of the yeast complex structures, the peptide bond between Val-15 and Asp-16, equivalent to Val-16 and Asp-17 in yeast eEF1A, is flipped almost 180°. The orientation of the bond in the yeast structures disrupts the hydrogen-bonding interaction between the backbone amide of Asp-17 and one of the β-phosphate oxygens of GDP. The carbonyl oxygen in this peptide bond is positioned close to two β-phosphate oxygens, 2.61 and 2.81 Å away, respectively. This is energetically unfavorable, and it favors the exclusion of the phosphate moiety from the P-loop, in accordance with the mechanism suggested for GDP release in EF-Tu (31). Although the peptide was flipped in the yeast structure, the position of the β-phosphate was very similar to the one in archael EF1A-GDP. The fact that there was density for the β-phosphate in the structure presented here argues strongly for the importance of Lys-205 in the nucleotide exchange reaction in yeast. When comparing the eEF1Bα K205A mutant GDP structure with the native structure, it was clear that Lys-205 does not superimpose with the β-phosphate of GDP (Fig. 1C). It therefore seems likely that the function of Lys-205 is to disrupt the Mg$^{2+}$ and phosphate binding sites.

**The Lethal Mutation eEF1Bα K205A Suppresses an eEF1Bα-deficient Strain**—To further investigate the lethal K205A eEF1Bα mutant, we analyzed its in vivo effects using an *S. cerevisiae* strain where the essential *TEF5* gene encoding eEF1Bα gene was deleted but the cells were supplemented with an extra copy of the *TEF2* gene encoding eEF1A gene on a plasmid. This eEF1Bα-deficient strain (Fig. 2A, pRS315), has two chromosomal and one plasmid-borne eEF1A-encoding genes, and thus increased eEF1A protein levels compensate for the loss of eEF1Bα. This strain exhibits cold- and temperature-sensitive growth defects (12). The addition of wild-type eEF1Bα in this strain results in enhanced growth compared with the eEF1Bα-deficient strain (Fig. 2A, WT). Surprisingly, this increased growth was equivalent when the lethal K205A eEF1Bα mutant

**FIGURE 1. The structural mechanism of eEF1Bα Lys-205 function in nucleotide exchange.** A, electron density of the K205A region in the mutant apo complex with the final model superimposed (domain I of eEF1A is shown in gray and eEF1Bα in violet). B, stereo view of the $\alpha$-weighted $2F_o - F_i$ electron density map contoured at 1.5 σ around GDP after the final refinement. The GDP molecule omitted from the map calculation has been superimposed on the density and shown in yellow (carbon), blue (nitrogen), red (oxygen), and green (phosphorus). C, superposition of the native apo complex with that of the K205A GDP-containing complex showing the relative position of Lys-205 to GDP. The native structure is shown in wheat and slate for eEF1A and eEF1Bα, respectively, and the mutant complex is colored as above.
was added to the eEF1β-deficient strain (Fig. 2A, K205A). Doubling time assay results at the permissive temperature confirmed the enhanced growth rate of an eEF1β-deficient strain in the presence of wild-type eEF1β (4.2 ± 0.03 h) or the K205A mutant form of eEF1β (4.4 ± 0.14 h) compared with a rate of 7.15 ± 0.41 h for the eEF1β-deficient strain. The K205A mutant protein was stably expressed in the eEF1β-deficient strain as confirmed by Western blot analysis (data not shown).

Because an eEF1β-deficient strain was also previously demonstrated to have hypersensitivity to inhibitors of translation elongation (12), we determined whether the addition of wild-type or K205A mutant eEF1β had the ability to suppress the translational defects conferred by the eEF1β-deficient strain. The sensitivity to the translation elongation inhibitor cycloheximide in this strain was suppressed equally in the presence of K205A mutant compared with wild-type eEF1β (data not shown). This supports that suppression is likely due to the presence of residual activity of K205A eEF1β.

The eEF1β K205R Mutant Is Functional in Vivo—To investigate the potential of occupying the Mg²⁺ binding site constitutively, we replaced Lys-205 with Arg, which maintains a positive charge but extends one carbon further toward the Mg²⁺ binding site. The K205R mutant, unlike K205A, is functional in vivo as the only form of eEF1β, and the strain exhibits comparable growth to a wild-type strain on YEPD media at permissive and non-permissive temperatures (Fig. 2B). However, the doubling times at permissive temperature in liquid media reveal that the K205R mutation causes an 18% reduction of growth over a wild-type strain (3.9 ± 0.07 h versus 3.1 ± 0.12 h). To test the possibility that K205R might result in a translational defect, we tested the sensitivity to drugs that inhibit translation elongation. K205R eEF1β does not affect sensitivity to the translational inhibitors hygromycin B, cycloheximide, or paromomycin (data not shown). The presence of the K205R eEF1β mutant also fully suppresses the growth defect (4.2 ± 0.11 h) and sensitivity to cycloheximide observed in an eEF1β-deficient strain (Fig. 2A and data not shown). These results indicate that the K205R mutant allele is not significantly defective in vivo.

eEF1β-catalyzed Nucleotide Exchange on eEF1A—To assess the nucleotide binding and exchange properties of eEF1A, the GDP nucleotide analog, mant-GDP, was used. This nucleotide contains a fluorophore, which increases its fluorescence signal in response to hydrophobicity. The increase in fluorescence indicates the binding of the nucleotide to protein. Upon the addition of mant-GDP to eEF1A, a considerable increase in fluorescence was observed by fluorescence resonance energy transfer from tryptophans or tyrosines of eEF1A to the mant group of GDP using the fluorometric titration assay (Fig. 3A).

EFE1B-catalyzed Nucleotide Exchange on eEF1A—To assess the nucleotide binding and exchange properties of eEF1A, the GDP nucleotide analog, mant-GDP, was used. This nucleotide contains a fluorophore, which increases its fluorescence signal in response to hydrophobicity. The increase in fluorescence indicates the binding of the nucleotide to protein. Upon the addition of mant-GDP to eEF1A, a considerable increase in fluorescence was observed by fluorescence resonance energy transfer from tryptophans or tyrosines of eEF1A to the mant group of GDP using the fluorometric titration assay (Fig. 3A).

The eEF1B K205R Mutant Is Functional in Vivo—To investigate the potential of occupying the Mg²⁺ binding site constitutively, we replaced Lys-205 with Arg, which maintains a positive charge but extends one carbon further toward the Mg²⁺ binding site. The K205R mutant, unlike K205A, is functional in vivo as the only form of eEF1B, and the strain exhibits comparable growth to a wild-type strain on YEPD media at permissive and non-permissive temperatures (Fig. 2B). However, the doubling times at permissive temperature in liquid media reveal that the K205R mutation causes an 18% reduction of growth over a wild-type strain (3.9 ± 0.07 h versus 3.1 ± 0.12 h). To test the possibility that K205R might result in a translational defect, we tested the sensitivity to drugs that inhibit translation elongation. K205R eEF1β does not affect sensitivity to the translational inhibitors hygromycin B, cycloheximide, or paromomycin (data not shown). The presence of the K205R eEF1β mutant also fully suppresses the growth defect (4.2 ± 0.11 h) and sensitivity to cycloheximide observed in an eEF1β-deficient strain (Fig. 2A and data not shown). These results indicate that the K205R mutant allele is not significantly defective in vivo.

To test if mant-GDP and non-fluorescent GDP possess equivalent affinities for eEF1A, the equilibrium dissociation constant (Kd) of eEF1A for mant-GDP in the presence of 5 mM Mg²⁺ was obtained by using a FluoroMax-2 spectrofluorometer. Both mant-GDP and non-labeled GDP bind to eEF1A with similar affinities. The Kd for mant-GDP binding to eEF1A is 1.8 × 10⁻⁷ M (Fig. 3A), whereas the published Kd of GDP for eEF1A is 10 × 10⁻⁷ M (16). Thus, the change in mant-GDP fluorescence provides an accurate depiction of the eEF1A-GDP interaction.

To study spontaneous GDP dissociation from eEF1A in the absence of its known GEF, we determined the dissociation rate constant of the mant-GDP-eEF1A complex using stopped-flow kinetics. eEF1A bound to mant-GDP was rapidly mixed with excess GDP in 5 mM Mg²⁺ to displace the nucleotide analog causing a decrease in fluorescence. The average dissociation rate constant was 0.17 s⁻¹ (Fig. 3B). We examined the rate of GDP release catalyzed by eEF1Bα in 5 mM Mg²⁺ by monitoring the dissociation of mant-GDP from eEF1A over time in the presence of excess GDP and wild-type eEF1Bα protein. The dissociation rate of mant-GDP was accelerated 7 × 10²-fold. These results support the significance of catalyzing the nucleotide exchange reaction by eEF1Bα, so sufficient GTP-bound eEF1A can be available to sustain the overall rate of elongation. Two exponential phases of the dissociation of mant-GDP from eEF1A were observed in eEF1Bα-catalyzed reactions (Fig. 3C). The faster dissociation rate constant was 70 times more rapid and accounted for ~90% of the total amplitude (Fig. 3C). The two phases could be a result of several effects. First, a biphasic effect related to the presence of two isomers in the mant-GDP mixture was previously observed for the dissociation of mant-GDP from Ras complexed with its GEF, Cdc25 (32). Second, the effect could be attributed to subpopulations of eEF1A unable to be activated by eEF1Bα or different conformations of eEF1Bα that have differing effects on nucleotide dissociation reaction, thereby causing a much slower reaction.
FIGURE 3. **eEF1Bα catalyzes nucleotide exchange on eEF1A-GDP.** A, aliquots of mant-GDP were added to binding buffer with or without eEF1A (1 μM) containing 5 mM Mg2+. Increased fluorescence of mant-GDP was observed by fluorescence resonance energy transfer, which excited tryptophans or tyrosines of eEF1A at 280 nm and used the emission wavelength (440 nm) for the mant moiety. ○, fluorescence intensity of mant-GDP in the presence of eEF1A; ●, fluorescence intensity of mant-GDP alone. Data were fit to a hyperbolic curve to give a $K_d$ value of $1.8 \times 10^{-7}$ M. Using stopped-flow kinetics, eEF1A (1 μM):mant-GDP (1 μM) complex in binding buffer containing 5 mM Mg2+ was rapidly mixed with a solution containing excess GDP (45 μM) without (B) or with 12 μM eEF1Bα (C). Change in fluorescence intensity was observed over time. Data (○) were fitted to a single or double exponential decay equation (solid line) to calculate dissociation rate constants (s⁻¹) and amplitudes (arbitrary fluorescence unit): B, $k_1 = 0.17 \pm 0.006, A_1 = 1.2 \pm 0.002$; C, $k_1 = 92.4 \pm 0.1, A_1 = 1.33 \pm 0.006, k_2 = 1.31 \pm 0.07, A_2 = 0.17 \pm 0.003$. Residual plots were prepared to detect experimental error for the fitted data subsets.
The Role of Lys-205 eEF1Bα in the Nucleotide Exchange Activity of eEF1A—To test the proposed involvement of the Lys-205 residue of eEF1Bα in the nucleotide exchange mechanism, we measured the rate of mant-GDP dissociation catalyzed by the K205A and K205R forms of eEF1Bα by stopped-flow kinetics. mant-GDP dissociated from eEF1A at a rate of 7.4 ± 0.03 s⁻¹ when catalyzed by K205A eEF1Bα (Fig. 4A). This is a 13-fold reduction compared with the mant-GDP dissociation rate in the presence of wild-type eEF1Bα (Fig. 3C). Nevertheless, the nucleotide dissociation rate constant was stimulated 43 times by the mutant K205A eEF1Bα. This result indicates that the K205A eEF1Bα protein is not a complete loss of function mutant but is defective in its catalytic activity to release GDP from eEF1A. The K205R eEF1Bα protein also displayed a decreased mant-GDP dissociation rate of 49 ± 0.4 s⁻¹ (Fig. 4B); however, this rate was more comparable with that of the wild-type eEF1Bα, 92 ± 0.1 s⁻¹ (Fig. 3C). The rate of mant-GDP dissociation from eEF1A catalyzed by the K205R eEF1Bα protein stimulated the mant-GDP dissociation rate by ~7-fold compared with the K205A mutant (Fig. 4). These results support the contribution of Lys-205 eEF1Bα in catalysis of GDP dissociation from eEF1A.

To determine the maximal dissociation rate constant of the mant-GDP·eEF1A complex, experiments were performed at varying concentrations of eEF1Bα. A time course of the fluorescence intensity was monitored for each eEF1Bα concentration to obtain the dissociation rate constants by fitting the data to a single or double exponential decay curve. The dissociation rate constants were plotted against the eEF1Bα concentration and fitted to a hyperbolic curve and the apparent equilibrium dissociation constant (K_d) and the maximal dissociation rate constant (k_cat) values were obtained. The maximal dissociation rate constant of the mant-GDP·eEF1A complex under saturating conditions of wild-type eEF1Bα was 122 ± 8 s⁻¹, and the apparent K_d value was 4 ± 0.8 μM, whereas the K205A eEF1Bα mutant caused the maximal rate to be 15-fold slower (8 ± 0.3 s⁻¹, Fig. 5, A and B). Previous data demonstrated the critical function of Lys-205 by the lethality of a mutation to alanine in vivo, and this was not due to the loss of the ability to bind its substrate, eEF1A (15). Interestingly, K205A exhibited an extremely low apparent K_d value of 0.4 ± 0.1 μM compared with the wild-type protein (Fig. 5B). K205R eEF1Bα caused the maximal nucleotide dissociation rate of eEF1A to be lowered to 68 ± 6.6 s⁻¹ (Fig. 5C). The apparent K_d value of the K205R for the mant-GDP·eEF1A complex was 2.4 ± 0.8 μM, which was essentially the same as the wild-type eEF1Bα protein. Therefore, we conclude that the conserved Lys-205 residue contributes to the catalytic activity of eEF1Bα.

Mg²⁺ and Lys-205 Affect eEF1Bα Exchange Activity

The Role of Lys-205 eEF1Bα in the Nucleotide Exchange Activity of eEF1A—To determine the effects of Mg²⁺ on the guanine nucleotide exchange of eEF1Bα in the absence or presence of a wild-type or K205A and K205R mutant forms of eEF1Bα, we used stopped-flow kinetics at varying concentrations of Mg²⁺. eEF1A bound to mant-GDP was rapidly mixed with excess GDP and a saturating amount of eEF1Bα at different Mg²⁺ concentrations. Using a single or double exponential equation, the dissociation rate constants and amplitudes were calculated (Table 3). The Mg²⁺ concentration and GDP dissociation rate constant for eEF1A with wild-type eEF1Bα had an inverse relationship (Fig. 6A), however, the spontaneous exchange activity of eEF1A did not show an inhibitory effect with Mg²⁺ (Fig. 6A). Consequently, we fitted the dissociation rate constants to a hyperbolic decay curve to obtain the apparent K_M value that reflects Mg²⁺ binding to eEF1A and the maximal nucleotide dissociation rate constant in the absence of Mg²⁺. In the absence of Mg²⁺, the dissociation rate of the eEF1A-mant-GDP complex with wild-type eEF1Bα protein accelerated to 260 ± 6.5 s⁻¹ compared with the maximal dissociation rate constant, 122 ± 8 s⁻¹ in the presence of 5 mM Mg²⁺ (Fig. 6B). These data support the model that physiological levels of Mg²⁺ inhibit the rate at which GDP is released from the eEF1A·eEF1Bα complex (17). However, the dissociation rate constant of the eEF1A·mant-GDP complex without eEF1Bα in the absence of Mg²⁺ was 0.17 s⁻¹, which was equivalent to the dissociation rate constant in the presence of 5 mM Mg²⁺ (Fig. 6A). Therefore, cellular Mg²⁺ levels did not affect the spontaneous release of GDP from eEF1A in the absence of eEF1Bα. The apparent K_M for Mg²⁺ to eEF1A-wild-

Figure 4. The effect of Lys-205 eEF1Bα mutants on nucleotide dissociation from eEF1A. Using stopped-flow kinetics, eEF1A (1 μM)-mant-GDP (1 μM) complex in binding buffer containing 5 mM Mg²⁺ was rapidly mixed with a solution containing excess GDP (45 μM) with 6 μM K205A eEF1Bα (A) or 6 μM K205R eEF1Bα (B). Change in fluorescence intensity over time was monitored. Data (○) were fitted to a double exponential decay equation (solid line) to calculate dissociation rate constants (s⁻¹) and amplitudes (arbitrary fluorescence unit): B, k₁ = 7.4 ± 0.03, A₁ = 1.3 ± 0.003; C, k₁ = 49 ± 0.4, A₁ = 1.92 ± 0.01, k₂ = 10.13 ± 0.4, A₂ = 0.4 ± 0.003. Residual plots were prepared to detect experimental error for the fitted data subsets.
FIGURE 5. K205A eEF1Bα exhibits reduced exchange activity of eEF1A compared with the WT and K205R forms. Using stopped-flow kinetics, an eEF1A (1 μM)-mant-GDP (1 μM) complex in binding buffer containing 5 mM Mg2+ was rapidly mixed with a solution containing excess GDP (45 μM) and various concentrations of eEF1Bα to reach saturating conditions: WT (A), K205A (B), or K205R (C). A time course of fluorescence intensity was monitored for each eEF1Bα concentration, and data were fitted to a single or double exponential decay equation to calculate the dissociation rate constants (Φ). A hyperbolic equation was used to fit the given dissociation rate constants to calculate the dissociation rate constants: WT (A), 4 ± 0.8 and k\text{eff} = 122 ± 8 (A), K205A (B), 0.4 ± 0.1 and k\text{eff} = 8 ± 0.3 (B), and K205R (C), 2.4 ± 0.8 and k\text{eff} = 68 ± 6.6 (C). Residual plots were prepared to detect experimental error for the fitted data subsets.
**TABLE 3**

Nucleotide exchange rates

Dissociation rate constants, $k_{on}$, of mant-GDP in the presence of excess unlabeled GDP at various added concentrations of Mg$^{2+}$ were determined as described under "Experimental Procedures" by measuring the fluorescence of mant-GDP over time. Shown are the averages of more than seven experiments.

| Protein complex | Mg$^{2+}$ | $k_{on}$ |
|-----------------|-----------|---------|
|                  | mM        | s$^{-1}$ |
| eEF1A           | 0         | 0.17 ± 0.0008 |
|                 | 1         | 0.18 ± 0.0009 |
|                 | 5         | 0.17 ± 0.0009 |
|                 | 10        | 0.15 ± 0.0006 |
| eEF1A-WT eEF1Bα | 0         | 260.80 ± 6.42 |
|                 | 1         | 212.74 ± 3.62 |
|                 | 5         | 119.90 ± 3.33 |
|                 | 10        | 74.55 ± 0.81 |
| eEF1A-K205A eEF1Bα | 0       | 135.10 ± 1.77 |
|                  | 1         | 15.91 ± 0.39 |
|                  | 5         | 14.41 ± 0.87 |
|                  | 10        | 5.70 ± 0.03 |
| eEF1A-K205R eEF1Bα | 0       | 53.90 ± 0.70 |
|                  | 1         | 55.61 ± 1.77 |
|                  | 5         | 49.90 ± 0.46 |
|                  | 10        | 41.10 ± 0.49 |

The $k_{on}$ is significantly reduced as an effect of Mg$^{2+}$. The presence of Mg$^{2+}$ demonstrates that Mg$^{2+}$ is a key factor for the nucleotide exchange from the Mg$^{2+}$-bound eEF1Bα complex. The K205A mutant causes the apparent $K_{1/2}$ for mant-GDP to increase considerably to 27 ± 13 mM, suggesting that the Mg$^{2+}$-ion is not binding to eEF1A as well as in the absence of Mg$^{2+}$. Therefore, Mg$^{2+}$ does not affect the catalytic activity of this mutant (Fig. 6D). In the absence of Mg$^{2+}$, the maximal GDP dissociation rate constant from the eEF1A-K205R eEF1Bα complex is 62.6 ± 3.4 s$^{-1}$ (Fig. 6D), which is equivalent to the maximal dissociation rate constant in 5 mM Mg$^{2+}$, 68 ± 6.6 s$^{-1}$ (Fig. 5C). These results support the hypothesis that Arg at position 205 inserts into the Mg$^{2+}$ binding pocket to reduce Mg$^{2+}$ binding to eEF1A. Thus, the Lys-205 residue contributes to the catalytic function of eEF1Bα by displacing Mg$^{2+}$ from its binding site, allowing the nucleotide exchange activity of eEF1A.

**DISCUSSION**

A GEF acts as a catalyst to accelerate the rate of GDP release from a G-protein (17). The mechanism of guanine nucleotide exchange has some conserved aspects, such as a high conservation of the nucleotide binding domains of G-proteins and a proposed key role for Mg$^{2+}$ in the affinity for the nucleotide. This is in contrast to the vast diversity of the size, structure, and sequence of GEF proteins themselves. However, a general model for GEF function is to overcome the inhibitory potential of Mg$^{2+}$ allowing release of GDP from G-proteins (17). eEF1Bα is a component of the eEF1 complex and functions as an essential GEF required to sustain sufficient translation elongation in euukaryotes. To gain insight into the guanine nucleotide exchange mechanism of the eEF1A-eEF1Bα complex, we have examined the roles of Mg$^{2+}$ and a key lysine of eEF1Bα in catalysis.

Using an eEF1Bα-deficient strain that survives due to the presence of excess eEF1A (12) allows for in vivo analysis of the normally lethal eEF1Bα K205A mutant. The addition of the K205A mutant to an eEF1Bα-deficient strain causes suppression of a growth defect of that strain. This is a strong indication that either the residual activity in exchange is above the threshold required for viability or eEF1Bα might possess other cellular functions. Because K205A eEF1Bα also suppresses sensitivity to a translation elongation inhibitor, the mutant likely has residual translation activity in vivo. One possible explanation for this finding is that the introduction of the wild-type eEF1Bα in the eEF1Bα-deficient strain is now an eEF1A overexpression strain. eEF1A overexpression compared with a wild-type strain shows slow growth and actin cytoskeleton disruption phenotypes (35). The addition of the K205A mutant in the eEF1Bα-deficient strain may not behave the same as an eEF1A overexpression strain, due to decreased catalytic GEF activity and higher affinity for eEF1A. This may allow less eEF1A to bind actin (9) and consequently avoid the negative consequences on growth. Thus, the eEF1Bα K205A mutant may prevent eEF1A from inappropriate association with the actin cytoskeleton and thus avoid the negative consequences seen with eEF1A overexpression (35). Because eEF1A binding to K205A eEF1Bα is maintained, the mutant may also function in channeling of reactants in protein synthesis, such as the suggested role for eEF1Bα in aa-tRNA binding (7). Actin binding by eEF1A is mutually exclusive with aa-tRNA binding (9). Conceivably, a fine balance between eEF1A functions in protein synthesis versus actin binding must be achieved for proper cellular homeostasis.
**Mg\(^{2+}\) and Lys-205 Affect eEF1B\(\alpha\) Exchange Activity**

The fluorescent nucleotide analog, mant-GDP, has a similar \(K_d\) for eEF1A compared with unmodified GDP. Using mant-GDP and stopped-flow kinetics to investigate the guanine nucleotide exchange rate of eEF1A in vitro, we determined that the spontaneous GDP dissociation rate constant is probably too slow to sustain viability. Thus eEF1B\(\alpha\) causes the rate of GDP release to accelerate by a factor of 700, which is 9-fold slower than that of the prokaryotic GEF, EF-Tu (18). Previous kinetic studies show the maximal dissociation rate constant of mant-GDP from the prokaryotic EF-Tu:EF-Ts complex is 125 ± 25.5 s\(^{-1}\) (18), which is equivalent to the yeast eEF1A:eEF1B\(\alpha\) complex (122 ± 8 s\(^{-1}\) shown in Fig. 5A). However, in the absence of its GEF, the spontaneous GDP dissociation of eEF1A is 85 times more rapid than that of EF-Tu (18). Interestingly, removal of \(Mg^{2+}\) by EDTA did not alter the rate of spontaneous nucleotide dissociation of eEF1A (Fig. 6A), whereas the spontaneous release of GDP from EF-Tu accelerates by a factor of 150–300 (18). Upon the addition of eEF1B\(\alpha\), \(Mg^{2+}\) affected the exchange activity of eEF1A and elimination of \(Mg^{2+}\) accelerated GDP by 2-fold (Fig. 6B). This confirms that the displacement of \(Mg^{2+}\) is not the only factor responsible for the eEF1B\(\alpha\)-catalyzed nucleotide exchange of eEF1A. Although \(Mg^{2+}\) is an important element for tight binding of the nucleotide to most G-proteins, other nucleotide binding elements must also be disrupted by formation of GTPase-GEF complex to allow proper nucleotide exchange activity (reviewed in Ref. 28). On the other hand, increasing the \(Mg^{2+}\) concentrations in vitro decelerates GDP release from the eEF1A:eEF1B\(\alpha\) complex.

Unlike the K205A eEF1B\(\alpha\) mutation, a strain with K205R as the only form of the protein is viable. K205R displays a slightly reduced GDP dissociation rate and growth defect, possibly due to the amino groups of the arginine side chain interacting with the oxygens of the \(\alpha\)- or \(\beta\)-phosphate of the nucleotide to stabilize tighter binding of the nucleotide, which will cause a decrease in the rate of GDP release. Interestingly, the \(Mg^{2+}\) concentration did not affect the rate of GDP release from eEF1A by K205R eEF1B\(\alpha\). These data support the hypothesis that the arginine can interrupt \(Mg^{2+}\) binding to eEF1A by extending into the \(Mg^{2+}\) pocket. Overall, the striking differences in the \(Mg^{2+}\) response of the different forms of eEF1B\(\alpha\) support the model that eEF1B\(\alpha\) binds to eEF1A and inserts the Lys-205 residue into the \(Mg^{2+}\) binding pocket to displace the ion (Fig. 6). Thus, the interaction of eEF1B\(\alpha\) and \(Mg^{2+}\) is biologically critical for nucleotide exchange of eEF1A and continued protein synthesis.
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