F-actin Sequesters Elongation Factor 1α from Interaction with Aminoacyl-tRNA in a pH-dependent Reaction

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Abstract. The machinery of eukaryotic protein synthesis is found in association with the actin cytoskeleton. A major component of this translational apparatus, which is involved in the shuttling of aa-tRNA, is the actin-binding protein elongation factor 1α (EF-1α). To investigate the consequences for translation of the interaction of EF-1α with F-actin, we have studied the effect of F-actin on the ability of EF-1α to bind to aa-tRNA. We demonstrate that binding of EF-1α:GTP to aa-tRNA is not pH sensitive with a constant binding affinity of ∼0.2 μM over the physiological range of pH. However, the sharp pH dependence of binding of EF-1α to F-actin is sufficient to shift the binding of EF-1α from F-actin to aa-tRNA as pH increases. The ability of EF-1α to bind either F-actin or aa-tRNA in competition binding experiments is also consistent with the observation that EF-1α's binding to F-actin and aa-tRNA is mutually exclusive. Two pH-sensitive actin-binding sequences in EF-1α are identified and are predicted to overlap with the aa-tRNA–binding sites. Our results suggest that pH-regulated recruitment and release of EF-1α from actin filaments in vivo will supply a high local concentration of EF-1α to facilitate polypeptide elongation by the F-actin–associated translational apparatus.

IN the current model of the elongation cycle of eukaryotic protein translation, elongation factor 1α (EF-1α)[1] plays a role in transporting aminoacyl-tRNA to the ribosome during protein synthesis. Binding of EF-1α with the nucleotide exchange factors EF-1βγ leads to the replacement of GDP with GTP, which switches on the ability of EF-1α to interact with aminoacyl-tRNA. Subsequently, the binding of EF-1α:GTP:aa-tRNA ternary complex with the ribosome triggers the GTPase activity on EF-1α and the resultant EF-1α:GDP dissociates from the ribosome, ready for the next cycle (Riis et al., 1990).

EF-1α is a ubiquitous protein with homologues (EF-Tu) in prokaryotic systems. It is a very abundant protein that constitutes about 1–2% of the total protein in normal growing cells. Large increases in mRNA levels for EF-1α are observed in rapidly proliferating cultured cells, embryos, and a variety of human tumors, suggesting a correlation of EF-1α expression level with the rate of cell growth and proliferation (for review see Condeelis, 1995). The first evidence that EF-1α is an actin-binding protein was obtained in *Dictyostelium* (Demma et al., 1990; Yang et al., 1990). Subsequently, EF-1α has been shown to colocalize with actin filaments and this colocalization changes with chemoattractant stimulation in *Dictyostelium* and adenocarcinoma cells (Dharmawardhane et al., 1991; Okazaki and Yumura, 1995; Edmonds et al., 1996). In fibroblasts, EF-1α is found to colocalize at actin filament junctions and EF-1α from carrot root cells bundles actin filaments (Yang et al., 1993; Bassell et al., 1994). Owen et al. (1992) demonstrated that EF-1α cross-links F-actin into bundles with a unique cross-bridge bonding rule that would tend to exclude other actin cross-linking proteins. This unique cross-bridge structure may represent a special property of EF-1α that is important in the stability of the cytoskeleton and the transport, anchorage, and translation of mRNA (Condeelis, 1995). Apart from binding to actin filaments, binding to calmodulin, bundling, and/or severing of microtubules by EF-1α from carrot, *Trypanosome*, *Xenopus*, rabbit liver, and human (recombinant) have been reported (Durso and Cyr, 1994a; Kaur and Ruben, 1994; Shiina et al., 1994).

In addition to EF-1α, an increasing number of protein synthesis components have been observed to associate with the cytoskeleton. The association of mRNA with the cytoskeleton has been well documented (for review see St Johnston, 1995), and there is correlation between this association and protein synthesis (see Nielsen et al., 1983; Singer, 1993). In addition, ribosomes and initiation factor 2 (eIF-2) have been shown to associate with the cytoskele-

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1. *Abbreviations used in this paper.* EF-1α, elongation factor 1α; EtBr, ethidium bromide; GST, glutathione-S-transferase; 3-D, three-dimensional.

ton (Howe and Hershey, 1984; Gavrilova et al., 1987; Zambetti et al., 1990; Hamill et al., 1994; Hesketh et al., 1991). Interestingly, the other elongation factor (EF-2) has been demonstrated to bind directly to actin filaments (Bektas et al., 1994). Colocalization of these components with the cytoskeleton supports the speculation that protein synthesis in vivo is channeled, i.e., the components are organized in a high degree of spatial order and intermediates are transferred from one enzyme to another without mixing with the surrounding cytoplasm (Stapulionis and Deutscher, 1995).

A correlation between cytoplasmic alkalinization and increases in protein synthesis has been observed in a number of different cell types (for review see Grinstein et al., 1989). In sea urchins, elevation of intracellular pH serves as a primary signal in the activation of protein synthesis at fertilization (Winkler et al., 1980). Measurements of cytosolic pH in sea urchin eggs before fertilization indicate that protein synthesis is restricted below pH 6.8 but not at pH 7.1 (Rees et al., 1995). In fibroblasts, intracellular pH may play a determinant role in the control of cell division by controlling the rate of protein synthesis (Chambard and Pouyssegur, 1986). In *Dictyostelium*, stimulation of cells with cAMP induces cytoplasmic alkalinization, and artificially raising the intracellular pH can trigger a severalfold increase in the rate of DNA and protein synthesis (Aerts et al., 1985, 1987). The interaction of *Dictyostelium* EF-1α with F-actin is pH-dependent with a transition from tight to loose bundling between pH 6.7 and 7.6 (Edmonds et al., 1995). It has been proposed that pH may regulate the association of EF-1α with the cytoskeleton in such a way as to regulate, both spatially and temporally, its activity as an elongation factor (Liu et al., 1996). This is potentially important for developing organisms like *Dictyostelium*, in which, during early development, the mean cytoplasmic pH can range from 6.0 to 7.2 (Furukawa et al., 1990).

To understand the physiological significance of the interaction of EF-1α with actin filaments, we investigated the interaction of EF-1α with F-actin and aa-tRNA in vitro. We demonstrate that the abilities of EF-1α to bundle and bind to F-actin are blocked by the formation of EF-1α: GTP:Phe-tRNA ternary complex in a pH-dependent manner. To understand the mechanism of the blockade, we chose to map the F-actin–binding sites on EF-1α. Using truncated recombinants of EF-1α, we have identified two F-actin–binding domains that exhibit different pH sensitivities for F-actin binding. Structural comparison by using EF-Tu:GTP:Phe-tRNA complex as a model (Nissen et al., 1995) suggests that the proposed F-actin–binding domains on EF-1α may overlap with those for the EF-1α/Phe-tRNA interaction. These observations provide clues in explaining how pH may, by modulating the interaction of EF-1α with F-actin, influence the dynamics of the cytoskeleton and the rate of protein translation in the cells.

Materials and Methods

Construction of Expression Vectors for Glutathione-S-Transferase (GST)–EF-1α Fusion Proteins

Full-length *Dictyostelium* EF-1α cDNA sequence (Yang et al., 1990) was subcloned into pGEX-KG vector (Guan and Dixon, 1991) at NcoI and XhoI sites. Construct pGEX-Dd-dmI, encoding amino acids 1–221, was generated by PCR from *Dictyostelium* EF-1α cDNA with primers GGC GGA ATT CTA ATG GAA TTT CCG GAA TCC GAA AAA ACA CAT and GCG AAG CTT ATT CTA ATA AAG TTG GAC CTTT and inserting the PCR product into pGEX-KG vector at EcoRI and HindIII sites. Similarly, construct pGEX-Dd-dmII (encoding amino acids 222–320 of *Dictyostelium* EF-1α) was generated with primers CGC GGA ATT CTA GCC CTC GAT GCC ATC GTC GAA and CGCA AGC TTA GGC GTC ACC AGC GAC CAT, and construct pGEX-Dd-dmIII (encoding amino acids 321–456 of *Dictyostelium* EF-1α) was generated with primers AGC GGA ATT CTA AAA AAC GAT CCA CCA CAA GAA and CG CGA AGC TTA TTT CTT CTT TGA TGG AGC AGC. A construct of mouse EF-1α (Lu and Werner, 1989) in pGEX-KG vector was a gift from Dr. E. Richard Stanley (Albert Einstein College of Medicine). Construct pGEX-mouse-dmI (encoding amino acids 1–230 of mouse EF-1α) was made by taking advantage of a HindIII site on the mouse EF-1α sequence near residue 230 to remove the coding sequence for amino acids 231–461 from the construct pGEX-mouse-EF-1α and religating the rest of the construct. All the constructs were validated by DNA sequencing and Western blotting with antibodies against EF-1α.

Protein Purification

Dictyostelium EF-1α was purified as previously described (Edmonds et al., 1995). Rabbit skeletal muscle actin was prepared from acetone powder by the method of Spudich and Watt (1971) and further purified by G-150 gel filtration (Bresnick et al., 1990). *Dictyostelium* actin was isolated and purified by the method of Bresnick and Condeelis (1990).

The GST fusion proteins of EF-1αs and their truncates were expressed and purified by the method modified from Smith and Johnson (1988). In brief, host cells (XL1-Blue or JM109) containing the desired construct were allowed to grow overnight in LB medium with 100 μg/ml ampicillin at 30°C. When cell density reached $OD_{600} = 1$, the expression of fusion protein was induced by addition of 0.1–0.5 mM of IPTG for 4 to 6 h at 30°C (depending on which fusion protein was induced). At the end of induction, the cells were harvested and cell pellet was washed once with wash buffer (10 mM Tris, 1 mM DTT, pH 7.5) and then resuspended with lysis buffer (20 mM NaPO₄, 150 mM NaCl, 20 μg/ml leupeptin, 20 μg/ml pepstatin A, 20 μg/ml chymostatin, 3% [vol/vol] aprotinin, 1 mM DTT and 1 mM EDTA, 1% Triton X-100, pH 8.0). After sonication and centrifugation at 50,000 g for 30 min, the supernatant was incubated with glutathione (GSH)-conjugated beads at room temperature for 30 min. The beads were washed with PBS (pH 8.0) and bound GST fusion proteins were eluted with elution buffer (10 mM GSH, 200 mM NaCl, 120 mM Tris, pH 9.0).

Right Angle Light Scattering to Study EF-1α Cross-linking of F-actin

The loading of GTP to EF-1α was performed by incubating 1 μM EF-1α with 1 mM GTP for 30 min at room temperature. Nucleotide binding was confirmed by nitrocellulose filtration assay (Nagata et al., 1976) or Mant-GTP fluorescence. Right angle light scattering was used to study the EF-1α–mediated formation of F-actin bundles. In an assay buffer containing 20 mM Pipes, 50 mM KCl, 5 mM MgCl₂, 2 mM EGTA, 1 mM DTT, 1 mM ATP, and 15% glycerol, preformed *Dictyostelium* F-actin (3 μM) was mixed with *Dictyostelium* EF-1α (1 μM) that was incubated with 1 mM GTP for 30 min and then 1 μM [³H]Phe-tRNA for an additional 20–30 min to form ternary complex at room temperature. The assays were performed by using a fluorescence spectrophotometer (model F-2000; Hitachi Sci. Instrs., Mountain View, CA) with 600-nm excitation and emission wavelength at a band pass of 5 nm. Data were collected and analyzed by using the computer software SpectraCalc and GRAMS/386 (Galactic Industries Corp., Salem, NH). After light scattering analysis, the reaction mixtures were collected for actin cosedimentation assay.

Actin Cosedimentation Assay

Actin cosedimentation assay was used to test the actin-binding activity of the fusion proteins of EF-1α. Each fusion protein was mixed with G-actin in sedimentation buffer (20 mM Pipes, 50 mM KCl, 2 mM EGTA, 2 mM MgCl₂, 1 mM DTT, 1 mM ATP) at preset pH and then incubated at 0–4°C for 18–20 h. This buffer contains physiological concentrations of monovalent salts that have been measured in amebae as ~50 mM (Marin and Rothman, 1980). The reaction mixture was centrifuged and samples of su-

pernatants and pellets were analyzed by SDS-PAGE and densitometry. Fusion proteins were soluble under these assay conditions in the absence of F-actin.

A differential actin sedimentation assay was applied to study the effect of Phe-tRNA on EF-1α bundling and binding to F-actin. Samples collected after light scattering assays were allowed to incubate at room temperature for 2 h. The samples then were centrifuged by using an airfuge at 50,000 g for 2.5 min to pellet F-actin bundles (low speed pellet), and the supernatants were transferred and further centrifuged at 130,000 g for 40 min to pellet single actin filaments (high speed pellet) as demonstrated previously (Demma et al., 1990; Edmonds et al., 1995). Aliquots of reaction mixture, supernatants, and pellets were quantified by SDS-PAGE for protein contents, ethidium bromide (EtBr) fluorescence for tRNA contents, and liquid scintillation counting for [3H]Phe-tRNA.

Electrophoresis

SDS-PAGE was performed according to the method of Laemmli (1970). The amounts of actin and the fusion proteins were determined by scanning Coomassie blue–stained gels using Molecular Dynamics' Computing Densitometer and Image Quant software (Eugene, OR).

Quantitation of tRNA by EtBr Fluorescence

Samples containing tRNA or [3H]Phe-tRNA were quantified by EtBr fluorescence as described by Gallagher (1994). A fluorescence spectrophotometer (model F-2000; Hitachi Sci. Instrs.) was used at a wave length of 302 nm for excitation and 590 nm for emission. In brief, the emission of a EtBr solution containing 10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 250 mM NaCl, and 5 μg/ml EtBr was read as blank. 1/3 vol of actin sedimentation buffer or tRNA-containing sample was added into the blank and the emission was recorded. Standard curves demonstrated a linear relationship of fluorescence emission and concentration of tRNA over a range of 0–10 μM. ATP or GTP (up to 1 mM), or actin or BSA (up to 8 μM) in the sample showed no obvious interference in this assay.

Synthesis of [3H]Phe-tRNA

The method that we used to synthesize [3H]Phe-tRNA is essentially the same as that reported by Schreier et al. (1977) except that we used tRNA stocks rich in tRNA^{Phe}. Using a method modified from Merrick (1979), tRNA synthetases were isolated from rabbit reticulocyte lysate (Promega Corp., Madison, WI) by centrifugation at 95,000 rpm for 20 min at 4°C using a rotor (model TLA 100; Beckman Instrs., Fullerton, CA). The pellet was resuspended and then centrifuged in a buffer containing 20 mM Tris-HCl, pH 7.5, 1 mM DTT, 0.1 mM EDTA, 0.25 M sucrose, and 0.5 M KCl. Both supernatants contain tRNA synthetases for the aminoacylation of tRNA. For each bulk preparation, a small scale of tRNA aminoacylation was conducted for optimal conditions. Usually, 80–90% of tRNA^{Phe} was aminoacylated based on the amount of [3H]Phe incorporation.

Detection of EF-1α:GTP:Phe-tRNA Ternary Complex by G75 Gel Filtration Chromatography

The formation of EF-1α:GTP:Phe-tRNA ternary complex at pH 6.5 and 7.0 was detected by gel filtration according to the method of Nagata et al. (1976), except that the ammonium sulphate precipitation step was omitted. Briefly, 10 μM of Dictyostelium EF-1α and 100 μM of [32P]-γ-GTP were incubated in column buffer containing 20 mM Pipes, 5 mM MgCl₂, 5 mM β-mercaptoethanol, 100 mM NH₄Cl, and 15% glycerol at pH 6.5 or 7.0 for 30 min at room temperature and then incubated with 3 μM [3H]Phe-tRNA for 10 min. The concentration of EF-1α and Phe-tRNA was used such that their final concentration in the fraction after about 10-fold dilution in the column was within 0.2–1 μM, which is close or above the predicted K_d at these pH's based on a reported 0.24 μM K_m of calf EF-1α for Phe-tRNA at pH 7.5 (Crechet and Parmeggiani, 1986). 100 μl of incubation mixture was loaded onto a Sephadex G75 column (4.6 × 28.5 mm) and eluted with the same column buffer at a flow rate of 5 ml/h. Fractions (each 150 μl) were collected and 20 μl of each fraction was mixed with 5 ml of Cytoscin scintillant (ICN Biomedicals, Inc., Costa Mesa, CA) and counted for [32P]γ-GTP and [3H]Phe-tRNA. EF-1α concentration in the fractions was measured by immunoblotting using affinity-purified anti-Dictyostelium EF-1α polyclonal antibody (Demma et al., 1990).

Estimation of Binding Affinity of EF-1α:GTP with Phe-tRNA

The determination of Dictyostelium EF-1α:GTP binding affinity for Phe-tRNA at pH 6.5 and 7.0 was performed by using intrinsic tryptophan fluorescence technique. In sedimentation buffer (plus 1 mM GTP and 15% glycerol), 0.5 μM of Dictyostelium EF-1α was incubated with various amounts of Phe-tRNA. A fluorescence spectrophotometer (model F-2000; Hitachi Sci. Instrs.) was used at a wavelength of 290 nm for excitation and 337 nm for emission. We used the method of Birdsall et al. (1983) to correct the inner filter effect caused by tRNA. Tryptophan was used as standard fluorophore to establish calibration curves. Under our conditions, 3.9 μM of tryptophan and 0.5 μM of Dictyostelium EF-1α gave equal absorption at 290 nm and fluorescence emission at 337 nm. The calibration curve obtained from each pH was then fitted by nonlinear least squares analysis to the polynomial

$$F_{obs} = (F_{corr} + F_{blank}) \frac{(e^{-aL_Td} - e^{-aL_T})}{aL_T(1-d)}$$

where F_{obs} and F_{corr} represent observed and corrected fluorescence, respectively. F_{blank} is fluorescence from source other than the compound of interest and L_T is the concentration of tRNA. a and d are constants that can be determined from the curve fitting and were used to correct fluorescence data of EF-1α:GTP binding to Phe-tRNA by using the above equation. To obtain the dissociation constant (K_d), the corrected binding titration data were curve-fit by nonlinear least squares to a bimolecular binding isotherm according to the expression:

$$Y = \left(\frac{X}{K_d + X}\right) P1$$

where Y is the fraction of bound EF-1α and X is the free aa-tRNA concentration and $P1$ represents the maximal change of fluorescence. Given ~2,000-fold molar excess of GTP to EF-1α and a low intrinsic GTPase activity of EF-1α in the absence of ribosomes, almost all the EF-1α is loaded with GTP. The maximal quenching of fluorescence by aa-tRNA (after correction of inner filter effect) was set to 1 and the data was replotted as fractional approach to this number as shown in Fig. 2.

Homology Modeling of Dictyostelium EF-1α

Molecular model of Dictyostelium EF-1α was constructed first from coordinates of a 2.5 Å crystal structure of EF-Tu, which was retrieved from the Protein Data Bank (Brookhaven, NY) (Kjeldgaard et al., 1993). Cα backbone was constructed using the Homology module of the Insight-II molecular modeling pakage (BioSym Technologies, Inc., San Diego, CA). Cα atoms of all EF-Tu amino acids located in SCR boxes were replaced by corresponding Cα atoms for amino acids from the modeled molecule. New loops were searched or generated and assigned for all deletion/insertion areas. The backbone and side chain atoms were either added later to the constructed Cα chain in the BioPolymer module of Insight-II or, in cases of high homology, replaced together with the Cα atoms. Manual and automatic side chain rotamers were used to avoid conflicts between side chains. The last 19 amino acids at the COOH terminus of EF-1α were omitted in the constructed model because of a lack of corresponding sequence on EF-Tu for replacement.

The energy minimization of the model was done in X-PLOR (Brunger, 1992) by first prep-stage relaxation and further slow-cool refinements. The quality of the model was evaluated using Procheck (Laskowski et al., 1993) and visual analysis.

Results

To understand the physiological consequences of the interaction of EF-1α with both F-actin and aa-tRNA, we have studied these interactions in vitro. In previous studies (Edmonds et al., 1995), it was found that increasing pH over the physiological range (pH 6.2–7.8) causes a loss of EF-1α–mediated F-actin bundling and single filament binding. The K_d for binding of EF-1α to F-actin increases from ~0.2 μM to >2 μM over this pH range. In the present study, we have investigated how the binding of EF-1α to F-actin is affected by aa-tRNA.

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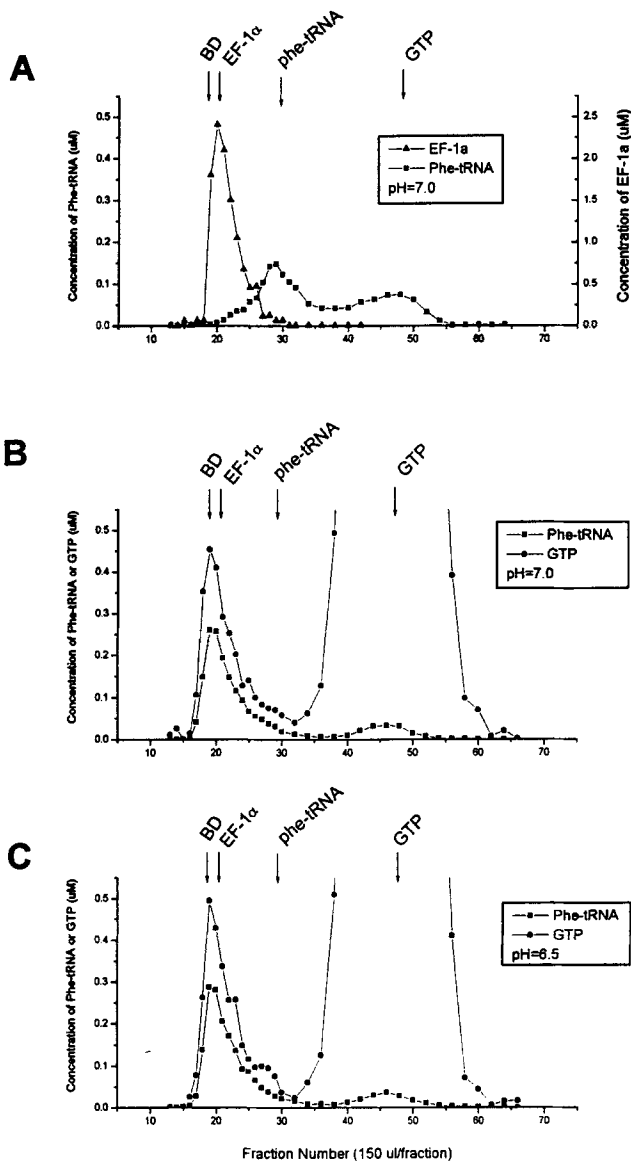
Figure 1. Detection of EF-1α:GTP:Phe-tRNA complex at pH 6.5 and 7.0 by Sephadex G75 gel filtration chromatography. Details of analysis are described in Materials and Methods. Arrows indicate peak of each component when it was run individually on the same column (not shown); BD is for blue dextran. Elution of ternary complex is indicated as a shift in Phe-tRNA from fraction 29 to fraction 19. (A) EF-1α and [³H]Phe-tRNA were incubated in the absence of GTP and analyzed by Sephadex G75 gel filtration at pH 7.0. (B) EF-1α, [³²P]γ-GTP, and [³H]Phe-tRNA were incubated and analyzed at pH 7.0. (C) EF-1α, [³²P]γ-GTP and [³H]Phe-tRNA were incubated and analyzed at pH 6.5.

Formation of the EF-1α:GTP:aa-tRNA Complex at pH 6.5 and 7.0

Like its prokaryotic counterpart EF-Tu, EF-1α must bind GTP to form a stable complex with aa-tRNA. The GTP-dependent formation of the EF-1α:GTP:aa-tRNA ternary complex at physiological pH was studied here using gel filtration. As shown in Fig. 1 A, when EF-1α was incubated with Phe-tRNA in the absence of GTP, there was no detectable ternary complex formed, confirming the depen-
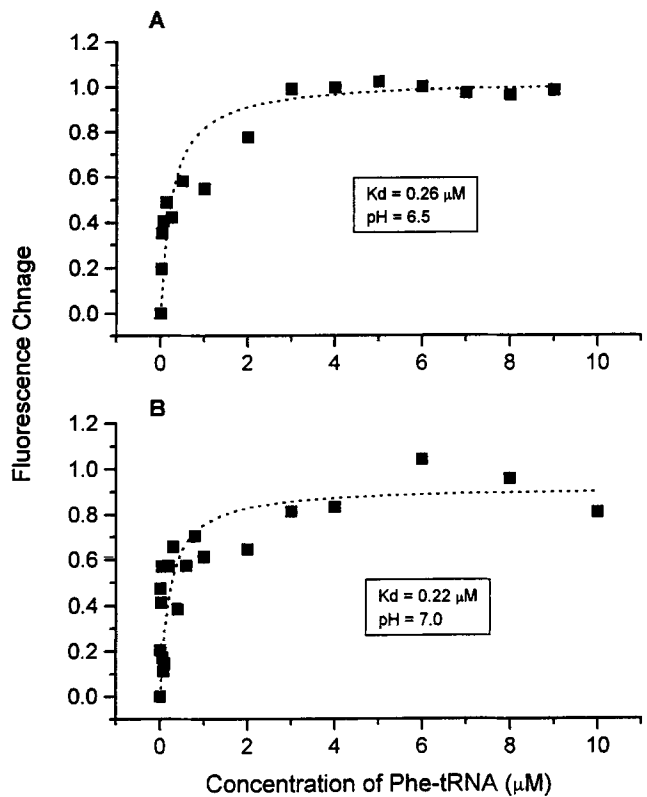
Figure 2. The binding affinity of EF-1α:GTP to Phe-tRNA is unchanged between pH 6.5 and 7.0. Details of correction of inner filter effect and estimation of binding affinity are described in Materials and Methods. Constant 0.5 μM of Dictyostelium EF-1α was incubated with various amounts of Phe-tRNA as indicated.

dence on GTP for the formation of the complex. In contrast, in the presence of GTP, at both pH 6.5 and 7.0 (Fig. 1, B and C), ternary complexes were formed as detected in the void volume in the gel filtration assays. It is worth noting that during the incubation and G75 gel filtration, some of the Phe-tRNA was deacylated in the absence of ternary complex as some of the [³H]Phe was found with GTP (Fig. 1 A). Such deacylation was dramatically reduced when ternary complexes were formed (Fig. 1, B and C), suggesting that without binding to EF-1α, aa-tRNA is relatively unstable in solution at physiological pH.

Binding Affinity of EF-1α:GTP for Phe-tRNA at pH 6.5 and 7.0

Having qualitatively demonstrated that the ternary complex can be formed at pH 6.5 and 7.0, we quantitated the EF-1α:GTP interaction with Phe-tRNA at these pHs. In eukaryotes, the affinity of calf brain EF-1α:GTP for aa-tRNA at pH 7.5 is ∼0.24 μM as estimated from the stimulation of the GTPase activity of EF-1α by aa-tRNA (Crechet and Parmeggiani, 1986). As an alternative approach, we studied changes in the intrinsic tryptophan fluorescence of EF-1α as a method to investigate the interaction of Dictyostelium EF-1α:GTP with Phe-tRNA. As shown in Fig. 2, under our experimental conditions, the binding of EF-1α: GTP to Phe-tRNA has a K_d of 0.26 μM at pH 6.5 and 0.22 μM at pH 7.0.
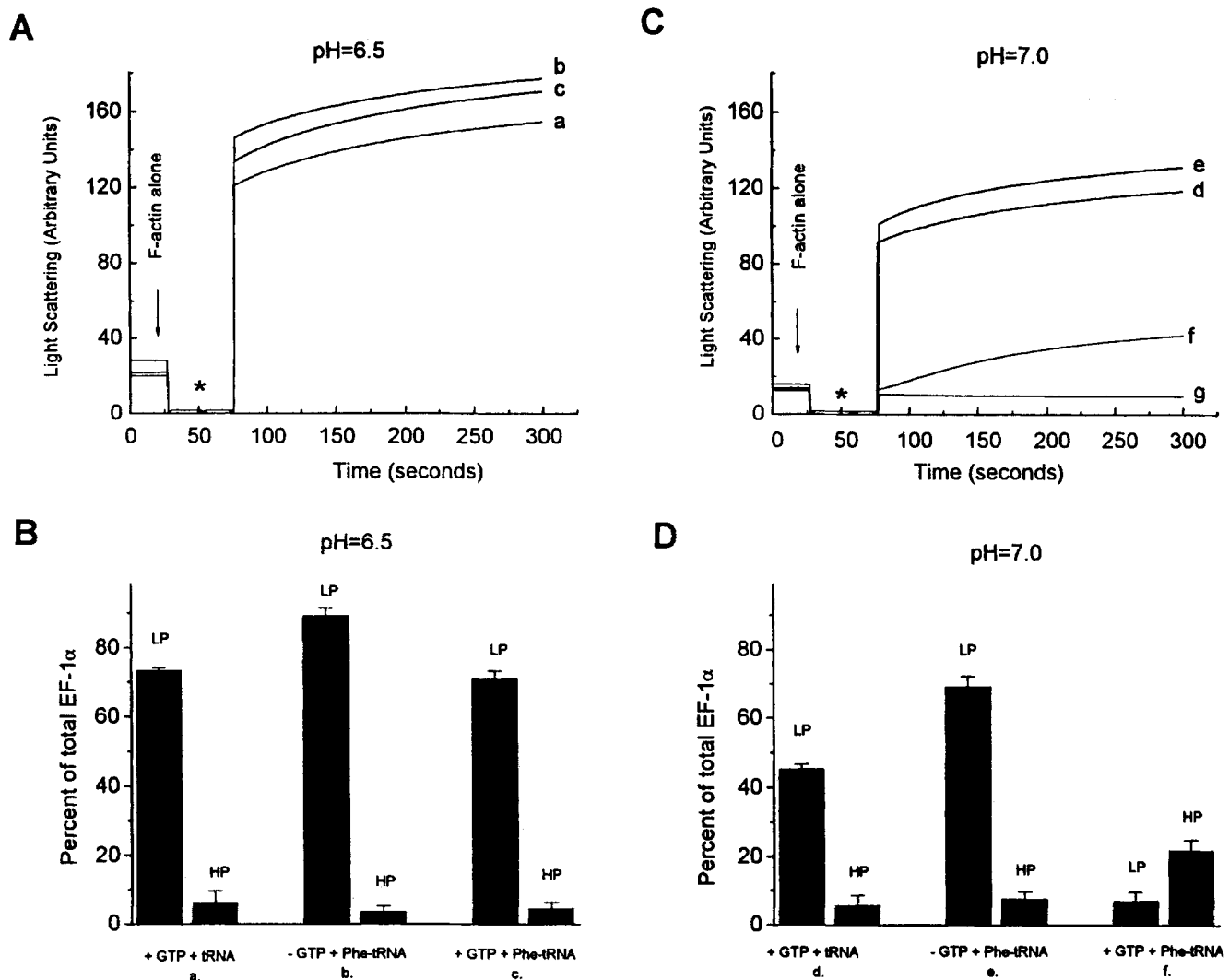
Figure 3. Phe-tRNA blocks the F-actin–bundling and –binding activities of EF-1α:GTP in a pH-dependent manner. (*A* and *C*) Detection of F-actin bundles by right angle light scattering. *, Shutter closed during sample mixing. (*a* and *d*) 3 μM F-actin + 1 μM EF-1α + 1 mM GTP + 1 μM tRNA. (*b* and *e*) 3 μM F-actin + 1 μM EF-1α + 1 μM [³H]Phe-tRNA in the absence of GTP. (*c* and *f*) 3 μM F-actin + 1 μM EF-1α + 1 mM GTP + 1 μM [³H]Phe-tRNA. (*g*) 3 μM F-actin + 1 mM GTP + 1 μM [³H]Phe-tRNA in the absence of EF-1α. (*B* and *D*) F-actin cosedimentation assay. *LP*, low speed pellet; *HP*, high speed pellet.

Table I. EF-1α Bound to Actin Filaments Is Not in Ternary Complex

	EF-1α in pellets* (percentage of total 1 μM)	tRNA or [³H]Phe-tRNA in pellets (percentage of total 1 μM)
I. At pH 7.0		
a. (+GTP +tRNA)	50.8 ± 4.3 SD (*n* = 3)	9.9 ± 1.6 SD (*n* = 3)
b. (−GTP + Phe-tRNA)	76.7 ± 1.8 SD (*n* = 3)	4.8 ± 0.36 SD (*n* = 3)
c. (+GTP + Phe-tRNA)	28.7 ± 4.1 SD (*n* = 3)	3.2 ± 0.14 SD (*n* = 3)
d. (+GTP + Phe-tRNA, no EF-1α)	N/A	2.5 ± 0.88 SD (*n* = 2)
II. At Ph 6.5		
e. (+GTP + tRNA)	79.4 ± 2.60 SD (*n* = 2)	17.0 ± 4.60 SD (*n* = 2)
f. (−GTP + Phe-tRNA)	92.7 ± 0.65 SD (*n* = 2)	8.2 ± 0.35 SD (*n* = 2)
g. (+GTP + Phe-tRNA)	75.6 ± 3.80 SD (*n* = 2)	7.0 ± 0.88 SD (*n* = 2)

Samples are from the same experiments shown in Fig. 3. Concentration of tRNA or [³H]-Phe-tRNA was measured by ethidium bromide fluorescence and liquid scintillation counting as described in Materials and Methods. I. (pH 7.0) Groups *a–d* are the same as in Fig. 3 *B*. II. (pH 6.5) Groups *e–g* are the same as in Fig. 3 *D*. *, Pellets = low speed pellet + high speed pellet.
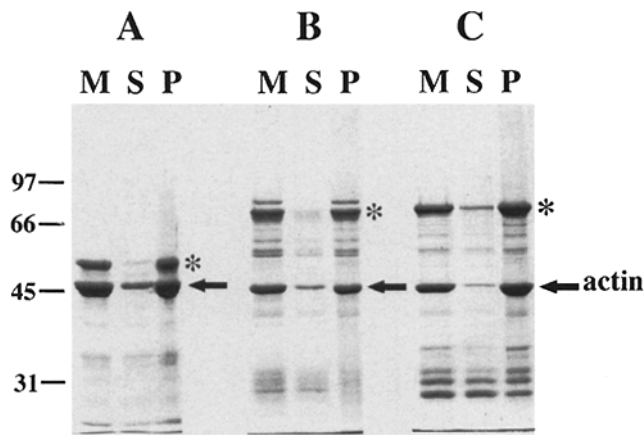
Figure 4. GST–EF-1α fusion proteins bind to F-actin. Actin cosedimentation assays analyzed by 10% SDS–polyacrylamide gels. (Bands with lower molecular weights are proteolytic fragments of the fusion proteins.) Assays were performed at pH 6.5. *M*, reaction mixture before centrifugation; *S*, supernatant; *P*, pellet; *, EF-1α or GST–EF-1α. (*A*) Native *Dictyostelium* EF-1α (2 μM) with rabbit actin (5 μM). (*B*) GST-Dicty-EF-1α (1.5 μM) with *Dictyostelium* actin (3 μM). (*C*) GST-mouse-EF-1α (1.5 μM) with rabbit actin (3 μM).

Binding of EF-1α to F-actin in the Presence of aa-tRNA

The affinity of EF-1α for F-actin weakens as the pH increases over the physiological range while the affinity of EF-1α for aa-tRNA remains constant between pH 6.5 and 7.0. This may cause the binding of EF-1α to aa-tRNA to become favored as the affinity of EF-1α for F-actin decreases. To test this hypothesis, we reconstituted the EF-1α:GTP:Phe-tRNA ternary complex to determine if EF-1α in the ternary complex is capable of bundling and binding to F-actin as well. As controls we used deacylated tRNA, which has a lower affinity for EF-1α:GTP, and guanine nucleotide–free EF-1α, which cannot bind to aa-tRNA (see Moon and Weissbach, 1972; Nagata et al., 1976; Slobin and Möller, 1976; and Fig. 3 *A*). As shown in Fig. 3, *A* and *B*, addition of EF-1α in the form of ternary complex at pH 6.5 has little effect on the ability of EF-1α to bind and cross-link F-actin as detected by light scattering (Fig. 3 *A*) and F-actin cosedimentation (Fig. 3 *B*). Analysis of the amount of Phe-tRNA in the relation to EF-1α in these F-actin pellets revealed that little Phe-tRNA was found above background in the pellets (Table I), in spite of the fact that stable ternary complex had formed (Figs. 1 and 2). These results indicate that EF-1α in the pellets is no longer bound to Phe-tRNA and that binding of EF-1α to F-actin is favored over binding to Phe-tRNA at pH 6.5.

At pH 7.0, a dramatically different result was obtained. As shown in Fig. 3, *C* and *D*, addition of EF-1α in the form of ternary complex to F-actin reduced the ability of EF-1α to bind and cross-link F-actin as detected by a reduction in light scattering (Fig. 3 *C*) and cosedimentation with F-actin (Fig. 3 *D*). The amount of Phe-tRNA in the pellets was again at background levels, indicating that the EF-1α that cosedimented with F-actin was no longer in the form of ternary complex (Table I).

Interaction of GST–EF-1α Fusion Proteins with Actin Filaments

To gain insight into the mechanism by which the binding of EF-1α to F-actin is blocked by aa-tRNA in a pH-dependent manner, mapping of F-actin binding site(s) on EF-1α was conducted. As shown in Fig. 4, *A* and *B*, the native *Dictyostelium* EF-1α and affinity-purified recombinant *Dictyostelium* EF-1α cosediment with actin filaments, indicating that the recombinant EF-1α retains F-actin binding activity. Under the same conditions, GST itself did not cosediment with actin filaments (data not shown). To see if vertebrate EF-1αs would also bind to actin filaments, a mouse EF-1α GST fusion protein was purified and found to bind to actin filaments (Fig. 4 *C*).

Interaction of Truncated GST–EF-1α Fusion Proteins with Actin Filaments

Specific protein–protein interactions can be dependent on primary sequence or secondary structure. In the latter case, it is important to avoid disruption of the secondary structure of the protein while trying to identify the specific binding site(s) by truncation/deletion. Although currently the three-dimensional (3-D) structure of EF-1α is not available, the crystal structure of EF-Tu, a prokaryotic homologue of EF-1α, has been elucidated (Jurnak, 1985; Clark et al., 1990; Berchtold et al., 1993; Kjeldgaard et al., 1993). EF-Tus share sequence homology with EF-1α's and have the same function in protein translation. Assuming that the 3-D structure of EF-1α is similar to that of EF-Tu, we constructed a homology 3-D model of *Dictyostelium* EF-1α using the *Thermus aquaticus* EF-Tu structure as template and defined three regions of the protein as domain I, do-
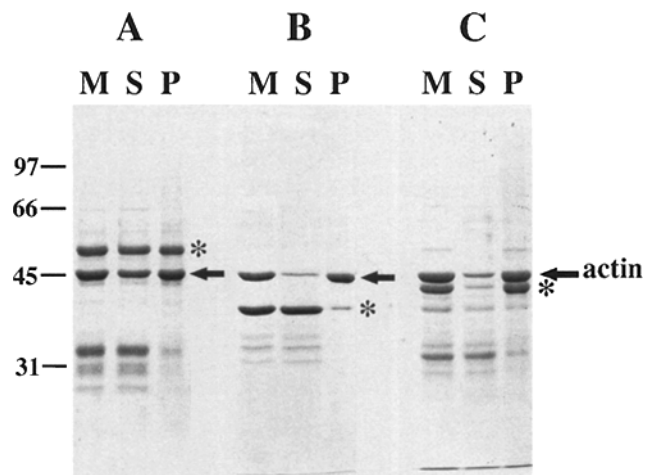
Figure 5. F-actin binding activity is located in domains I and III but not in domain II. The cosedimentation assays were performed in molar ratio of fusion protein/actin = 1:2 (1.5 μM of fusion protein to 3 μM actin or 2 μM of fusion protein to 4 μM actin). Results of actin cosedimentation assays analyzed by 10% SDS–polyacrylamide gels. (Bands with lower molecular weights are proteolytic fragments of the fusion proteins.) *M, S,* and *P* as in Fig. 4. *, GST–EF-1α truncate. (*A*) GST-Dicty-dm I (amino acid 1–221) with *Dictyostelium* actin. (*B*) GST-Dicty-dm II (amino acid 222–320) with *Dictyostelium* actin. (*C*) GST-Dicty-dm III (amino acid 321–456) with *Dictyostelium* actin.

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Dicty.  --MEFPESEKTHINIVVIGHVDAGKSTTTGHLIYK--CGGIDKRVIEKYEKEASEMGKQSFKYAWVMDKLKAERERGITI 76
Mouse   -----MGK.........I....S..I..........--.......T...F....A....G.......L............. 73
EF-Tu   -AKGKFIRT.P.V.VGT.I....H..T.L.---------AALTYVAAAENPNVEVKDYGD-------I..APE..A..... 63
H-ras   ---------M.EYKL..VI.AGGV..IAL.IQ..QNHFVDEY.PT-..DSY.QVVID.ETCL---LDILDTAGQE.YSAMR 67

Dicty.  DIALWKFETSKYYFTIIDAPGHRDF--IKNMITGTSQADCAVLVIASPTGEFEAGIAKNGQTREHALLAYTLGVKQMIVA 154
Mouse   ..SQR........V...ES..I....---.............IV.AGV.......S.................L..G 151
EF-Tu   NT.HVEY..A.RHYSHV.C..I.A.Y--.......AA.M.G.I..VSAAD.PM-------P.....I...RQV..PYIV.F 134
H-ras   .QYMRTG.GFLCV.A.NNTKSFE.IHQYREQ.KRVKDS.DVPMVLVGNKCDLA.RTVESR.AQD---..RSY.IPYIETS 144

                    |----- A -----|  |--- B ---|
Dicty.  INKMDEKSTNYSQARYDEIVKEVSSFIKKIGYNPEKVAFVPISGWNGDNMLERSDKMEWYKG------------PTLLEA 222
Mouse   V|...STEPP...K..E.......TY........DT.................P.AN.P.F..WKVTRKDGSAVA...... 231
EF-Tu   I..V.MVD---DPELL.LVEM..RDLLNQYEFPGDE.PVIRG.ALKA---..EMH.NPKT.RGENEWVDKIWELLDAIDE 208
H-ras   AKTRQGV-----EDAFYTL.R.IRQHKLRKLNP.DESGPGCM.CKCVLS                               188

              |----- C ----|
Dicty.  LDAIVEPKRPHDKPLRIPLQDVYKIGGIGTVPVGRVETGIIKPGMVVTFAPAGLS---TEVKSVEMHHEQLPEARPGDNV 299
Mouse   ..C.L-.P..T..............................VL.........VNVT---...........A.S..L..... 307
EF-Tu   --Y.PT.V.DV...FLM.VE..FT.T.R...AT..I.R.KV.V.DE.EIVGLAPETRR.V.TG....RKT.D.GIA.... 286

              |-- D --|
Dicty.  GFNVKNVSVKEIKRGMVAGDSKNDPPQETEKFVAQVIVLNHPGQIHAGYSPVLDCHTAHIACKFTEIVDKVDRRTGAVVA 379
Mouse   .........DVR..N..........M.AAG.T....I.......S...A..............A.LKE.I...S.---K 384
EF-Tu   .LLLRG..RE.VE..Q.LA---.PGSITPHT..E.S.YI.KKEE--GGRHTGFFTGYRPQFYFRT.DVTGV.R-------- 353

Dicty.  KEGTAAVVLKNGDAAMVELTPSRPMCVESFTEYPPLGRFAVRDMRQTVAVGVIKSTVKKAPGKAGDKKGAAAPSKKK   456
Mouse   .LEDGPKF..S....I.DMV.GK.......SD.....................AVD...A.AGKVT.S.QKAQ.A.    461
EF-Tu   -LPQGVEMVMP..NVTFTVELIK.VAL.EGL------...I.EGGR..GA..VTKILE                       405
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Figure 6. EF-1αs are highly conserved in eukaryotes but have less sequence homology with EF-Tu or *Ras* protein. ● indicates identical amino acid residue. Boxes are consensus sequences for guanine nucleotide binding; |-A-|, depactin-like; |-B-|, actin-like; |-C-|, actA-like; and |-D-|, actobidin-like. Sequences are from: (1) *Dictyostelium* EF-1α: Yang et al., 1990; (2) mouse EF-1α: Lu and Werner, 1989; (3) *T. aquaticus* EF-Tu: Voss et al., 1992; and (4) H-*ras*: Capon et al., 1983.

main II, and domain III (as shown in Fig. 8). These three domains were expressed as GST fusion proteins and purified for actin-binding assays. As shown in Fig. 5, *A* and *C*, GST domain I and GST domain III of *Dictyostelium* EF-1α cosediment with actin filaments. However, GST domain II of *Dictyostelium* EF-1α did not cosediment with F-actin above background under the same conditions (Fig. 5 *B*). In a similar way, we constructed and affinity-purified GST domain I of mouse EF-1α, which also bound to actin filaments in actin cosedimentation assays (data not shown).

Sequence comparison indicates that domain I of EF-1α, like that of EF-Tu, is the guanine nucleotide–binding domain that is conserved in the G-protein family (Woolley and Clark, 1989). This domain contains three consensus sequences, GxxxxGK, DxxG, and NKxD. Because we had identified an F-actin–binding activity in domain I of EF-1α, we investigated whether the consensus sequences of guanine nucleotide–binding domains (see boxed sequences in Fig. 6) and related secondary structure are sufficient for F-actin binding. Human H-ras is one of these G-proteins containing all the consensus sequences and is roughly the same size as domain I of EF-1α (with 19% of sequence identity and 44% of similarity). We tested the interaction of F-actin with H-ras wild-type GST fusion protein GST–H-*ras* Gly12 and a mutant GST–H-*ras* Val12 (gift from Dr. D. Bar-Sagi, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). Both of these recombinant ras proteins did not bind to actin filaments (data not shown).

Interaction of Truncated GST Fusion Proteins with Actin Filaments Is pH Dependent

It has been demonstrated that the binding of *Dictyostelium* and vertebrate EF-1α to actin filaments is pH dependent and increases in pH reduce the F-actin–binding affinity (Edmonds et al., 1995, 1996). As two F-actin–binding domains on *Dictyostelium* EF-1α have been identified, it was important to know whether these domains show pH dependence for F-actin binding like the native protein and, if so, whether they exhibit equal pH sensitivities. To

answer these questions, we performed actin cosedimentation assays over the physiological pH range (see Furukawa et al., 1988, and references therein) between pH 6.0 to 7.6 by using these truncates of EF-1α. As illustrated in Fig. 7, the interactions of F-actin with recombinant domains I and III show a pH dependence generally in agreement with the native *Dictyostelium* EF-1α (Edmonds et al., 1995). Recombinant domain II was used as a control and shows no binding to F-actin throughout the tested pH range. A close comparison of pH-dependent curves of domains I and III indicates that although both domains exhibit pH-dependent F-actin binding, domain I is more pH sensitive than domain III such that at pH 6.6, domain I has lost most F-actin–binding activity while domain III still
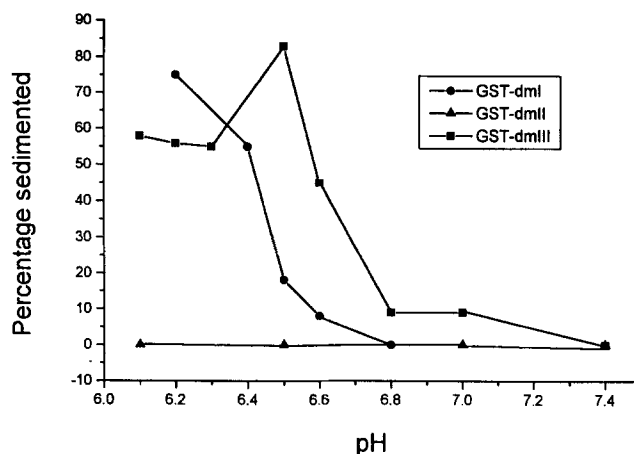

Figure 7. Domains I and III have different pH sensitivities for F-actin binding. 1.5 μM (or 2 μM) of each GST fusion protein was allowed to interact with 3 μM (or 4 μM) of *Dictyostelium* actin at the indicated pH. The amount of F-actin and fusion protein that sedimented were quantified as described in Materials and Methods. Fusion proteins did not sediment in the absence of F-actin over the pH range tested under these assay conditions. The results are representative of three separate experiments.
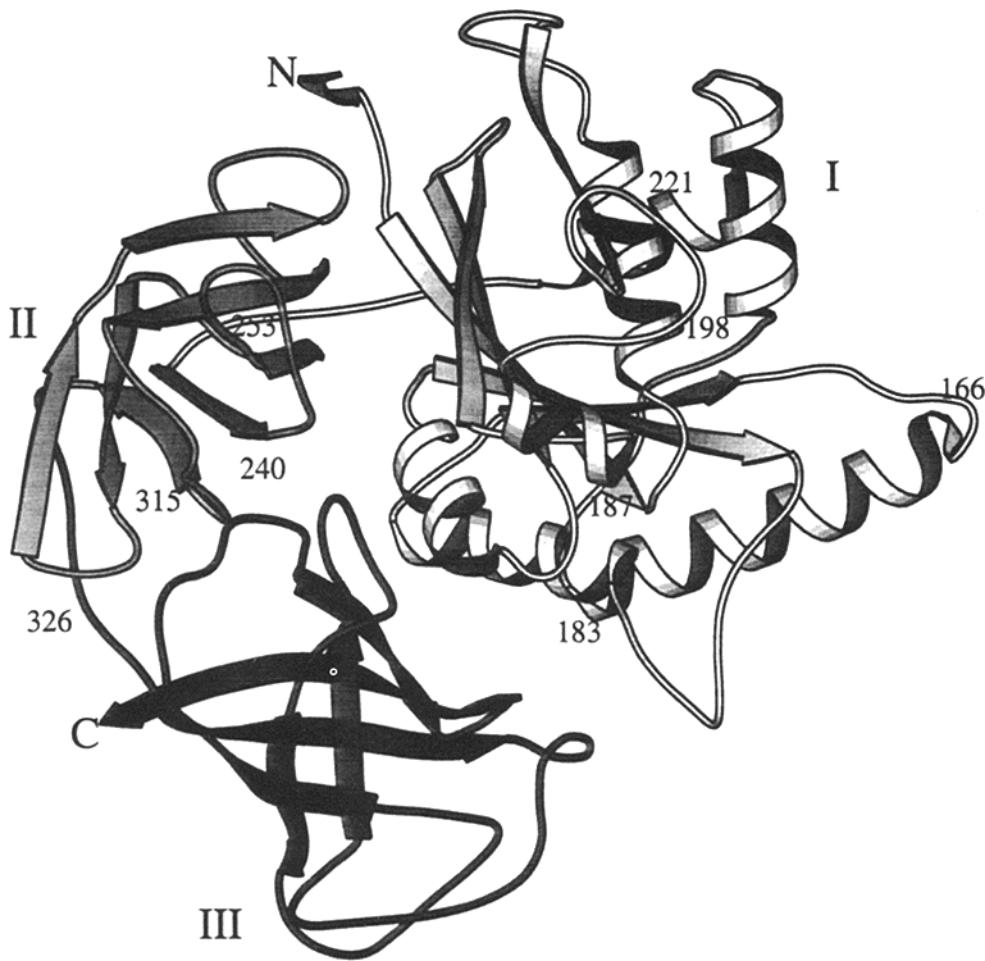
Figure 8. A three-dimensional model of *Dictyostelium* EF-1α. The model was constructed by using molecular modeling software and is in a GTP-binding conformation (for details, see Materials and Methods). The domain with α-helixes is domain I. The gray-shaded region is domain II, and the dark-shaded region is domain III. The aa-tRNA binding sites are on the surface of the molecule facing the reader. The actin binding sites in domains I and III are postulated to be on the same surface.

binds to F-actin substantially. Both domains I and III require lower pH to bind to F-actin compared to that for the native full-length EF-1α (Edmonds et al., 1995). This may be due to the collaborative effect of the two actin-binding sites in the native protein.

Discussion

Interaction of Domains I and III with F-actin

In contrast to the rich structural information about EF-Tu and its effectors (see Jurnak, 1985; Clark et al., 1990; Berchtold et al., 1993; Kjeldgaard et al., 1993; Nissen et al., 1995; Kawashima et al., 1996), the crystal structure of EF-1α is not yet available. As an elongation factor participating in protein synthesis, both EF-Tu and EF-1α bind to a variety of ligands including factors such as guanine nucleotides, exchange factors, aa-tRNAs, and ribosomes. The property of multieffector binding and the common task of protein translation may have restrained EF-Tu and EF-1α into similar, if not identical, 3-D conformations during the evolution. Consistent with this prediction, during the homology modeling of *Dictyostelium* EF-1α, we found that all of the deletions and insertions occur in the loops. Our 3-D model of EF-1α, obtained from a combination of sequence alignment, sequence deletion/insertion, and energy mini-

mization, is very similar to that of EF-Tu (Kjeldgaard et al., 1993; Fig. 8).

To date, it has been observed that EF-1α's from *Dictyostelium*, carrot, rabbit, rat, and mouse bind to actin (Yang et al., 1990, 1993; Bektas et al., 1994; Edmonds et al., 1996; this study). Like domain I of *Dictyostelium* EF-1α, domain I of mouse EF-1α has also been shown to bind to F-actin. Given the fact that EF-1α is a very conserved family with more than 70% of sequence identity among eukaryotes, it is likely that all the EF-1α's bind to F-actin via the same actin-binding sites. Although other factors, such as modifications and interactions with other molecules, may play important roles in the regulation of binding of EF-1α to F-actin, pH appears to be a major factor in this aspect (Edmonds et al., 1995; Liu et al., 1996).

Possible actin binding sites have been predicted for EF-1α based on sequence homology to other known actin-binding proteins (Yang et al., 1990; Edmonds, 1993). As illustrated in Figs. 6 and 8, residue 166–183 (sequence A) is depactin-like with 39% identity to depactin 3–20 (Sutoh and Mabuchi, 1989); residue 187–198 (sequence B) is actin-like with 40% identity to actin 213–222 (Vandekerckhove and Weber, 1980); residue 240–253 (sequence C) is *listeria* actA–like with 43% identity to actA 237–240, and residue 315–326 (sequence D) is actobindin-like with 50% identity to actobindin 30–39 (Kocks et al., 1992; Vandekerckhove

et al., 1990). Of these four proposed sites, sequences C and D are located in domain II that shows no F-actin–binding activity. Although the other two homologous sequences (A and B) fall in domain I, they are not predicted as the actin-binding sites on EF-1α because they locate on the opposite side of the molecule involving aa-tRNA binding (Fig. 8). Nevertheless, their validity as actin-binding sites on EF-1α awaits further definition of actin-binding sites at higher resolution.

EF-1α is a very abundant protein that represents about 1–2% of total protein in most cells. Stoichiometric studies indicate that there is 17–35-fold molar excesses of EF-1α to ribosomes and sevenfold to EF-1β (Slobin, 1980). In addition to binding to actin, EF-1α has been reported to bind to and sever microtubules, activate phosphotidylinositol-4-kinase, and bind to calmodulin (Yang et al., 1993; Durso and Cyr, 1994a; Kaur and Ruben, 1994; Shiina et al., 1994). These observations have led to the suggestion that EF-1α may regulate cytoskeletal function independent of translation (Durso and Cyr, 1994b; Condeelis, 1995). *Dictyostelium* EF-1α cross-links actin filaments with a unique cross-bridge bonding rule (Owen et al., 1992). EF-1α also regulates the rate and extent of actin polymerization in vitro and these activities are correlated with its F-actin cross-linking activity (Murray et al., 1996). The high concentration of EF-1α in *Dictyostelium* (about 75 μM) makes it a likely predominant F-actin bundler, a conclusion that is consistent with its colocalization with F-actin in vivo (Dharmawardhane et al., 1991; Edmonds et al., 1995) and its association with actin bundles in situ (Liu et al., 1996).

Because two F-actin–binding domains have been identified in EF-1α, one would ask how EF-1α cross-links actin filaments? That is, do these two domains bind to the same or different regions on the actin monomers in neighboring filaments? We conducted sequence comparisons of the two F-actin–binding domains and the results indicate that they share only 22% sequence identity. Furthermore our preliminary data suggest that these two actin–binding domains do not compete with each other for binding to F-actin. These observations suggest that the two actin–binding domains of EF-1α probably bind to different regions on the actin monomer. Consistent with this prediction is the unique bonding rule of EF-1α where it cross-links actin filaments that are rotated by 90° relative to each other (Owen et al., 1992). Therefore, the two actin–binding domains of an EF-1α must interact with different regions of actin monomers in neighboring actin filaments.

An intriguing aspect of the binding of domains I and III of *Dictyostelium* EF-1α to F-actin is their different pH sensitivities (Fig. 7). Therefore, increasing pH would first dissociate domain I from the actin filament while domain III would remain bound to an actin filament. With the continuing elevation of pH, domain III would eventually dissociate from the actin filament, leaving EF-1α free. In fact, such a transition of EF-1α from bundling, to single filament binding, and finally dissociation from actin filaments was observed for native *Dictyostelium* EF-1α by Edmonds et al. (1995). Therefore, the differential pH sensitivities of domains I and III over the physiological range of pH would regulate whether EF-1α is free or bound to F-actin in a bivalent or monovalent interaction. This could be physiologically important in terms of how the interaction of EF-1α with actin filaments affects protein synthesis as discussed next.

Spatial Relationships of the F-actin and aa-tRNA–binding Sites on EF-1α

The recent elucidation of the crystal structure of EF-Tu:GDPNP:Phe-tRNA ternary complex has resolved the puzzle of how EF-Tu interacts with aa-tRNA (Nissen et al., 1995). In the crystal model, the phenylalanylated CCA end and the phosphorylated 5' end of Phe-tRNA are located in a clef formed by interfaces of all three domains, in agreement with an earlier prediction by using ¹H-NMR spectroscopy (Förster et al., 1993). In addition, the T stem of Phe-tRNA interacts with the surface of the β-barrel in domain III. Because our 3-D model of EF-1α has similar topology to that of EF-Tu and both elongation factors bind aa-tRNA, this information is extremely useful in predicting F-actin–binding domains on EF-1α. For instance, the blockade of EF-1α binding to F-actin by Phe-tRNA predicts that actin-binding sites on EF-1α are probably located on the same side of the EF-1α molecule and overlap, at least partially, with the aa-tRNA–binding sequences.

pH Regulates the Ability of Phe-tRNA to Block the Binding of EF-1α to F-actin

As described by Edmonds et al. (1995), the interaction of EF-1α and F-actin is pH dependent with $K_d > 2.2$ μM at pH 7.8 and 0.2 μM at pH 6.5. In contrast to the strong influence of pH on actin-binding affinity, little effect of pH on the affinity of EF-1α binding to Phe-tRNA was observed under our experimental conditions as the binding constants were estimated as 0.26 μM at pH 6.5 and 0.22 μM at pH 7.0, respectively (Fig. 2). In addition, Crechet and Parmeggiani (1986) reported an apparent binding constant of 0.24 μM of Phe-tRNA for calf brain EF-1α at pH 7.5. Given the experimental error for these binding constants, it is likely that the binding affinity of EF-1α for aa-tRNA is little affected by the changes of pH over the physiological range, at least not an order of magnitude change as is the binding of EF-1α to F-actin. By using the above binding constants and aa-tRNA, EF-1α, and F-actin at concentrations of 1, 1, and 3 μM, respectively, we have simulated a binding competition between F-actin and aa-tRNA for EF-1α. The resultant prediction is that as pH changes from 6.5 to 7.0, the amount of EF-1α bound to F-actin would decrease from ~70 to ~40%. These theoretical values are very close to those observed in real experiments (Fig. 3). Although the binding affinity of EF-1α to aa-tRNA presumably remains unchanged within this pH range, due to a weakening binding affinity of EF-1α for F-actin as pH changes from 6.5 to 7.0, the amount of EF-1α bound to aa-tRNA would double from ~23 to ~44%.

Protein Synthesis and the Binding of EF-1α to F-actin

The role of EF-1α in translation is to transport aa-tRNA from tRNA synthetases to the ribosomes. Because free aa-tRNA is unstable at physiological pH and aa-tRNA is not freely diffusible in the cell (Negrutskii and Deutscher, 1991), minimally an equal molar concentration of EF-1α is required to transport aa-tRNA to the ribosomes to ac-

count for the fast rate of peptide elongation in vivo. The intracellular molar ratio of EF-1α to total tRNA has been estimated variously as 13:1 and 1:6 in rabbit reticulocytes, respectively (Burka, 1968; Slobin, 1980), and about 1:1.5 in rat liver (Blobel and Potter 1967; Edmonds et al., 1996), and about 90% or more of tRNA is aa-tRNA (Allen et al., 1969; Vaughan and Hansen, 1973; Ogilvie et al., 1979). Therefore, the general assumption is that the molar ratio of EF-1α to aa-tRNA in eukaryotes is about 1:1, which is the documented molar ratio of EF-Tu to aa-tRNA in bacteria (Gouy and Granthan, 1980; Pingoud et al., 1983). Because significant amounts of EF-1α are bound tightly to actin (both monomers and filaments) in vivo (Dharmawardhane et al., 1991; Edmonds et al., 1995, 1996; Murray et al., 1996), most of EF-1α would be bound to actin rather than to aa-tRNA in resting cells with low-resting pH. In some intracellular compartments where significant amounts of EF-1α are sequestered in actin bundles, the relative concentration of EF-1α that is capable of binding to aa-tRNA would be so low that it may become a rate-limiting factor in peptide elongation.

Having identified two F-actin–binding domains on EF-1α with different pH sensitivities, it is possible to speculate how changes in pH over the physiological range might influence protein synthesis and the organization of the cytoskeleton through its effects on the interaction of EF-1α with F-actin. The pH of cytoplasm in *Dictyostelium* cells has been measured using a variety of methods that demonstrate that pH ranges from 6 to 8 in vivo (summarized in Furukawa et al., 1988). The mean pH in resting cells has been measured as 6.7 by NMR and between 6.0 and 7.2, depending on stage in development (Satre et al., 1986; Furukawa et al., 1990, respectively). Aerts et al. (1987) have measured a pH increase of 0.2 U upon stimulation of resting cells with cAMP. Therefore, changes in pH from 6.0 to 7.2 are expected to occur routinely in *Dictyostelium* amebae and this is the pH range over which large changes in the interaction between EF-1α and F-actin occur.

Our results predict that in resting cells at low pH , EF-1α on the cytoskeleton is inactive in protein synthesis because it binds to actin filaments, which prevents EF-1α from binding to aa-tRNA. When cytoplasmic pH increases as a result of hormone stimulation, domain I of EF-1α dissociates from the actin filaments because of its greater pH sensitivity compared to domain III. This transition of EF-1α from bivalent to monovalent interaction with actin filaments may have dual effects. First, the decrease of cross-linking provides a more dynamic environment for the reorganization of the cytoskeleton (Murray et al., 1996), including the translational machinery that is associated with the cytoskeleton (Singer, 1993). This reorganization of the actin cytoskeleton and its associated EF-1α, triggered by hormone stimulation, has been well documented in many types of cells (Dharmawardhane et al., 1991; Edmonds et al., 1995, 1996). Second, the release of EF-1α from actin binding by increases in pH would make EF-1α accessible to aa-tRNA and binding of aa-tRNA to newly exposed EF-1α would release the resultant ternary complex from association with the actin filaments, supplying a very high local concentration of "active" EF-1α to facilitate peptide elongation upon initiation. Since mRNA, aa-tRNA synthetases, and polyribosomes are associated with actin fila-

ments (Bassell et al., 1994; Mirande, 1991), the high local concentration of EF-1α supplied by the dissociation of the EF-1α/F-actin complex would be proximal to these translational components, and this may be an important consequence of localizing the translational apparatus on actin filaments in cells.

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