Binding of Elongation Factor eEF1A2 to Phosphatidylinositol 4-Kinase β Stimulates Lipid Kinase Activity and Phosphatidylinositol 4-Phosphate Generation*

Received for publication, March 29, 2006, and in revised form, November 6, 2006. Published, JBC Papers in Press, November 6, 2006, DOI 10.1074/jbc.M602955200

Sujeever Jeganathan and Jonathan M. Lee

From the Department of Biochemistry, Microbiology, and Immunology, University of Ottawa, Ottawa, Ontario K1H 8M5, Canada

Eukaryotic protein translation elongation factor 1α 2 (eEF1A2) is an oncogene that transforms mammalian cell lines and increases their tumorigenicity in nude mice. Increased expression of eEF1A2 occurs during the development of breast, ovarian, and lung cancer. Here, we report that eEF1A2 directly binds to and activates phosphatidylinositol 4-kinase III β (PI4KIIIβ), an enzyme that converts phosphatidylinositol to phosphatidylinositol 4-phosphate. Purified recombinant eEF1A2 increases PI4KIIIβ lipid kinase activity in vitro, and expression of eEF1A2 in rat and human cells is sufficient to increase overall cellular phosphatidylinositol 4-kinase activity and intracellular phosphatidylinositol 4-phosphate abundance. siRNA-mediated reduction in eEF1A2 expression concomitantly reduces phosphatidylinositol 4-kinase activity. This identifies a physical and functional relationship between eEF1A2 and PI4KIIIβ.

eEF1A2 is one of two members of the eEF1A family of proteins (eEF1A1 and eEF1A2). During protein translation elongation, eEF1A proteins bind amino-acylated tRNA and facilitate their recruitment to the ribosome (1). Aside from their canonical role in protein translation, eEF1A proteins have other functions, including binding actin and inducing rearrangements of the actin and tubulin cytoskeleton (2, 3). The inactivation of the mouse eEF1A2 homolog, Eef1a2, leads to immunodeficiency and death by 30 days of age (4, 5).

Mammalian eEF1A2 mRNA can be detected only in normal mammalian heart, brain, and skeletal muscle tissues (6–8). However, high levels of eEF1A2 protein and mRNA are observed in a 30–60% fraction of ovarian, breast, and lung tumors (9–12). We have previously reported that eEF1A2 has transforming properties; ectopic expression of wild type human eEF1A2 in mammalian cells enables anchorage-independent growth and enhances tumorigenicity in nude mice (9). Thus, eEF1A2 has an important role in promoting tumor development. However, the mechanism by which eEF1A2 promotes oncogenicity remains unclear.

It has previously been reported that an eEF1A-like protein purified from carrots, PIK-A49, binds and activates carrot phosphatidylinositol 4-kinase (PI4K) (13, 14). This suggests an important relationship between translation elongation and phosphatidylinositol generation. Phosphatidylinositol 4-kinases are negatively charged, membrane-bound phospholipids that serve as regulators of multiple signaling pathways (15–18). Phosphatidylinositol 3-kinases, PI4K, and phosphatidylinositol 5-kinases phosphorylate the D3, D4, and D5 carbons. Specific kinase families are responsible for phosphorylation at each of these sites. Phosphatidylinositol 3-kinases, PI4K, and phosphatidylinositol 5-kinases phosphorylate the D3, D4, and D5 inositol carbons, respectively (15–17).

PIK-A49 showed in vitro translation elongation factor activity and an ability to activate in vitro PI4K lipid kinase activity (13, 14). However, PIK-A49 does not have complete amino acid sequence identity with wild-type carrot eEF1A and has yet to be cloned as a full-length cDNA. It is therefore unclear whether PIK-A49 is a bona fide eEF1A protein; nor is it known whether wild-type carrot eEF1A or eEF1A proteins from nonplant species participate in PI4K activation. In addition, there are three identified subfamilies of PI4K proteins, phosphatidylinositol 4-kinase III α, phosphatidylinositol 4-kinase III β (PI4KIIIβ), and PI4KII (19, 20), and it is unclear which PI4K isoform(s) is activated by PIK-A49 or other eEF1A proteins. Moreover, in vitro PI4K activation by PIK-A49/eEF1A has unknown physiological significance.

Here we report that human eEF1A2 can directly bind and activate PI4KIIIβ. Ectopic expression of eEF1A2 in rodent and human cells increases overall PI4K activity and cellular phosphatidylinositol 4-phosphate (PI4P) generation. Furthermore, eEF1A2 ablation reduces endogenous PI4K activity. This suggests that eEF1A2 is a physiological regulator of PI4KIIIβ.

EXPERIMENTAL PROCEDURES

Cell Lines—MCF7, BT549, and Rat2 cells were purchased from the American Type Culture Collection (Manassas, VA) and grown according to their instructions.

* This work was supported by the National Cancer Institute of Canada (with funds from the Canadian Cancer Society) and the Canadian Breast Cancer Research Alliance (to J. L.) and a studentship from the Canadian Institutes of Health Research (to S. J.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Adenoviral Vectors—eEF1A2 was subcloned into the pShuttle-IREs plasmid (EcoRV/Xhol) with a FLAG epitope tag. eEF1A2 and GFP virus were manufactured by the Adenoviral Core Facility of the University of Ottawa. For viral transduction, BT549 and Rat2 cells were infected with Ad-eEF1A2 or Ad-GFP at a multiplicity of infection (MOI) of 200 (BT549) or 500 (Rat2) in complete media. Cells were incubated with virus for a minimum of 24 h.

Antibodies—Antibodies used for experiments were as follows: human PI4KIIIβ (Upstate Cell Signaling Solutions, Charlottesville, VA), β-actin (Sigma), horseradish peroxidase-conjugated goat anti-mouse IgG (Upstate Cell Signaling Solutions), and horseradish peroxidase-conjugated anti-rabbit IgG (Cell Signaling Technology, Danvers, MA). The generation of the rabbit polyclonal eEF1A2 antibody and its validation in Western blotting, immunoprecipitation, and immunohistochemistry is described elsewhere (10).

GST Fusion Proteins—eEF1A2 cDNA was cloned into the EcoRI/NotI site of pGEX-4T2 (Amersham Biosciences). GST-eEF1A2 was transformed into Escherichia coli BL21DE3 and grown in Luria-Bertani media with 100 μg/ml ampicillin to A600 = 0.7. 0.5 mM isopropyl 1-thio-β-D-galactopyranoside was added for 2 h at 25 °C. Bacteria were lysed in 25 mM HEPES, pH 7.9, 100 mM KCl, 2 mM EDTA, 20% glycerol, 2 mM dithiothreitol, and 1× protease inhibitor mixture (Roche Applied Sciences). Glutathione-Sepharose 4B beads (Amersham Biosciences) were equilibrated in lysis buffer and mixed with sonicated suspensions. PI4KIIIβ in pGEX-6P-3 was a gift of T. Balla (21, 22). The GST-PI4KIIIβ fusion protein was purified as described (22). GST-C/EBPβ was a kind gift from N. Wiper-Bergeron (University of Ottawa). To remove the GST moiety, 100 μg of GST-eEF1A2 was incubated overnight with 1 unit of thrombin (Amersham Biosciences) in 1× PBS at room temperature. PI4KIIIβ was generated by cleaving 100 μg of GST-PI4KIIIβ with 1 unit of PreScission Protease (Amersham Biosciences) in 50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, pH 7.0, at 4 °C.

Lipid Kinase Assay—10 μl of recombinant protein in PBS, at concentrations indicated in the figure legends, was added to 35 μl of kinase buffer (1 mM EDTA, 30 mM Hepes, pH 7.4, 100 mM NaCl, 2 mM MgCl2, and 0.2% Triton X-100), 3 mM PI (or as indicated in the figure legend), and 5 μl of 10 mM ATP containing 10 μCi of [32P]ATP, and incubated for 60 min. The reaction was stopped by the addition of 60 μl of 1 N HCl. Phospholipids were extracted by adding 160 μl of CHCl3/MeOH (1:1, v/v). After a brief vortex, samples were centrifuged for 10 min at 10,000 × g. Aliquots of the organic phase (10–20 μl) were spotted onto TLC plates (Sigma) and placed in a pre-equilibrated tank containing CHCl3/acetone/MeOH/HOAc/water (46:17:15:14:8, v/v/v/v/v). Prior to use, TLC plates were precoated with 1% potassium oxalate, 3 mM EDTA in methanol/water (2:3, v/v) for 1 h and allowed to air dry overnight. Plates were activated by baking for 1 h at 110 °C. Phosphatidylinositol standards were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). PI4P spots were scraped and dissolved in 1–2 ml of water. Aliquots were then diluted in Betamax scintillation fluid (ICN Biomedicals, Irvine, CA), and scintillation counts were taken using the Wallac 1414 liquid scintillation counter (Fisher). Values of $K_m$, $V_{max}$, and $K_{cat}$ were determined using the GraphPad Prism software (San Diego, CA).

Cross-linking Studies—Purified eEF1A2 and/or PI4KIIIβ (without GST) were incubated in PBS and cross-linked with 30 mM dimethyl pimelimidate (Pierce) in 0.2 mM ethanolamine, pH 8.0, at room temperature. Reactions were stopped by the addition of glacial acetic acid at a 1:4 (v/v) ratio to the sample. Proteins were concentrated to a final volume of 50 μl with Microcon centrifugal filter devices (Millipore, Billerica, MA), solubilized using 5% (w/v) sucrose in water, and electrophoresed in a continuous, nondenaturing 5% (29:1 acrylamide/bisacrylamide) polyacrylamide gel using 100 mM sodium phosphate (pH 7.0) running buffer as previously described (23).

Cell Lysis and Co-immunoprecipitation—For co-immunoprecipitation, cells were grown to 80–95% confluence in 100-mm cell culture plates. Cells were lysed by sonication on ice in detergent-free buffer (137 mM NaCl, 8 mM KH2PO4 (pH 7.5), 2.7 mM KCl, 2.5 mM EDTA, 1% aprotinin, 1 mg/ml leupeptin, 50 mM NaF, 1 mM Na3VO4, 10 μg/ml pepstatin, 1 mM phenylmethylsulfonyl fluoride) and centrifuged at 10,000 × g for 20 min to remove membranes. Supernatants were collected, and protein levels were quantified using a Bradford assay (Bio-Rad) according to the manufacturer’s instructions. 100 μg of total protein was precleared with protein G-Sepharose (Amersham Biosciences) for 1 h at 4 °C. Following this, 2–4 μg of PI4KIIIβ, FLAG, or eEF1A2 antibody coupled to beads were added and incubated overnight at 4 °C. Beads were washed three times in PBS, centrifuged, and boiled for 5 min in sample buffer, and the supernatant was subjected to SDS-PAGE. The covalent coupling of the eEF1A2 antibody to protein A-agarose beads was performed using a previously described protocol (24). Western blot detection was according to the manufacturer’s instructions or as described for the eEF1A2 antibody (10).

For Western blotting, cells were lysed in radioimmune precipitation buffer (50 mM Tris-Cl, pH 7.4, 1% Triton X-100, 1 mM sodium deoxycholate, 0.1% SDS, 1 mM EDTA, pH 7.0, 150 mM NaCl, 1% aprotinin, 1 mg/ml leupeptin, 50 mM NaF, 1 mM Na3VO4, 10 μg/ml pepstatin in ethanol, and 1 mM phenylmethylsulfonyl fluoride in Me2SO).

siRNA Transfections—Sequences of the eEF1A2 siRNAs are 5′-UGGUCCUUUUGUCAAUCACtt-3′ (siRNA 1) and 5′-UCGAACUUUCAUGGUGUCCtt-3′ (siRNA 2). The negative control siRNA was purchased from Ambion (catalog number 4611; Austin, TX). siRNA transfections were performed using siPORT Lipid (Ambion) according to the manufacturer’s instructions.

Immunofluorescence—To detect PI4P levels, Rat2 and BT549 cells were transfected with either eEF1A2-pcDNA3.1 (9) or GFP-pcDNA3.1 using Lipofectamine2000 (Invitrogen) according to the manufacturer’s instructions. 5 h post-transfection, transfection medium was removed, and complete medium was added. The next day, cells were fixed and permeabilized as above. Cells were then blocked with 10% goat serum, PBS for 30 min at 37 °C, and anti-PI4P IgM antibody was added (1:100 in PBS overnight; Echelon Biosciences Inc., Salt Lake City, UT). Goat anti-mouse IgM, (R)-phycocerythrin (Caltag Laboratories, Carlsbad, CA) was then added at 1:100 in PBS for 30 min.
eEF1A2 was detected with a monoclonal anti-V5 antibody (1:500 in PBS, 1 h; Sigma) followed by an AlexaFluor 488 goat anti-mouse IgG (1:450 in PBS, 1 h; Invitrogen). Cell nuclei were stained with Hoescht 33258 (20 μg/ml; Sigma) for 10 min at room temperature. Slides were viewed with either a Leica DM-IL fluorescence microscope and deconvoluted using Improvision 3.1 software (Richmond Hill, Canada) or an Olympus Fluoview FV1000 laser-scanning confocal microscope. Fluorescence was quantified with the confocal microscope using Olympus software (FV1000, version 01.04a, Center Valley, PA).

RESULTS

eEF1A2 Increases PI4KIIIβ Lipid Kinase Activity—To determine whether eEF1A2 could activate PI4KIIIβ, we purified recombinant GST-eEF1A2 and GST-PI4KIIIβ. The proteins were isolated both with and without their GST moiety. A Coomassie-stained gel of the purified proteins is shown in Fig. 1A. The predicted molecular mass of full-length, wild-type eEF1A2 is ~54 kDa, and that of PI4KIIIβ is ~100 kDa.

We first determined whether eEF1A2 could increase PI4K activity in cell lysates. As shown in Fig. 1B, the addition of GST-eEF1A2 to a cell lysate of BT549 breast carcinoma cells reproducibly doubled in vitro PI4P generation compared with the addition of GST alone or GST coupled to the C/EBP transcription factor. A representative TLC plate is also shown in the right panel of Fig. 1B.

Bacterially expressed PI4KIIIβ has previously been reported to be active in in vitro lipid kinase assays (21, 22). As shown in Fig. 1C, our GST-PI4KIIIβ was active in vitro, and its kinase activity was inhibitable by 0.2 μM wortmannin. Wortmannin inhibition indicates that we are measuring the lipid kinase activity of a type III PI4K, the family of PI4Ks to which PI4KIII belongs. To investigate whether purified eEF1A2 could directly activate PI4KIIIβ, we next added recombinant eEF1A2 (without GST) to purified PI4KIIIβ (without GST). As shown in Fig. 1D, eEF1A2 increased PI4KIIIβ lipid kinase activity in a dose-dependent manner. PI4KIIIβ activity was increased ~2-fold. 100–200 nM eEF1A2 is required to maximally activate a 100 nM solution of PI4KIIIβ. No enhancement of PI4KIIIβ activity was observed in the presence of bovine serum albumin.

In order to further determine how eEF1A2 affected PI4KIIIβ activity, we experimentally determined the PI4KIIIβ K_m, V_max, and K_cat for phosphatidylinositol with and without eEF1A2. Bovine albumin served as the control. As shown in Fig. 1E, eEF1A2 (without GST) reproducibly increased the V_max of PI4KIIIβ (without GST) from 0.62 to 1.11 μmol/min/mg. This doubling of V_max was also mirrored in the increase of K_cat from 0.82 to 1.48 s^-1. The PI4KIIIβ K_m was unchanged.

eEF1A2 Binds PI4KIIIβ—We next tested whether eEF1A2 could directly interact with PI4KIIIβ in a cell-free system. As shown in Fig. 2A, purified PI4KIIIβ (without GST) was immunoprecipitated by GST-eEF1A2, and eEF1A2 (without GST) was immunoprecipitated by GST-PI4KIIIβ. Neither PI4KIIIβ nor eEF1A2 was immunoprecipitated by GST alone. We next investigated the stoichiometry of eEF1A2/PI4KIIIβ interaction. We incubated recombinant eEF1A2 and PI4KIIIβ (both without GST) and chemically cross-linked the resulting complex. As seen in Fig. 2B, after 1 h of incubation, most of the added PI4KIIIβ and eEF1A2 were found in a complex with an apparent molecular mass of 165 kDa. The formation of the complex was time-dependent, and a greater amount of complex was detected at 60 min of incubation relative to the 30 min time point. eEF1A2 has an apparent molecular mass of ~60 kDa, and that of PI4KIIIβ is ~100 kDa. The ~165-kDa molecular mass of the complex indicates that eEF1A2 and PI4KIIIβ associate with a 1:1 stoichiometry. Neither eEF1A2 nor PI4KIIIβ formed detectable multimers on their own. We were unable to detect protein complexes with a molecular mass greater than 165 kDa, indicating that multimers containing more than one molecule each of eEF1A2 or PI4KIIIβ do not form.

eEF1A2 Activates PI4KIIIβ in Mammalian Cell Lines—To determine whether eEF1A2 and PI4KIIIβ associate in cells, we first identified mammalian cells that express eEF1A2. In rodents and humans, eEF1A2 shows detectable mRNA and protein expression only in the tissues of the heart, brain, and skeletal muscle (6–8). We have also observed eEF1A2 expression in many breast cancer cell lines (10). As shown in Fig. 3A, we detected eEF1A2 protein expression in the MCF7 human breast carcinoma cell line but neither in BT549 breast carcinoma cells nor in the nontransformed Rat2 rat fibroblast cell line.

We then used co-immunoprecipitation to determine whether wild-type eEF1A2 and PI4KIIIβ associate in MCF7 cells. We used detergent-free cell lysis for these experiments. As shown in Fig. 3B, eEF1A2 detectably immunoprecipitated with PI4KIIIβ, and PI4KIIIβ reciprocally immunoprecipitated with eEF1A2. Neither protein bound to the FLAG antibody control. Although immunoprecipitation is only semiquantitative, we estimate that ~10% of endogenous PI4KIIIβ associated with eEF1A2 under detergent-free lysis conditions. A similar ratio of eEF1A2 was associated with PI4KIIIβ.

Both PI4KIIIβ and eEF1A2 have been previously reported to be diffusively cytoplasmic, although PI4KIIIβ has some concentration in the Golgi apparatus (9, 19, 20). We attempted to visualize eEF1A2 and PI4KIIIβ co-localization via immunofluorescence confocal microscopy but were unable to detect significant co-localization between PI4KIIIβ and eEF1A2 (not shown). Co-localization detection is complicated by the diffuse cytoplasmic staining pattern of the two proteins and the limited extent to which the two proteins co-precipitate. Nonetheless, the detectable amount of eEF1A2 that co-precipitates with in vivo PI4KIIIβ indicates that PI4KIIIβ-eEF1A2 complexes do exist in MCF7 cells.

eEF1A2 Is Sufficient to Increase Cellular PI4K Activity—To determine whether eEF1A2 expression was sufficient to increase PI4K activity in mammalian cells, we used an adenovirus to express eEF1A2 in BT549 and Rat2 cells. Neither cell line detectably expresses eEF1A2 without transduction (Fig. 3A). At a low MOI of 20 virus particles/cell, infection had little effect on PI4K activity; lipid phosphorylation was similar between eEF1A2-infected cells and those infected with GFP (Fig. 4A). However, increasing the MOI to 200 reproducibly doubled cellular PI4K activity in eEF1A2-infected cells relative to those infected with GFP (Fig. 4A). Similarly, infection of Rat2 cells with the eEF1A2 adenovirus doubled PI4K activity relative to
FIGURE 1. eEF1A2 activates PI4KIIIβ lipid kinase activity. A, Coomassie stain of purified recombinant eEF1A2 and PI4KIIIβ proteins with and without their GST moiety. 30 μg of each sample were loaded on a 10% SDS-polyacrylamide gel. B, left, the addition of 10 μM GST-eEF1A2 to a protein lysate from BT549 cells increases total PI4K lipid kinase activity relative to the addition of GST alone or GST-C/EBPβ. Activation is calculated relative to the GST alone control and is the mean and S.D. of three independent experiments with triplicate scintillation counts. Significant activation (p < 0.05, Student’s t test) is indicated by an asterisk. Right, a representative TLC plate from one of the kinase assays. C, purified recombinant GST-PI4KIIIβ protein has lipid kinase activity (open circles) that is inhibited by 0.2 μM wortmannin (closed circles). Fold increase is calculated relative to no protein control. The reaction contained 20 mM phosphatidylinositol. D, PI4KIIIβ (GST-free) activity is increased by eEF1A2 (GST-free) addition (open circles) but not by bovine albumin (closed circles). Lipid kinase assays are the mean and S.D. of triplicate experiments with 3 mM phosphatidylinositol and 100 nM PI4KIIIβ (GST-free). E, eEF1A2 increases the Vmax of the reaction but not the Kcat. Top, a representative kinase assay using 100 nM PI4KIIIβ (GST-free) and either 2.5 μM eEF1A2 (GST-free) or 2.5 μM bovine albumin. Bottom, summary of PI4KIIIβ kinetic parameters. Values are the means and S.D. from three independent experiments. eEF1A2 significantly increases the Vmax and Kcat values (* and †, respectively; p < 0.0005, unpaired t test with Welch correction) but not the Kcat, BSA, bovine serum albumin.
eEF1A2 Activates PI4KIIIβ

**FIGURE 2.** eEF1A2 and PI4KIIIβ interact in a cell-free system. A, 30 μg of purified eEF1A2 or PI4KIIIβ, both GST-free, were incubated with 30 μg of GST, GST-PI4KIIIβ, or GST-eEF1A2. Precipitated proteins were then detected by Western blotting (WB) with PI4KIIIβ or eEF1A2 antibodies (indicated). B, the eEF1A2-PI4KIIIβ complex consists of a 1:1 molar ratio of eEF1A2 and PI4KIIIβ. 10 μM each of eEF1A2 or PI4KIIIβ (both without GST) were incubated for the indicated times and cross-linked with dimethyl pimelimidate. Purified eEF1A2 and PI4KIIIβ have apparent molecular masses of 60 and 100 kDa, respectively. Incubating a mixture of eEF1A2 and PI4KIIIβ results in a complex with an apparent molecular mass of ~165 kDa.

**FIGURE 3.** eEF1A2 and PI4KIIIβ interact in human cells. A, eEF1A2 protein expression is detected in MCF7 cells but not in BT549 or Rat2 cells. Actin is the loading control. B, antibodies for either eEF1A2 or PI4KIIIβ were used for immunoprecipitation (IP) in membrane-free whole cell lysates (WCL) of MCF7 cells made by sonication in detergent-free lysis buffer. An anti-FLAG antibody is used as a specificity control. Co-immunoprecipitating proteins were detected by Western blotting (WB) using eEF1A2 and PI4KIIIβ antibodies. The WCL lane contains 25 μg of total cellular protein, and each immunoprecipitation was performed using 25 μg of protein lysate.

**DISCUSSION**

Phosphatidylinositol lipids are well documented participants in pathways that regulate organelle biogenesis, cell morphology, proliferation, and oncogenesis (25). In this report, we show that eEF1A2 binds PI4KIIIβ, increases its in vitro lipid kinase activity and can increase intracellular PI4P generation in rat and human cells.

We find that purified eEF1A2 can increase recombinant PI4KIIIβ activity in vitro. Two other direct PI4KIIIβ activators have been previously identified: ADP-riboseylation factor and neuronal calcium sensor-1 (26, 27). ADP-riboseylation factor, a small GTPase, regulates Golgi function through PI4KIIIβ activation (27). Neuronal calcium sensor-1 controls IgE-mediated exocytosis in mast cells through PI4KIIIβ (28). Since we found that eEF1A2-directed siRNA reduced PI4K activity and that ectopic eEF1A2 expression increased this activity, it is likely that eEF1A2 is a novel and physiological PI4KIIIβ activator.

In human cells, the bulk of PI4K activity is associated with the plasma membrane and the membranes of the nucleus, lysosome, Golgi, and endoplasmic reticulum (20, 29–32). PI4Ks are also known to localize and be active in several specialized organelles and secretory vesicles (32). The yeast homolog of eEF1A2 is a Physiological PI4K Regulator—To determine whether eEF1A2 had a physiological role in regulating PI4K activity, we reduced eEF1A2 protein levels in MCF7 cells. MCF7 cells express abundant eEF1A2 (Fig. 3A). For this purpose, we designed two eEF1A2-specific siRNAs. As shown in Fig. 5, both siRNAs substantially reduce eEF1A2 protein levels compared with those cells treated with a control siRNA or untreated cells. Some residual eEF1A2 remains, however. The siRNA-mediated decrease in eEF1A2 protein levels led to a concomitant 2-fold decrease in PI4K activity relative to controls.

eEF1A2 Increases Cellular PI4P Generation—We next determined whether eEF1A2 could increase intracellular PI4P abundance. To this end, we transiently transfected Rat2 and BT549 cells with an eEF1A2 plasmid and stained these cells with an antibody specific for PI4P. As shown in Fig. 6A, BT549 and Rat2 cells transiently expressing eEF1A2 showed PI4P staining that is visibly brighter than in untransfected cells, indicating greater abundance of PI4P upon eEF1A2 expression. The increase in PI4P occurs throughout the cytosol (Fig. 6A). Transfection with GFP had no visible effect on PI4P levels (Fig. 6B). To quantify the eEF1A2-dependent increase in PI4P generation, we divided each transfectant pool into groups based on expression of the transfected protein and quantified PI4P fluorescence in individual cells from each group. As shown in Fig. 6C, cells expressing moderate or high levels of eEF1A2 each have a significantly higher level of PI4P than those that express no or low eEF1A2 (p < 0.001, Mann-Whitney). This increase of PI4P generation occurred in both Rat2 and BT549 cells transfected with eEF1A2. GFP had no effect on PI4P generation. Thus, eEF1A2 expression increases intracellular PI4P abundance.
mammalian PI4KIIIβ, PIK1, has been studied in great detail (33–38). Pik1p, the protein encoded by PIK1, is essential for normal secretion, Golgi and vacuole membrane dynamics, and endocytosis (33–38). Pik1p localizes to the nucleus and the trans-Golgi (34, 38). Pik1ts cells exhibit a defect in secretion of Golgi-modified secretory pathway cargoes (36). Mammalian PI4KIIIβ is concentrated in the Golgi apparatus (39, 40), although detectable protein is found in the non-Golgi cytoplasm. However, we have not seen appreciable eEF1A2 protein in the Golgi, and we have observed no consistent Golgi defects in eEF1A2-expressing cells (data not shown). This suggests that the capacity of eEF1A2 to increase PI4P formation is regulating some non-Golgi aspect of cell physiology. Active PI4K is observed in the non-Golgi cytosol (41), and we find that eEF1A2 expression increases PI4P generation throughout the cell.
The molecular mechanism by which eEF1A2 activates PI4KIIIβ is currently unknown. eEF1A proteins bind and hydrolyze GTP and bind tRNA. They have not been shown to have phosphotransferase activity or other capacity to covalently modify proteins. Thus, it is unlikely that eEF1A2 affects PI4KIIIβ activity by post-translationally modifying it. Because eEF1A2 expression does not increase steady-state PI4KIIIβ protein levels (Fig. 4C), it is unlikely that eEF1A2 expression increases cellular PI4P generation by increasing the amount of PI4KIIIβ message being translated. We propose that direct interaction between eEF1A2 and PI4KIIIβ leads to a conformational change in PI4KIIIβ that increases its catalytic activity. This hypothesis is consistent with our observations that eEF1A2 increases the overall PI4KIIIβ catalytic rate ($K_{cat}$) and makes PI4KIIIβ a more efficient kinase ($V_{max}/K_{m}$). Although the possibility of this conformational change remains an open one, we do not believe that eEF1A2 is a general phosphatidylinositol kinase activator. We have been unable to detect physical interaction between eEF1A2 and phosphatidylinositol 3-kinases, nor have we observed activation of this kinase by eEF1A2 (data not shown).

Our *in vitro* results suggest that eEF1A2 and PI4KIIIβ bind in a 1:1 stoichiometry, and the majority of recombinant eEF1A2 and PI4KIIIβ are competent to physically interact with each other. However, up to a 2-fold molar excess of recombinant eEF1A2 is required to maximally increase *in vitro* PI4KIIIβ activity. Thus, it is possible that some post-translational modification of eEF1A2 may enhance its ability to activate PI4KIIIβ. Yang and Boss (14) showed that dephosphorylation of PIK-A49 prevented it from activating PI4KIIIβ and that activation could be restored by *in vitro* phosphorylation of PIK-A49 by a calcium-dependent protein kinase. The possibility of post-translational modification of eEF1A2 enhancing its ability to enhance PI4KIIIβ activity is intriguing. However, no post-translational modifications of eEF1A2 have yet been identified, and our results indicate that post-translational modification of eEF1A2 is not obligatory for PI4KIIIβ activation. Post-translational modification could also regulate PI4KIIIβ/eEF1A2 interaction. Because only a small fraction (≤10%) of total eEF1A2 is in a complex with PI4KIIIβ in MCF7 cells (and *vice versa*), post-translational modification of eEF1A2 could enhance cellular complex formation and therefore PI4P generation.

**eEF1A2 is highly expressed in a 30–60% fraction of breast, ovary, and lung tumors (9–12).** We have previously reported that eEF1A2 has transforming properties (9) and that its inactivation in mice increases lymphoid apoptosis (42). This identifies eEF1A2 as an important human oncogene. We hypothesize that eEF1A2 activates oncogenesis through PI4K activation and PI4P generation. Our observation that eEF1A2 expression is sufficient to increase both PI4K activity and PI4P levels indicates that high eEF1A2 levels in tumors probably lead to an overall increase in cellular PI4P production. We propose that increased production of PI4P could increase the abundance of PI(4,5)P$_2$ and PI(3,4,5)P$_3$. Both of these phospholipids are second messengers that regulate actin cytoskeletal organization, intracellular vesicular trafficking, and proliferation (15, 20, 43–47). Of particular importance to oncogenesis would be PI(3,4,5)P$_3$, the abundance of which controls cell growth, apo-
ptosis, and cell invasiveness. Although phosphatidylinositol 3-kinase activity has long been thought to be the rate-limiting step in the production of PI(3,4,5)P₃, this may not be the case in tumors that have high phosphatidylinositol 3-kinase activity through oncogenic mutation of the kinase or have inactivating mutations in the phosphatase and tensin homolog (PTEN) lipid phosphatase (48). In those tumors with constitutively active phosphatidylinositol 3-kinase or inactive PTEN, increased PI4K activity would be predicted to increase overall PI(3,4,5)P₃ abundance and activate PI(3,4,5)P₃-dependent signaling. This idea is consistent with a recent report by Pendaires et al. (49), who show that the phosphatidylinositol 5-phosphate produced by the Shigella pathogen is sufficient to activate Akt, a serine threonine kinase whose activity is dependent on PI(3,4,5)P₃.

The idea is consistent with a recent report by Pendaires et al. (49), who show that the phosphatidylinositol 5-phosphate produced by the Shigella pathogen is sufficient to activate Akt, a serine threonine kinase whose activity is dependent on PI(3,4,5)P₃. This may not be the case in tumors with high phosphatidylinositol 3-kinase activity through oncogenic mutation of the kinase or have inactivating mutations in the phosphatase and tensin homolog (PTEN) lipid phosphatase (48). In those tumors with constitutively active phosphatidylinositol 3-kinase or inactive PTEN, increased PI4K activity would be predicted to increase overall PI(3,4,5)P₃ abundance and activate PI(3,4,5)P₃-dependent signaling. This idea is consistent with a recent report by Pendaires et al. (49), who show that the phosphatidylinositol 5-phosphate produced by the Shigella pathogen is sufficient to activate Akt, a serine threonine kinase whose activity is dependent on PI(3,4,5)P₃.

In summary, we have identified eEF1A2 as a direct activator of PI4KIIIβ. Ectopic expression of eEF1A2 increases cellular PI4K activity and increases cellular PI4P abundance. Furthermore, siRNA-mediated eEF1A2 inactivation decreases PI4K activity. This is consistent with the idea that eEF1A2 functionally and physiologically regulates PI4K function. We propose that PI4KIIIβ activation has an important role in eEF1A2-mediated tumorigenesis and transformation.

Acknowledgments—We thank Farahnaz Noei for assistance with the fluorescent deconvolution microscopy, Heidi McBride for assistance with the quantitative confocal microscopy, Meenakshi Sundaram for help with the lipid kinase assays, Jessica Rousseau and Anahita Amiri for the eEF1A2 adenovirus, and Geeta Kulkarni for GST-eEF1A2 and the eEF1A2 antibody. We also thank T. Balla for the PI4KIIIβ plasmids and Nadine Wiper-Bergeron for the GST-C/EBPβ construct. We thank Dixie Pinke, Anne Morrow, and Nadine Wiper-Bergeron for critical reading of the manuscript.

REFERENCES

1. Hershey, J. W. (1991) Annu. Rev. Biochem. 60, 717–755
2. Condeelis, J. (1995) Trends Biochem. Sci. 20, 169–170
3. Shultz, L. D., Sweet, H. O., Davisson, M. T., and Coman, D. R. (1982) Science 266, 282–285
4. Shultz, L. D., Sweet, H. O., Davisson, M. T., and Coman, D. R. (1982) Nature 297, 402–404
5. Chambers, D. M., Peters, J., and Abbott, C. M. (1998) Proc. Natl. Acad. Sci. U.S.A. 95, 4643–4648
6. Knudson, S. M., Frydenberg, J., Clark, B. F., and Leffers, H. (1993) Eur. J. Biochem. 215, 549–554
7. Lee, S., Wolfram, L. A., and Wang, E. (1993) J. Biol. Chem. 268, 24453–24459
8. Collins, C., Gray, J. W., Diebold, J., Demetruck, D. J., and Lee, J. M. (2002) Nat. Genet. 31, 301–305
9. Bukh, J., and Spear, P. (1997) Annu. Rev. Microbiol. 51, 523–553
10. Postel-Vinay, S., and Sigalas, M. (2002) Mol. Microbiol. 43, 1029–1038

JANUARY 5, 2007 • VOLUME 282 • NUMBER 1 JOURNAL OF BIOLOGICAL CHEMISTRY 379
42. Potter, M., Bernstein, A., and Lee, J. M. (1998) Cell Immunol. 188, 111–117
43. Balla, T. (1998) Biochim. Biophys. Acta 1436, 69–85
44. Gehrmann, T., and Heilmeyer, L. M., Jr. (1998) Eur. J. Biochem. 253, 357–370
45. Huijbregts, R. P., Topalof, L., and Bankaitis, V. A. (2000) Traffic 1, 195–202
46. Maruta, H., He, H., Tikoo, A., Vuong, T., and Nur, E. K. M. (1999) Microsc. Res. Tech. 47, 61–66
47. Insall, R. H., and Weiner, O. D. (2001) Dev. Cell 1, 743–747
48. Wymann, M. P., and Marone, R. (2005) Curr. Opin. Cell Biol. 17, 141–149
49. Pendaries, C., Tronchere, H., Arbibe, L., Mounier, J., Gozani, O., Cantley, L., Fry, M. J., Gaits-Iacovoni, F., Sansonetti, P. J., and Payrastre, B. (2006) EMBO J. 25, 1024–1034
50. Downward, J. (2004) Semin. Cell Dev. Biol. 15, 177–182