COOH-terminal coiled-coil region, which are shared with other SNX proteins, are most likely responsible for its SNX function. The presence of both activities in one molecule makes RGS-PX1 an ideal bridge between G protein signaling and regulation of vesicular trafficking.

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4. Initially we identified a human cDNA clone KIAA0713 (GenBank accession number BAA34433) and several expressed sequence tags encoding an RGS protein through a BLAST search of GenBank (National Center for Biotechnology Information). A full-length cDNA (RGS-PX1, GenBank accession number AF420470) was obtained by screening a human heart cDNA library (Stratagene) with a fragment corresponding to the putative RGS domain of KIAA0713 and subsequent 5′ rapid amplification of cDNA ends with human heart Marathon-ready cDNA (Clontech). RGS-PX1 appears to be an alternatively spliced form of KIAA0713, with a longer COOH-terminal region. The COOH-terminal region of RGS-PX1 encoding the last 362 residues is identical to a partial cDNA encoding SNX13 (GenBank accession number AAD27835). Domain analysis was done with the SMART database (*5*). Sequence alignment and shading were conducted with the ClustalW and MacBoxshade programs, respectively. MacVector software (Oxford Molecular, Madison, WI) was used for transmembrane region prediction.
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7. The RGS domain of RGS-PX1 (residues 327 through 526) or full-length RGS4 was expressed in *Escherichia coli* as GST- or His-tagged protein with the pGEX-KG (Pharmacia) and pET28 (Novagen) vectors, respectively. Recombinant proteins were purified as described (*22*). For pulldown assays on brain lysates, GST-RGS-PX1, GST-RGS4, or GST (~5 μg) on glutathione-agarose beads (Sigma) were incubated overnight with ~10 mg of bovine brain lysate in buffer A [20 mM tris-HCl (pH 8.0), 100 mM NaCl, 2 mM $MgSO_4$, 5 mM β-mercaptoethanol, 0.05% C12E10, and 5% glycerol] containing 100 μM GDP in the presence of 30 μM $AlCl_3$ and 10 μM NaF at 4°C as described (*23*). Bound proteins were analyzed by immunoblotting with affinity-purified antibody to $G\alpha_{i3}$ (EC) and $G\alpha_q$ (QL) and with protein A–purified anti-$G\alpha_s$ and anti-$G\alpha_{12}$ immunoglobulin G's (IgG's) (Calbiochem).
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10. Two micrograms of purified His-tagged RGS domain of RGS-PX1 or full-length RGS4 bound to Ni-NTA-agarose beads and 0.1 μg of $G\alpha_s$ or $G\alpha_{i1}$ were incubated at room temperature for 5 min in 500 μl of buffer B [20 mM tris-HCl (pH 8.0), 0.5 mM EDTA, 20% glycerol, 1 mM dithiothreitol (DTT), 0.2% NP-40, and 0.5 mM phenylmethylsulfonyl fluoride] containing 100 μM GDP in the presence of 30 μM $AlCl_3$ and 10 μM NaF as described (*24*). The reaction mixture was then washed (three times) with 1 ml of buffer B with 100 mM KCl. Bound $G\alpha_s$ and $G\alpha_{i1}$ were analyzed by immunoblotting with specific antibodies (*7*).
11. Hydrolysis of GTP and purification of $G\alpha_s$ and $G\alpha_{i1}$ were done as described (*24*, *25*). $G\alpha_s$ or $G\alpha_{i1}$ (200 nM) was loaded with [γ-^{32}P]GTP by incubation for 15 min at 30°C in 50 mM NaHepes (pH 8.0), 10 mM EDTA, 5 mM DTT, and 2 μM [γ^{32}-P]GTP (~10,000 counts per min per pmol) in the absence of Mg^{2+} and presence of EDTA to prevent GTP hydrolysis. We typically obtained 15 to 30% loading. GTPase assays were performed on ice in an attempt to capture the initial phase of GTP hydrolysis (*25*). One round of hydrolysis of the prebound [γ-^{32}P]GTP was started by addition of $MgSO_4$ and unlabeled GTP to final concentrations of 20 mM and 100 μM, respectively. Excess unlabeled GTP was included to prevent rebinding of [γ-^{32}P]GTP. Final concentrations of $G\alpha_s$ or $G\alpha_{i1}$ were 80 and 60 nM, respectively. In some cases, 400 nM purified His-tagged RGS domain of RGS-PX1 or full-length RGS4 was included. At the indicated times, aliquots (10 μl) were removed and added to 260 μl of 5% w/v Norit A charcoal and [^{32}P]P_i was counted by liquid scintillation.
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13. HEK293T cells in six-well plates were transfected using calcium phosphate with 1 μg of pCMV-β2AR and 4 μg of pCDNA3-FLAG-RGS-PX1-RGS or pCDNA3. Forty-eight hours after transfection, cells were serum-starved overnight, treated with 1 μM isobutyl methylxanthine for 30 min, and stimulated with 50 μM isoproterenol or 100 μM forskolin for 3 min. Cells were then lysed and cAMP accumulation was measured with the cAMP enzyme immunoassay system (Amersham Pharmacia) according to the manufacturer's protocol. For assay of AC activity, membranes from neonatal rat cardiac myocytes were prepared as described (*26*). His-tagged RGS domain of RGS-PX1 (150 nM) or vehicle was added to membranes for 5 min on ice and diluted 1:3 (50 nM final concentration) with AC assay reactants. Reactants were incubated for 10 min at 30°C, boiled, and assayed for cAMP content by radioimmunoassay (*26*).
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18. HEK293 cells were transfected using calcium phosphate (>50% transfection efficiency) with GFP-RGS-PX1, RGS-PX1 (residues 257 through 957) in pEGFP-C1 vector (Clontech), or empty vector together with EGFR. Twenty-four hours after transfection, cells were serum-starved for 16 hours and lysed at different times after addition of 10 nM EGF (Molecular Probes). Fifty micrograms of cell lysate was analyzed by immunoblotting with antibodies to EGFR (*14*), actin (Sigma), phosphoERK1/2, and ERK1/2 (Cell Signaling Technology, Beverly, MA). Protein-lipid overlays were performed essentially as described (*19*) with the use of PIP-strips (Echelon Research Laboratories, Salt Lake City, UT), a GST fusion protein (0.5 μg/ml) of the PX domain of RGS-PX1 (residues 562 through 676), and monoclonal antibody to GST (Santa Cruz Biotechnology). For immunofluorescence, Cos-7 cells were transiently transfected with GFP-RGS-PX1 by means of Fugene 6 (Roche). Twenty-four hours after transfection, cells were fixed in 2% paraformaldehyde, permeabilized (0.1% Triton-X100), and incubated with antibody to EEA1 (Transduction Laboratories, Lexington, KY), followed by Alexa 594 goat anti-rabbit IgG as described (*22*).
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27. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

28. We thank T. Nagase (Kazusa DNA Research Institute, Japan) for the KIAA0713 cDNA clone and A. Spiegel (NIH) for anti-$G\alpha_i$ EC and anti-$G\alpha_q$ QL IgGs. Supported by NIH grants CA58689 and DK17780 (M.G.F.), AG14563 and GM56904 (X.-Y.H.), and HL53773 and HL63885 (P.A.I.) and a fellowship from the Canadian Institutes of Health Research (C.L.).

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Phosphatidic Acid–Mediated Mitogenic Activation of mTOR Signaling

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The mammalian target of rapamycin (mTOR) governs cell growth and proliferation by mediating the mitogen- and nutrient-dependent signal transduction that regulates messenger RNA translation. We identified phosphatidic acid (PA) as a critical component of mTOR signaling. In our study, mitogenic stimulation of mammalian cells led to a phospholipase D–dependent accumulation of cellular PA, which was required for activation of mTOR downstream effectors. PA directly interacted with the domain in mTOR that is targeted by rapamycin, and this interaction was positively correlated with mTOR's ability to activate downstream effectors. The involvement of PA in mTOR signaling reveals an important function of this lipid in signal transduction and protein synthesis, as well as a direct link between mTOR and mitogens. Furthermore, these studies suggest a potential mechanism for the in vivo actions of the immunosuppressant rapamycin.

The mammalian target of rapamycin (mTOR; also named FRAP or RAFT1) (*1*–*3*) belongs to the family of phosphatidylinositol kinase–like kinases (PIKK) (*4*). The mTOR homologs in *Saccharomyces cerevisae*, Tor1p and Tor2p, control a wide range of growth-related cellular processes, including transcription, translation, and reorganization of the actin cytoskeleton (*5*).

mTOR is likely to have similarly pleiotropic and essential roles in the regulation of mammalian cellular functions (*6*). mTOR regulates translation initiation (*7*); its best known downstream effectors include the ribosomal subunit S6 kinase 1 (S6K1) and the eukaryotic initiation factor 4E binding protein 1 (4E-BP1), two regulators of mitogen-stimulated translation initiation (*8, 9*). Both activation of S6K1 and phosphorylation of 4E-BP1 are stimulated by mitogens, and mTOR is required for their responses (*10, 11*), possibly because it senses amino acid sufficiency and supplies a permissive signal (*7, 9*). mTOR may also directly receive mitogenic signals to activate downstream pathways (*12, 13*), but the mechanism is unknown.

Phosphatidic acid (PA) is a lipid second messenger that participates in various intracellular signaling events and regulates a growing list of signaling proteins, including several protein kinases and phosphatases (*14*). PA has been implicated as a mediator of the mitogenic action of various growth factors and hormones in several types of mammalian cells. The normal molar concentration of PA in cellular membranes is low, less than 5% of that of phosphatidylcholine (PC) (*15*), and mitogenic stimulation leads to an increase in the amount of PA as a result of phospholipase D (PLD) activation (*14*). Exogenous PA added to cell culture media incorporates rapidly into cellular membranes and subsequently participates in cellular functions (*16, 17*). We observed that extracellular concentrations of 100 μM PA stimulated S6K1 activation and 4E-BP1 phosphorylation in serum-starved human embryonic kidney (HEK) 293 cells (Fig. 1A). This stimulation was abolished by rapamycin, implicating the involvement of mTOR. In addition, PA's stimulatory effect was absent in cells deprived of amino acids (Fig. 1B), suggesting that the action of PA requires a permissive signal from amino acids.

Stimulation of HEK293 cells with serum led to an acute increase in the amount of cellular PA within 5 min, which returned to its basal level after 45 min (Fig. 2A). PA is the lipid product of PLD. Alcohols compete with water to be the hydroxyl donor in the hydrolysis of phospholipids by PLD, resulting in the production of phosphatidylalcohol at the expense of PA (*18*). Treatment of HEK293 cells with 0.3% 1-butanol abolished serum-stimulated PA production, and 2-butanol also had an inhibitory effect (Fig. 2A). This is consistent with observations reported in other mammalian cells (*19*). 1-Butanol almost completely blocked serum-stimulated activation of S6K1, whereas 2-butanol had a partial inhibitory effect (Fig. 2B). Similarly, serum-stimulated 4E-BP1 phosphorylation was inhibited by 1-butanol, and to a lesser degree by 2-butanol (Fig. 2B). Serum-stimulated activation of the extracellular signal–regulated kinases (ERK1 and ERK2) and of the protein kinase Akt was not affected by butanol under identical conditions (Fig. 2B), confirming the specificity of butanol's effect on PA production and PA's involvement in the rapamycin-sensitive pathway. Thus, PA production through PLD appears to be an early event required for mitogenic activation of mTOR's downstream effectors.

Our previous studies (*20*) suggested the existence of a putative regulator interacting with the FK506-binding protein (FKBP12)–rapamycin-binding (FRB) domain in mTOR (*21*). The helical bundle structure of FRB (*22*) is reminiscent of the amphipathic helices in the lipid-binding domains of exchangeable apolipoproteins (*23*). Indeed, a purified FRB fragment bound small unilamellar vesicles (SUVs) (*24*) containing PA (Fig. 3A). A PA concentration as low as 10% in PC-based vesicles was sufficient to bind FRB (*25*). This binding was specific, because none of the other phospholipids tested bound FRB, including PC, phosphatidylserine, phosphatidylethanolamine, and phosphatidylinositol (PI) (Fig. 3A), as well as PI3P, PI3,4P_2, and PI3,4,5P_3 (*25*). Incubation with the FKBP12-rapamcyin complex effectively eliminated PA binding to FRB, whereas a rapamycin-resistant FRB mutant protein (S2035I) (*21*) displayed PA binding that was insensitive to rapamycin (Fig. 3B). These observations support the hypothesis that PA may regulate mTOR function by directly interacting with the FRB domain.

High ionic strength (500 mM NaCl) diminished FRB's affinity for PA (Fig. 3C), suggesting an electrostatic interaction between FRB and the head group of PA, consistent with observations in other PA-binding proteins (*15*). A group of positively charged residues (Arg^{2042}, Lys^{2095}, and Arg^{2109}) located at the opening of the hydrophobic rapamycin-binding

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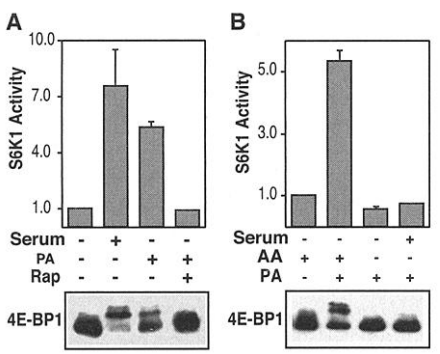


Fig. 1. Stimulation of mTOR signaling by PA. HEK293 cells treated under various conditions (*30*) were lysed. S6 kinase assays were performed with immunoprecipitated endogenous S6K1 as previously described (*29*). 4E-BP1 phosphorylation was assessed by mobility shift on protein immunoblots with a 4E-BP1 antibody (Zymed, San Francisco, California), with the slowest migrating band representing the fully phosphorylated state. (**A**) Serum-starved cells were stimulated with 100 μM PA or 10% serum for 30 min, with or without pretreatment by 20 nM rapamycin (Rap) for 30 min. (**B**) Serum-starved cells were deprived of amino acids (AA) with or without 10% dialyzed serum, followed by PA stimulation, as described above.

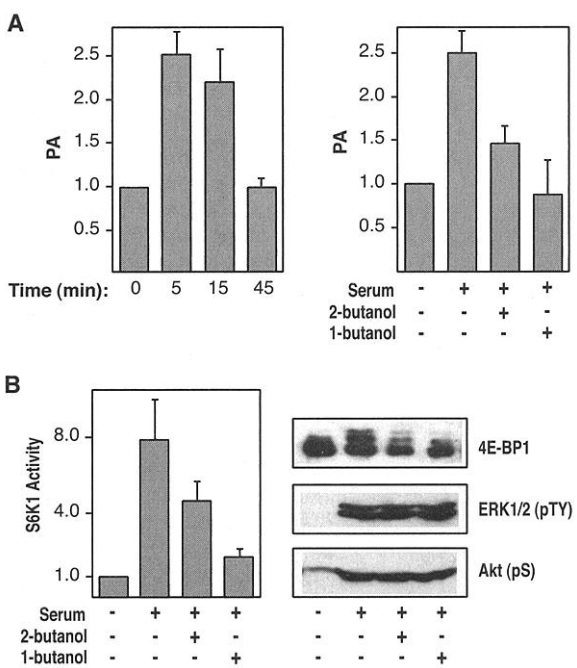


Fig. 2. Requirement of cellular PA production for mitogenic activation of mTOR signaling. (**A**) HEK293 cells serum-starved and metabolically labeled with ^{32}P-orthophosphate (*26*) were stimulated with 10% serum for various times (left). Cells were treated with 0.3% 1- or 2-butanol for 30 min before serum stimulation for 5 min (right). Cellular lipids were extracted and separated by thin-layer chromatography (*26*). PA was identified by co-spotting of standards and quantified by phosphorimaging. (**B**) Serum-starved cells were pretreated with 0.3% 1- or 2-butanol for 30 min before serum stimulation for 5 min. (Left) S6K1 activity was assayed as previously described (*30*). (Right) 4E-BP1 phosphorylation was examined by mobility shift on protein immunoblots with a 4E-BP1 antibody (Zymed). ERK1/ERK2 and Akt phosphorylation were determined by immunoblotting with phospho-p44/42 (T202Y204) (pTY) and phospho-Akt (S473) (pS) antibodies (Cell Signaling, Beverly, Massachusetts), respectively.

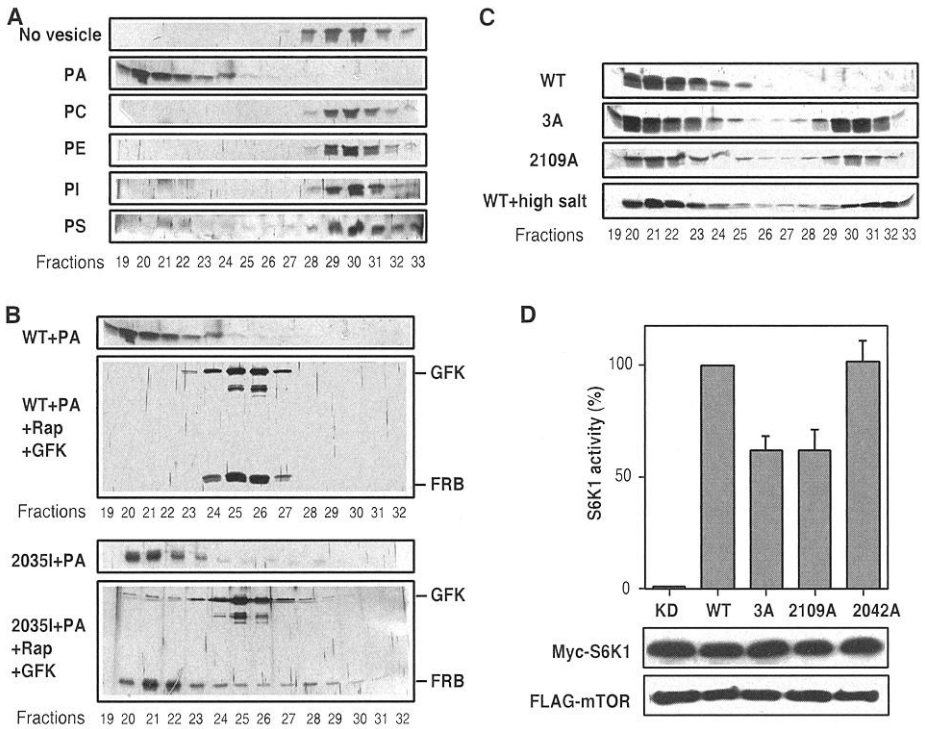
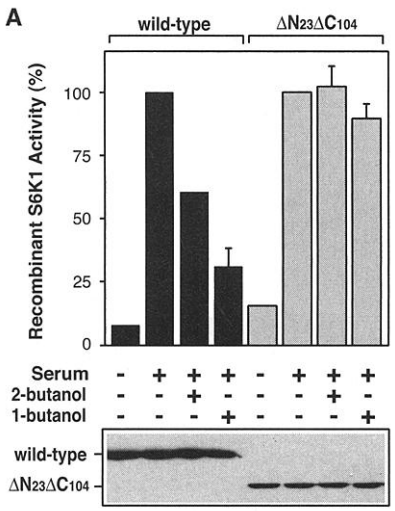


Fig. 3. Selective binding of PA to FRB; correlation between FRB-PA interaction and mTOR signaling. (**A**) SUVs were generated with various phospholipids (*24*), and lipid-binding assays using SUVs and the purified FRB protein (*20*) were done (*26*). The protein/vesicle mixtures were fractionated on a Sephacryl S-300 column, and the column fractions were analyzed on 13% SDS gels by silverstaining. Free FRB eluted between fractions 28 and 33, whereas vesicles eluted in the void volume (fractions 19 through 22). PC, phosphatidylcholine; PE, phosphatidylethanolamine; and PS, phosphatidylserine. (**B**) Wild-type or $Ser^{2035}Ile$ (2035I) mutant FRB was preincubated with rapamycin (Rap) and GST-FKBP12 (GFK) at a 1:1:1 molar ratio, followed by PA binding assays. The GFK protein solution used in the experiments contained some free GST, as revealed by the band immediately below GFK. (**C**) PA binding assays were done with the wild-type and various mutant FRB proteins under normal conditions. The assay was also done with wild-type FRB in the presence of 500 mM NaCl (high salt). (**D**) Various FLAG-mTOR cDNAs were coexpressed with Myc-S6K1 in HEK293 cells (*29, 31*). The transfected cells were treated with 100 nM rapamycin for 30 min before lysis. In vitro S6 kinase assays were performed with anti-Myc (9E10.2)–immunoprecipitated Myc-S6K1 (*30*). Expression of the recombinant proteins was monitored by immunoblotting with antibodies against epitope tags. Designations for FRB and mTOR constructs are as follows: KD, kinase-dead; WT, wild type; 3A, $Arg^{2042}Ala/Lys^{2095}Ala/Arg^{2109}Ala$; 2109A, $Arg^{2109}Ala$; 2042A, $Arg^{2042}Ala$. All full-length mTOR constructs, including WT, contained the S2035T mutation.



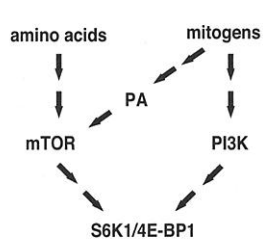


Fig. 4. PA signaling specifically through mTOR and not PI3K. (**A**) HEK293 cells were transiently transfected with Myc-tagged wild-type or $\Delta N_{23}\Delta C_{104}$ S6K1 (*31*), followed by serum starvation and stimulation by 10% serum for 15 min; 1- or 2-butanol was added 30 min before stimulation. Upon cell lysis, the recombinant proteins were immunoprecipitated with 9E10.2 antibody to Myc and S6 kinase assays were performed (*30*). Black bars, wild-type S6K1; gray bars, $\Delta N_{23}\Delta C_{104}$ S6K1. Expression of the recombinant proteins was monitored by immunoblotting with the antibody to Myc. (**B**) A model for mitogenic activation of S6K1/4E-BP1 is proposed.

pocket was identified after the crystal structure of FRB was examined (*22*). Mutating all three residues (designated 3A), or Arg^{2109} alone, to alanine diminished FRB's affinity for PA (Fig. 3C). Mutations at Arg^{2042} and Lys^{2095} alone had little effect on PA binding (*25*). Thus, Arg^{2109} appears to be a major contributor to the electrostatic interaction between FRB and PA. Because hydrophobic interactions also often contribute to this type of protein-lipid interaction (*15*), it is unlikely that PA binding could be completely eliminated without massive alteration of the protein structure.

The 289-kD mTOR protein is difficult to purify and forms a large complex of ~2 MD (*25*), precluding the assessment of lipid binding for the full-length protein. To probe the physiological relevance of FRB-PA interaction, we investigated the in vivo functional consequence of diminished PA binding by introducing $Arg^{2042}Ala$, $Arg^{2109}Ala$, and the 3A mutations into full-length mTOR protein expressed in HEK293 cells. The catalytic activity of mTOR was not affected by mutations disrupting PA binding (*26*), which was consistent with observations that neither PA nor butanol had any effect on mTOR kinase activity in vitro or in vivo (*25*). The signaling capacity of these mTOR mutants was assessed in the presence of a rapamycin-resistant mutation ($Ser^{2035}Thr$) (*10, 11*) and rapamycin. $Arg^{2109}Ala$, as well as 3A, prevented full activation of S6K1 in response to serum stimulation (Fig. 3D). These mutant mTORs displayed signaling activity at ~60% that of wild-type mTOR, in close correlation with the extent to which $Arg^{2109}Ala$ disrupted PA binding (Fig. 3C). $Arg^{2042}Ala$ mTOR behaved similarly to the wild-type protein. The ability of these mutants to activate phosphorylation of 4E-BP1 upon serum stimulation also correlated with S6K1 activation, meaning that $Arg^{2109}Ala$ diminished 4E-BP1 phosphorylation whereas $Arg^{2042}Ala$ had no effect (*25*). The correlation between FRB binding to PA and mTOR signaling indicates that PA binding to the FRB domain may be required to allow mTOR to activate downstream pathways. The incomplete disruption of signaling by $Arg^{2109}Ala$ may result from its partial disruption of FRB-PA binding. However, it is also possible that PA represents one of several mitogenic pathways that lead to S6K1 and 4E-BP1 activation.

Mitogenic activation of S6K1 and 4E-BP1 requires both the mTOR pathway and the PI3 kinase (P13K) pathway (*7, 9*). PA had no effect on the activity of PI3K (*26*), suggesting that PA signaling is unlikely to affect the PI3K path-

way. To confirm PA's specific involvement in the mTOR pathway, we used an S6K1 mutant ($\Delta N_{23}\Delta C_{104}$), the activity of which is resistant to rapamycin and sensitive to wortmannin (*27*). When transiently expressed in HEK293 cells, the rapamycin-resistant $\Delta N_{23}\Delta C_{104}$ mutant S6K1 activity was insensitive to butanol, whereas the recombinant wild-type S6K1 activity was inhibited by 1- and 2-butanol (Fig. 4A) to a similar extent as was the endogenous kinase (Fig. 2B). These observations support the hypothesis that PA signaling to S6K1 specifically goes through mTOR and not through PI3K . However, the specific PI3K inhibitor wortmannin abolished PA-stimulated S6K1 activation and 4E-BP1 phosphorylation (*26*), implying that PI3K is indispensable for the downstream response to PA. Based on the collective evidence, we propose a mechanism by which amino acid sufficiency sensed by the mTOR pathway, mitogenic stimulation of the mTOR pathway mediated by PA, and mitogenic stimulation of the PI3K pathway independent of PA are all required for full activation of S6K1 and 4E-BP1 (Fig. 4B). The observed PA stimulatory effect on these downstream effectors is likely dependent on the basal activity of PI3K in the absence of serum, which may also explain the fact that PA had a less potent stimulatory effect than did serum (Fig. 1).

Our findings reveal a mitogenic pathway upstream of S6K1 and 4E-BP1, which involves PA and probably its direct interaction with mTOR. The data suggest that rapamycin's inhibitory effect may derive from its competition with PA for binding to the FRB domain, preventing mTOR from activating downstream effectors but without inhibiting mTOR's catalytic activity. Another PIKK family member, DNA-PK, binds to inositol hexakisphosphate (IP_6), and its function in DNA double-strand break repair is regulated by IP_6 (*28*). The modulation of mTOR signaling by PA, together with DNA-PK stimulation by IP_6, may reveal a common theme of lipidlike molecules participating in regulation of PIKK proteins.

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30. HEK293 cells were maintained in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) at 37°C with 5% CO_2. Serum starvation of cells was carried out in serum-free DMEM for 24 hours. For amino acid deprivation, the cells were incubated in Dulbecco's phosphate-buffered solution containing 10% dialyzed FBS for 2 hours. Transient transfections were performed with SuperFect or PolyFect (Qiagen, Valencia, CA). The amount of plasmid DNA transfected per well in six-well plates was 1 μg of mTOR, 1 μg of S6K1, and 0.4 μg of 4E-BP1.
31. All bacterial expression plasmids were constructed in pGEX-2T (Pharmacia), and all mammalian expression plasmids were constructed in pCDNA3 (Invitrogen). pCDNA-FLAG-mTOR/S2035T, pCDNA-FLAG-mTOR/S2035T/D2357E (kinase-dead), pCDNA-Myc-S6K1 (p70s6k), pCDNA-FLAG-4E-BP1, pGEX-FRB (wild type), and pGEX-FRB/S2035I were previously described (*20*, *29*). $\Delta N_{23}\Delta C_{104}$ S6K1 (amino acids 24 to 398) cDNA was amplified by polymerase chain reaction (PCR) and inserted into pCDNA-Myc via Not I and Xba I sites. All point mutations were generated by nested PCR using Bam HI and Eco RI as the flanking sites for pGEX-FRB constructs and Kpn I and Hpa I for pCDNA-FLAG-mTOR constructs. Glutathione *S*-transferase (GST)–FKBP12 and various FRB proteins were expressed and purified from *Escherichia coli* strain BL21, as described previously (*20*).

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Regulation of Cell Survival by Secreted Proneurotrophins

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Neurotrophins are growth factors that promote cell survival, differentiation, and cell death. They are synthesized as proforms that can be cleaved intracellularly to release mature, secreted ligands. Although proneurotrophins have been considered inactive precursors, we show here that the proforms of nerve growth factor (NGF) and the proforms of brain derived neurotrophic factor (BDNF) are secreted and cleaved extracellularly by the serine protease plasmin and by selective matrix metalloproteinases (MMPs). ProNGF is a high-affinity ligand for $p75^{NTR}$ with high affinity and induced $p75^{NTR}$-dependent apoptosis in cultured neurons with minimal activation of TrkA-mediated differentiation or survival. The biological action of neurotrophins is thus regulated by proteolytic cleavage, with proforms preferentially activating $p75^{NTR}$ to mediate apoptosis and mature forms activating Trk receptors to promote survival.

The neurotrophin family of growth factors, including NGF, BDNF, and neurotrophins-3 and -4 (NT-3, NT-4) regulates neuronal survival and synaptic plasticity (*1*). They are synthesized as precursors (proneurotrophins) that are proteolytically cleaved to mature, biologically active neurotrophins (*2*). Because neurotrophins are normally expressed at low levels, little is known about their processing and secretion by neurons and non-neuronal cells in vivo. However, when expressed in heterologous cells, proneurotrophins are secreted as well as cleaved intracellularly by furin or proconvertases at a highly conserved dibasic amino acid cleavage site for secretion as stable, noncovalent dimers (*3*, *4*). Mature neurotrophins selectively bind to members of the Trk family of receptor tyrosine kinases, but they also interact with low affinity to a structurally distinct receptor, $p75^{NTR}$. Although $p75^{NTR}$ can increase the affinity and specificity of Trk-neurotrophin

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