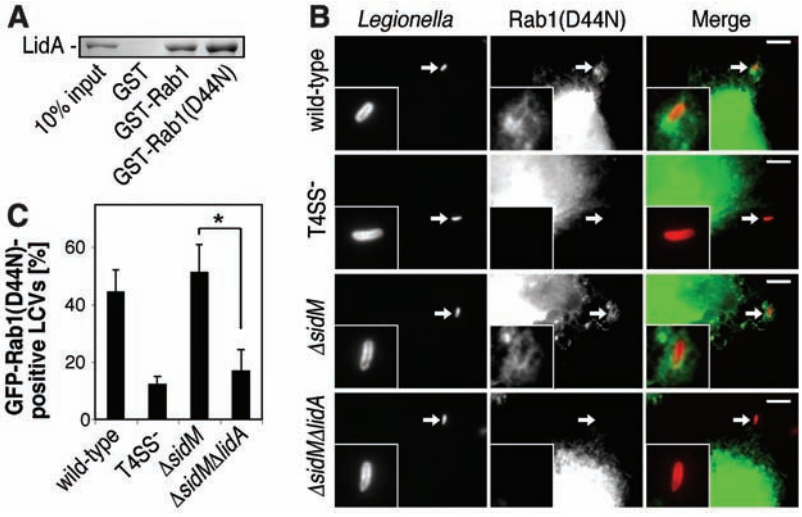

Fig. 4. LidA can recruit GDI-free Rab1(D44N) in the absence of SidM. (**A**) LidA can bind Rab1(D44N). Binding of LidA to bead-immobilized GST-Rab1 or GST-Rab1(D44N) but not GST was determined by SDS–polyacrylamide electrophoresis and Coomassie staining. The figure represents two repetitions. (**B**) Recruitment of GFP-Rab1(D44N) during *L. pneumophila* infection. Transiently transfected COS-1 cells producing GFP-Rab1(D44N) were infected for 30 min as indicated. Cells were fixed and stained for intracellular bacteria (left). (Middle) GFP-Rab1(D44N), (right) merged images with bacteria (red) and GFP-Rab1(D44N) (green). Arrows indicate the location of the LCV magnified threefold in the insets of each panel. Scale bar, 5 μm. Contrast was equally changed by linear adjustment. (**C**) Quantification of (B) showing that recruitment of GFP-Rab1(D44N) in the absence of SidM requires LidA. The graph represents pooled data (mean ± SD) from four independent experiments. *$P = 0.0001$ (Student's t test).

Rab1 about the LCV. Indeed, vacuoles containing *L. pneumophila* Δ*lidA* showed delayed Rab1 recruitment compared with wild-type LCVs (*20*).

SidM is a protein that has both GEF and GDF activity toward a Rab GTPase. This unique ability of SidM to link GDI displacement to Rab1 activation explains how the intravacuolar pathogen *L. pneumophila* can efficiently exploit host cell Rab1 even in the presence of GDI that naturally interferes with this process. The discovery of both GEF and GDF activity within SidM raises the intriguing possibility that eukaryotic GEF proteins may possess similar abilities to mediate membrane delivery and activation of Rab GTPases during intracellular vesicle transport.

References and Notes

1. M. Zerial, H. McBride, *Nat. Rev. Mol. Cell Biol.* **2**, 107 (2001).
2. C. C. Farnsworth *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 6196 (1991).
3. P. Chavrier, R. G. Parton, H. P. Hauri, K. Simons, M. Zerial, *Cell* **62**, 317 (1990).
4. R. Khosravi-Far *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 6264 (1991).
5. M. Peter, P. Chavrier, E. A. Nigg, M. Zerial, *J. Cell Sci.* **102**, 857 (1992).
6. B. T. Kinsella, W. A. Maltese, *J. Biol. Chem.* **267**, 3940 (1992).
7. T. Sasaki *et al.*, *J. Biol. Chem.* **265**, 2333 (1990).
8. T. Soldati, M. A. Riederer, S. R. Pfeffer, *Mol. Biol. Cell* **4**, 425 (1993).
9. O. Ullrich *et al.*, *J. Biol. Chem.* **268**, 18143 (1993).
10. M. D. Garrett, J. E. Zahner, C. M. Cheney, P. J. Novick, *EMBO J.* **13**, 1718 (1994).
11. S. Araki, A. Kikuchi, Y. Hata, M. Isomura, Y. Takai, *J. Biol. Chem.* **265**, 13007 (1990).
12. S. R. Pfeffer, A. B. Dirac-Svejstrup, T. Soldati, *J. Biol. Chem.* **270**, 17057 (1995).
13. A. B. Dirac-Svejstrup, T. Sumizawa, S. R. Pfeffer, *EMBO J.* **16**, 465 (1997).
14. T. Soldati, A. D. Shapiro, A. B. Svejstrup, S. R. Pfeffer, *Nature* **369**, 76 (1994).
15. U. Sivars, D. Aivazian, S. R. Pfeffer, *Nature* **425**, 856 (2003).
16. M. A. Horwitz, *J. Exp. Med.* **158**, 1319 (1983).
17. M. S. Swanson, R. R. Isberg, *Infect. Immun.* **63**, 3609 (1995).
18. L. G. Tilney, O. S. Harb, P. S. Connelly, C. G. Robinson, C. R. Roy, *J. Cell Sci.* **114**, 4637 (2001).
19. S. Ninio, C. R. Roy, *Trends Microbiol.* **15**, 372 (2007).
20. M. P. Machner, R. R. Isberg, *Dev. Cell* **11**, 47 (2006).
21. T. Murata *et al.*, *Nat. Cell Biol.* **8**, 971 (2006).
22. H. Plutner *et al.*, *J. Cell Biol.* **115**, 31 (1991).
23. E. J. Tisdale, J. R. Bourne, R. Khosravi-Far, C. J. Der, W. E. Balch, *J. Cell Biol.* **119**, 749 (1992).
24. O. Pylypenko *et al.*, *EMBO J.* **25**, 13 (2006).
25. Materials and methods are available as supporting material on *Science* Online.
26. S. Araki, K. Kaibuchi, T. Sasaki, Y. Hata, Y. Takai, *Mol. Cell. Biol.* **11**, 1438 (1991).
27. T. Musha, M. Kawata, Y. Takai, *J. Biol. Chem.* **267**, 9821 (1992).
28. P. Chavrier *et al.*, *Nature* **353**, 769 (1991).
29. C. Nuoffer, H. W. Davidson, J. Matteson, J. Meinkoth, W. E. Balch, *J. Cell Biol.* **125**, 225 (1994).
30. G. M. Conover, I. Derre, J. P. Vogel, R. R. Isberg, *Mol. Microbiol.* **48**, 305 (2003).
31. A. L. Wilson, R. A. Erdman, W. A. Maltese, *J. Biol. Chem.* **271**, 10932 (1996).

32. We thank C. Kumamoto, L. Feig, and D. Raychaudhuri for reviewing the manuscript; M. Barzik, V. Auerbuch, M. Bergman, E. Creasey, K.-L. Sheahan, Z. Li, T. O'Connor, A. Ensminger, M. Heidtman, and S. Mohammadi for critical comments and scientific advice; and A. Wandinger-Ness for providing reagents. This work was supported by the Howard Hughes Medical Institute and by National Institute of Diabetes and Digestive and Kidney Diseases, NIH, program grant P30DK34928. R.R.I is a Howard Hughes Medical Institute Investigator.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1149121/DC1
Materials and Methods
Figs. S1 to S5
Table S1
References

13 August 2007; accepted 9 October 2007
Published online 18 October 2007;
10.1126/science.1149121
Include this information when citing this paper.

Rheb Activates mTOR by Antagonizing Its Endogenous Inhibitor, FKBP38

Xiaochun Bai,[1,3] Dongzhu Ma,[1] Anling Liu,[1] Xiaoyun Shen,[1] Qiming J. Wang,[1] Yongjian Liu,[2] Yu Jiang[1]*

The mammalian target of rapamycin, mTOR, is a central regulator of cell growth. Its activity is regulated by Rheb, a Ras-like small guanosine triphosphatase (GTPase), in response to growth factor stimulation and nutrient availability. We show that Rheb regulates mTOR through FKBP38, a member of the FK506-binding protein (FKBP) family that is structurally related to FKBP12. FKBP38 binds to mTOR and inhibits its activity in a manner similar to that of the FKBP12-rapamycin complex. Rheb interacts directly with FKBP38 and prevents its association with mTOR in a guanosine 5′-triphosphate (GTP)–dependent manner. Our findings suggest that FKBP38 is an endogenous inhibitor of mTOR, whose inhibitory activity is antagonized by Rheb in response to growth factor stimulation and nutrient availability.

The mammalian target of rapamycin, mTOR, is a serine-threonine protein kinase that controls a wide spectrum of cellular events in response to various environmental cues, including stimulation by growth factors, changes in nutrient conditions, and fluctuations in energy levels (*1*, *2*). mTOR elicits its pleiotropic function in the context of two distinct multiprotein complexes termed mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (*3*). Rapamycin, in complex with FKBP12 (an FK506-binding protein), specifically interferes with mTORC1 function, and consequently, inhibits cell growth (*4*, *5*).

The major upstream regulators of mTORC1 are the TSC1 and TSC2 tumor suppressors. The two TSC proteins form a complex that displays

a guanosine triphosphatase (GTPase)–activating protein (GAP) activity toward Rheb, a Ras-like small GTPase (*6*–*8*). Like other small GTPases, the activity of Rheb is dictated by its guanine nucleotide binding states: it is active in its GTP-bound form and inactive in the guanosine diphosphate (GDP)–bound form (*9*). The TSC1-TSC2 complex stimulates the intrinsic GTPase activity of Rheb and, thus, negatively regulates Rheb function. Conversely, inactivation of the TSC1-TSC2 complex results in accumulation of GTP-bound Rheb, which activates mTORC1 (*10*, *11*).

FKBP38 (also known as FKBP8) belongs to the peptidyl prolyl cis/trans isomerase (PPIase) family of FKBPs. It contains a region, referred to as the FKBP-C domain, that is highly related to FKBP12 (*12*). FKBP38 also has a transmembrane domain at the very C terminus, which is unique among all the FKBP proteins and is required for targeting it to mitochondria (*13*, *14*). We isolated FKBP38 in a yeast two-hybrid screen designated to identify Rheb-interacting proteins (fig. S1) (*15*). The potential interaction between Rheb and FKBP38 in mammalian cells was confirmed by coimmunoprecipitation of endogenous Rheb with FKBP38 from human embryonic kidney (HEK293) cell lysates (Fig. 1A). Furthermore, purified bacterially expressed recombinant Rheb and FKBP38 interacted with each other in an in vitro binding assay (Fig. 1B). The interaction of Rheb with FKBP38 appeared to be dependent on its nucleotide binding states, because Rheb loaded with GTP-γ-S, a nonhydrolyzable GTP analog, guanosine 5′-*O*-(3′-thiotriphosphate), exhibited much higher binding affinity toward FKBP38 than did GDP-bound or untreated Rheb (Fig. 1C).

In cells overexpressing FKBP38, insulin-stimulated phosphorylation was largely prevented for several downstream targets of mTORC1, including ribosomal protein S6 kinase (S6K), ribosomal S6 protein (S6) and the eukaryotic initiation factor 4E–binding protein 1 (4E-BP1) (Fig. 2A and fig. S2). In contrast, FKBP38 overproduction had no detectable effect on phosphorylation of protein kinase Akt (Fig. 2A). The inhibitory effect of FKBP38 on mTORC1 activity was reversed if Rheb was overexpressed, which suggested that Rheb antagonizes FKBP38. Similar effects of FKBP38 overproduction on mTORC1 activity were observed when cells deprived of amino acids were exposed to amino acids (Fig. 2B and fig. S2).

In cells transiently transfected with FKBP38-specific small interfering RNA (siRNA) oligonucleotides, the amounts of FKBP38 were reduced by ~80% (Fig. 2C). Accompanying the decreased abundance of FKBP38 was an increase in mTOR-dependent phosphorylation in both S6K and 4E-BP1 but not of extracellular signal–regulated kinase (ERK) 1 or 2 (Fig. 2C). This effect on mTORC1 activity appeared to be specific, because a different FKBP38-specific siRNA oligonucleotide produced a similar effect (fig. S3). In addition to the enhancement in mTORC1 activity under normal growth conditions, decreased abundance of FKBP38 also reduced the rate of mTORC1 inactivation in response to serum or amino acid deprivation (fig. S4), which indicated that the affected cells were less sensitive to growth factor or nutrient limitation. The partial response of mTORC1 to changes in serum and nutrient conditions may result from the remaining FKBP38 in the cells. Alternatively, it may indicate the existence of FKBP38-independent mechanisms that regulate mTORC1. Collective-

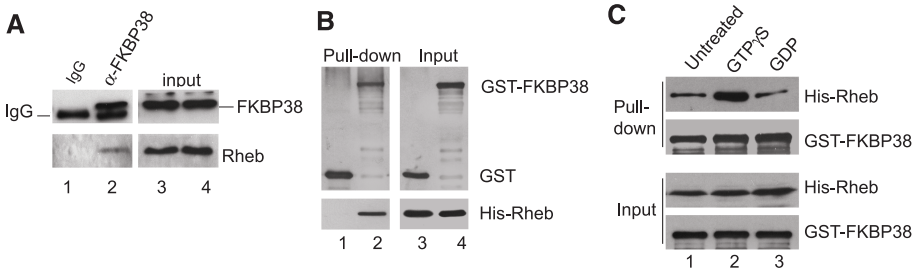

Fig. 1. GTP-dependent interaction of Rheb with FKBP38. (**A**) Coimmunoprecipitation of FKBP38 with Rheb. Lysates from HEK293 cells were immunoprecipitated with antibody to FKBP38 (lanes 2 and 4) or control IgG (lanes 1 and 3). FKBP38 (top) and Rheb (bottom) in the precipitates were detected by immunoblotting. (**B**) Direct binding between Rheb and FKBP38 in vitro. Histidine-tagged Rheb (His-Rheb) was incubated with glutathione *S*-transferase (GST) or GST-tagged FKBP38 (GST-FKP38) followed by precipitation with glutathione beads. The precipitates were immunoblotted for His-Rheb (bottom), GST, and GST-FKBP38 (top). (**C**) GTP-dependent binding of Rheb with FKBP38. Untreated His-Rheb (lane 1) GTP-γ-S–loaded His-Rheb (lane 2), and GDP-loaded His-Rheb (lane 3) were all incubated with GST-FKBP38 followed by precipitation with glutathione beads. The precipitates were immunoblotted for GST-FKBP38 and His-Rheb.

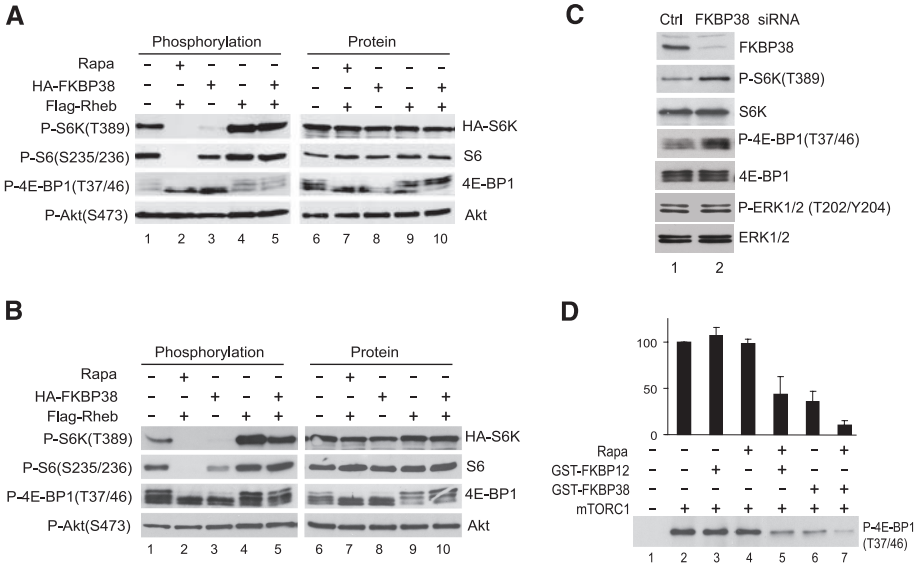

Fig. 2. Inhibition of mTORC1 activity by FKBP38. HEK293 cells were transfected with hemagglutinin (HA)–tagged S6K along with (+) or without (–) Flag-tagged Rheb and HA-tagged FKBP38 as indicated. (**A**) Effect of FKBP38 on insulin-stimulated phosphorylation. Cells were collected after being deprived of serum (0.5%) for 16 hours and treated with 100 nM insulin for 30 min. (**B**) Effect of FKBP38 on amino acid–stimulated phosphorylation. Cells were collected after being deprived of amino acids for 1 hour and then incubated with amino acids for 30 min. Rapamycin (20 nM) was added to the indicated samples 30 min before the addition of insulin or amino acids. (**C**) Effect of FKBP38 depletion on mTORC1 activity. HEK293 cells were transfected with FKBP38-specific (lane 2) or control (lane 1) siRNA. Cells were harvested and lysed 60 hours after the transfection. The indicated proteins (right panels) and their phosphorylation (left panels) were detected by immunoblotting. (**D**) In vitro kinase assay of mTORC1. Endogenously expressed mTOR was immunopurified from HEK293 cell lysates with antibody to mTOR and assayed for activity toward recombinant GST–4E-BP1 in the presence (+) or absence (–) of the indicated agents. Phosphorylation was detected by immunoblotting using antibody to phospho–4E-BP1(T37/46), and quantified by densitometry from three independent experiments (top).

[1]Department of Pharmacology, University of Pittsburgh School of Medicine, E1357 Biomedical Science Tower, 200 Lothrop Street, Pittsburgh, PA 15213, USA. [2]Department of Neurology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA. [3]Department of Cell Biology, School of Basic Medical Science, Southern Medical University, Guangzhou 510515, China.

*To whom correspondence should be addressed. E-mail: jiang@server.pharm.pitt.edu

ly, the above findings demonstrate that FKBP38 is a negative regulator of mTORC1.

FKBP38 inhibited the kinase activity of mTOR in a dose-dependent manner in vitro (Fig. 2D and fig. S5), and the extent of maximal inhibition was similar to that induced by the FKBP12-rapamycin complex (Fig. 2D). Although rapamycin alone had no effect on kinase activity of mTOR, it augmented the inhibitory effect of FKBP38, which suggests that rapamycin may interact with FKBP38 and may increase its inhibitory activity toward mTORC1.

Coimmunoprecipitation revealed that FKBP38 associated with the components of mTORC1, including mTOR itself, the GβL protein and raptor, but not rictor, a unique component of mTORC2 (Fig. 3A), which suggests that FKBP38 targets mTORC1 but not mTORC2. In addition, the association of FKBP38 with mTORC1 appeared to be regulated by nutrient conditions, because the association was increased in cells deprived of amino acids.

Rheb associates with mTOR through a region (amino acids 1967 to 2191) that overlaps with the FKBP12-rapamycin binding (FRB) domain (amino acids 2015 to 2114) (*16*). We tested the possibility that the association was mediated through FKBP38. Indeed, a recombinant peptide containing amino acids 1967 to 2191 of mTOR interacted directly with FKBP38 in an in vitro assay (Fig. 3B). On the other hand, no direct interaction was detected between Rheb and this FKBP38 binding (FKB) domain of mTOR (fig. S6). Deletion analysis of FKBP38 further revealed that its FKBP-C domain, a region highly similar to FKBP12, was sufficient for mTOR binding, which suggested that FKBP38 may bind to mTOR in a manner similar to that of the FKBP12-rapamycin complex (fig. S7B). In support of this, we found that the FKBP12-rapamycin complex competed with FKBP38 for mTOR binding in vitro (fig. S8), and rapamycin reduced the association of FKBP38 with mTOR in cells (Fig. 3C).

The interaction of FKBP38 with the FKB domain of mTOR appeared to be regulated by Rheb in a GTP-dependent manner, because the interaction was unaffected by the presence of GDP-bound Rheb but was abolished by the presence of the same amount of GTP-bound Rheb (Fig. 3D). The dissociation of FKBP38 from the FKB domain of mTOR was also accompanied by its binding to Rheb, which suggested that the interaction with Rheb interfered with FKBP38 binding to mTOR.

Because Rheb activity is regulated by growth factor and nutrient conditions (*6*, *17*, *18*), we examined whether Rheb controlled the interaction between endogenous FKBP38 and mTOR in a growth factor– and nutrient-dependent manner. FKBP38 interacted with mTOR in cells deprived of amino acids, and the interaction was reduced when amino acids were restored (Fig. 4A). Overexpression of wild-type Rheb also reduced the interaction of FKBP38 with mTOR in cells deprived of amino acids, and the interaction was further diminished when amino acids were restored. Accompanying the amino acid–induced decrease in the interaction of FKBP38 with mTOR was an increase in the binding of Rheb with FKBP38, which suggested that Rheb prevented FKBP38 from binding to mTOR in response to amino acid availability.

Rheb mutants, including an active allele, Q64L, in which leucine replaces glutamine at residue 64, and two inactive alleles, S20N and D60K (in which asparagine replaces serine at residue 20 and lysine is substituted for aspartic acid at residue 60), are defective for nucleotide binding, which renders the mutants largely insensitive to the GAP activity of the TSC1/TSC2 complex (*19*, *20*). In cells expressing the Q64L mutant, the interaction of FKBP38 with mTOR was barely detectable, regardless of the availability

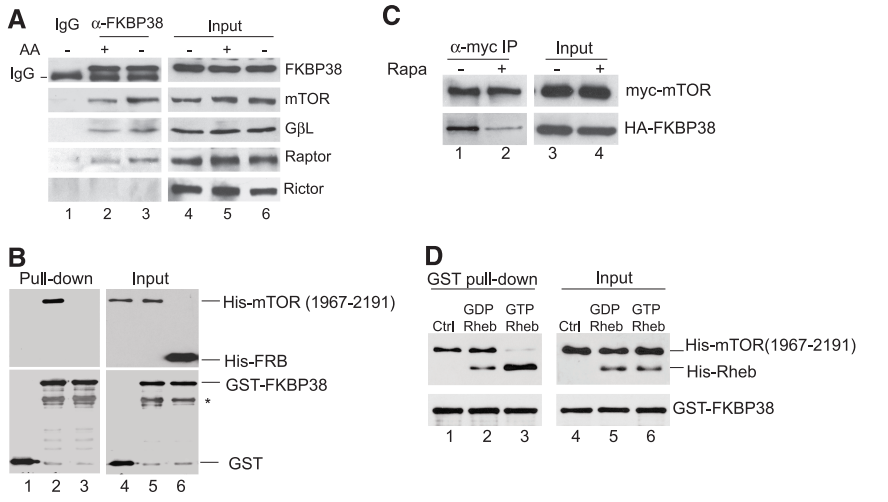

Fig. 3. Control of the interaction between FKBP38 and mTOR by Rheb. (**A**) Association of FKBP38 with mTORC1. HEK293 cells were deprived of amino acids for 1 hour and then incubated with amino acids for 30 min. Lysates were precipitated with antibody to FKBP38 or control IgG, and precipitates were blotted for the components of mTORC1 and mTORC2. (**B**) The FKBP38 binding domain in mTOR. Recombinant His-mTOR(1967–2191) or His-FRB domain of mTOR was incubated with GST or GST-FKBP38 followed by precipitation with glutathione beads. The precipitates were immunoblotted for GST (bottom, lane 1), GST-FKBP38 (bottom, lanes 2 and 3), His-mTOR (1967–2191) and His-FRB (top, lanes 1 to 3). Asterisk denotes a breakdown product of FKBP38. (**C**) Effect of rapamycin on the association of FKBP38 with mTOR. HEK293 cells expressing both myc-mTOR and HA-FKBP38 were treated with 20 nM rapamycin (lanes 2 and 4) or drug vehicle (lanes 1 and 3) for 1 hour. Lysates were precipitated with antibody to myc epitope. HA-FKBP38 and myc-mTOR in the precipitates were detected by immunoblotting. (**D**) Effect of Rheb on the interaction of FKBP38 with mTOR. Recombinant His-mTOR (1967–2191) and GST-FKBP38 were incubated together in the absence (lane 4) or presence of GDP-loaded His-Rheb (lane 5) or GTP-loaded His-Rheb (lane 6), followed by precipitation with glutathione beads. The precipitates were immunoblotted for GST-FKBP38 (bottom), His-mTOR(1967–2191) (top, top bands), and His-Rheb (top, lower bands).

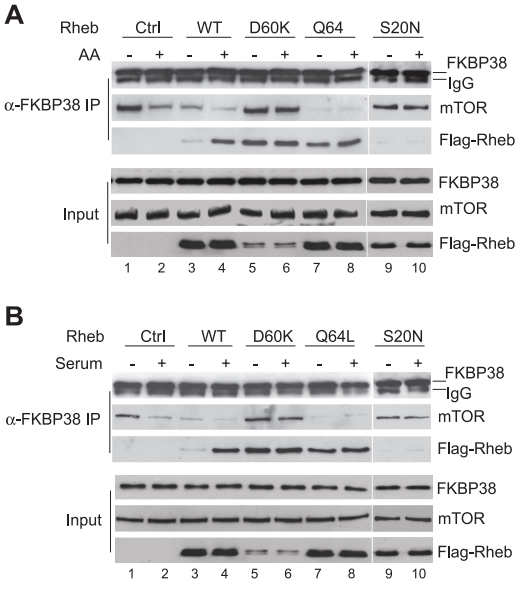

Fig. 4. Controlling interaction of FKBP38 with mTOR by Rheb in response to serum stimulation and nutrient availability. HEK293 cells were transfected with control vector or the indicated Rheb mutants and incubated for 24 hours. (**A**) Effect of amino acid conditions on the interaction of FKBP38 with mTOR. Cells were deprived of amino acids (AA) for 1 hour (–) followed by readdition of amino acids for 30 min (+). (**B**) Effect of serum conditions on the interaction of FKBP38 with mTOR. Cells were serum deprived (0.5% serum) for 16 hours, followed by treatment with 20% serum for 30 min (+) or no treatment (–). Lysates (input) were precipitated with antibody to FKBP38. The presence of endogenous FKBP38, mTOR, and ectopically expressed Rheb in the precipitates was detected by immunoblotting.

of amino acids, whereas the interaction of the expressed Rheb mutant with FKBP38 was strong (Fig. 4A and fig. S9). Despite this, the latter interaction was partially sensitive to amino acid starvation, consistent with a previous finding that the Q64L mutant retains a limited response to the GAP activity of the TSC1/TSC2 complex (*20*). In contrast, in cells expressing the S20N mutant, little Rheb was bound to FKBP38, and the interaction of FKBP38 with mTOR was strong and largely insensitive to changes in amino acid conditions. These observations indicate that these Rheb mutants block the amino acid–dependent regulation of the interaction between FKBP38 and mTOR, which suggests that amino acid conditions control the interaction of FKBP38 with mTOR through Rheb.

The effect of D60K on the interaction of FKBP38 with mTOR was similar to that of S20N. However, despite its failure to bind nucleotide and a low expression level (*19*), the D60K mutant interacted with FKBP38 more strongly than did wild-type Rheb, and the interaction was insensitive to changes in amino acid conditions. This observation suggests that the Asp to Lys (D to K) substitution at position 60 confers to Rheb a higher affinity for FKBP38 but impedes its action to release mTOR from FKBP38. The fact that the D60K mutant binds strongly to FKBP38 but does not displace it from mTOR interaction suggests that the binding of FKBP38 with Rheb and that with mTOR are not mutually exclusive.

Cells deprived of serum also showed increased interaction between FKBP38 and mTOR that was prevented by serum repletion or overexpression of wild-type Rheb (Fig. 4B). Similarly, overexpression of active Rheb mutant (Q64L) or inactive Rheb mutants (S20N and D60K) rendered the interaction of FKBP38 with mTOR insensitive to changes in serum conditions, which suggests that the interaction was regulated by Rheb in response to serum conditions.

The ability to bind and inhibit mTOR activity in the absence of rapamycin establishes FKBP38 as an endogenous inhibitor of mTOR. Under amino acid or serum starvation this mTOR inhibitor binds and interferes with mTORC1 function in a manner similar to that of the FKBP12-rapamycin complex. In response to growth factors or amino acid availability, Rheb prevents the interaction of FKBP38 with mTOR in a GTP-dependent manner, which leads to mTORC1 activation. This mechanism for the action of Rheb on mTOR is consistent with evidence that active Rheb associates less with mTOR than does the inactive form (*16*). In addition to mTOR, FKBP38 associates with Bcl-2 and calcineurin (*14*, *21*). It is thus possible that Rheb may also control Bcl-2–dependent apoptosis and calcineurin-dependent transcription through FKBP38.

References and Notes

1. D. E. Martin, M. N. Hall, *Curr. Opin. Cell Biol.* **17**, 158 (2005).
2. S. Wullschleger, R. Loewith, M. N. Hall, *Cell* **124**, 471 (2006).
3. R. Loewith *et al.*, *Mol. Cell* **10**, 457 (2002).
4. K. Hara *et al.*, *Cell* **110**, 177 (2002).
5. D. H. Kim *et al.*, *Cell* **110**, 163 (2002).
6. A. Garami *et al.*, *Mol. Cell* **11**, 1457 (2003).
7. K. Inoki, Y. Li, T. Xu, K. L. Guan, *Genes Dev.* **17**, 1829 (2003).
8. Y. Zhang *et al.*, *Nat. Cell Biol.* **5**, 578 (2003).
9. P. J. Aspuria, F. Tamanoi, *Cell. Signal.* **16**, 1105 (2004).
10. B. D. Manning, L. C. Cantley, *Trends Biochem. Sci.* **28**, 573 (2003).
11. Y. Li, M. N. Corradetti, K. Inoki, K. L. Guan, *Trends Biochem. Sci.* **29**, 32 (2004).
12. G. Fischer, T. Tradler, T. Zarnt, *FEBS Lett.* **426**, 17 (1998).
13. F. Edlich *et al.*, *EMBO J.* **24**, 2688 (2005).
14. M. Shirane, K. I. Nakayama, *Nat. Cell Biol.* **5**, 28 (2003).
15. Materials and methods are available as supporting material on *Science* Online.
16. X. Long, Y. Lin, S. Ortiz-Vega, K. Yonezawa, J. Avruch, *Curr. Biol.* **15**, 702 (2005).
17. X. Long, S. Ortiz-Vega, Y. Lin, J. Avruch, *J. Biol. Chem.* **280**, 23433 (2005).
18. M. Roccio, J. L. Bos, F. J. Zwartkruis, *Oncogene* **25**, 657 (2006).
19. A. P. Tabancay Jr. *et al.*, *J. Biol. Chem.* **278**, 39921 (2003).
20. Y. Li, K. Inoki, K. L. Guan, *Mol. Cell. Biol.* **24**, 7965 (2004).
21. F. Edlich *et al.*, *J. Biol. Chem.* **281**, 14961 (2006).

22. We thank K. Nakayama, K.-L. Guan, F. Tamanoi, N. Sonenberg, and J. Chen for plasmids and reagents; D. Alschuler for suggestions; and P. Houghton for critical reading of this manuscript. Supported by grants to Y.L. from the U.S. Department of Defense (W81XWH-06-1-0047) and to Y.J. from American Cancer Society (RSG-03-169-TBE) and NIH (GM068832).

Supporting Online Material

www.sciencemag.org/cgi/content/full/318/5852/977/DC1
Materials and Methods
Figs. S1 to S9
References

5 July 2007; accepted 14 September 2007
10.1126/science.1147379

Magnetic Resonance Spectroscopy Identifies Neural Progenitor Cells in the Live Human Brain

Louis N. Manganas,[1,3] Xueying Zhang,[1] Yao Li,[1] Raphael D. Hazel,[1,2] S. David Smith,[2] Mark E. Wagshul,[1] Fritz Henn,[2] Helene Benveniste,[1,2] Petar M. Djurić,[1] Grigori Enikolopov,[3]* Mirjana Maletić-Savatić[1,3]*

The identification of neural stem and progenitor cells (NPCs) by in vivo brain imaging could have important implications for diagnostic, prognostic, and therapeutic purposes. We describe a metabolic biomarker for the detection and quantification of NPCs in the human brain in vivo. We used proton nuclear magnetic resonance spectroscopy to identify and characterize a biomarker in which NPCs are enriched and demonstrated its use as a reference for monitoring neurogenesis. To detect low concentrations of NPCs in vivo, we developed a signal processing method that enabled the use of magnetic resonance spectroscopy for the analysis of the NPC biomarker in both the rodent brain and the hippocampus of live humans. Our findings thus open the possibility of investigating the role of NPCs and neurogenesis in a wide variety of human brain disorders.

The adult mammalian brain retains the ability to generate new neurons. These neurons are produced from neural stem and progenitor cells (NPCs), which reside in the hippocampus and the subventricular zone (*1*–*4*). NPCs possess the ability to self-renew and also to generate progeny that can give rise to mature cell types. The ability of NPCs to produce neurons, astrocytes, and oligodendrocytes in vitro and in vivo raises the prospect of harnessing them to repair nerve tissue damaged or lost to neurological disease or trauma (*1*, *2*, *4*). The realization of the curative potential of NPCs would benefit from the development of methods that would enable their identification and tracking in vivo. Currently, positron emission tomography, single-photon computed tomography scanning, and magnetic resonance imaging (MRI) are being examined toward this goal (*5*–*7*). These technologies require NPCs to be preloaded ex vivo with radiolabeled agents or superparamagnetic iron oxide–based derivatives, and therefore are not applied for the detection of endogenous NPCs in the human brain. We used proton magnetic resonance spectrosopy (^{1}H-MRS) to overcome the above limitations and to detect NPCs in the live human brain.

Proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) has been widely used for in vitro detection of low quantities of known metabolites and the identification of unknown compounds present in body fluids or tissues in vitro (*8*). ^{1}H-NMR can identify metabolites that are specific for neurons [such as *N*-acetyl aspartate (NAA)] or glia [such as choline (Cho) and myoinositol (mI)], and these compounds have been used as reliable biomarkers of the corre-

[1]SUNY Stony Brook, Stony Brook, NY 11794, USA. [2]Brookhaven National Laboratory, Upton, NY 11719, USA. [3]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA.

*To whom correspondence should be addressed. E-mail: enikolop@cshl.edu (G.E.); mmaleticsava@notes.cc.sunysb.edu (M.M.-S.).

Rheb Activates mTOR by Antagonizing Its Endogenous Inhibitor, FKBP38

Xiaochun Bai *et al.*

Science **318**, 977 (2007);
DOI: 10.1126/science.1147379

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.