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The Mammalian Target of Rapamycin (mTOR) Partner, Raptor, Binds the mTOR Substrates p70 S6 Kinase and 4E-BP1 through Their TOR Signaling (TOS) Motif*

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The mammalian target of rapamycin (mTOR) controls multiple cellular functions in response to amino acids and growth factors, in part by regulating the phosphorylation of p70 S6 kinase (p70S6k) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1). Raptor (regulatory associated protein of mTOR) is a recently identified mTOR binding partner that also binds p70S6k and 4E-BP1 and is essential for TOR signaling *in vivo*. Herein we demonstrate that raptor binds to p70S6k and 4E-BP1 through their respective TOS (conserved TOR signaling) motifs to be required for amino acid- and mTOR-dependent regulation of these mTOR substrates *in vivo*. A point mutation of the TOS motif also eliminates all *in vitro* mTOR-catalyzed 4E-BP1 phosphorylation and abolishes the raptor-dependent component of mTOR-catalyzed p70S6k phosphorylation *in vitro*. Raptor appears to serve as an mTOR scaffold protein, the binding of which to the TOS motif of mTOR substrates is necessary for effective mTOR-catalyzed phosphorylation *in vivo* and perhaps for conferring their sensitivity to rapamycin and amino acid sufficiency.

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The target of rapamycin (TOR)¹ proteins are protein kinases that were first identified in *Saccharomyces cerevisiae* through mutants that confer resistance to growth inhibition induced by the immunosuppressive macrolide rapamycin (1). In mammalian cells, rapamycin blocks phosphorylation of eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) (2, 3) and p70 S6 kinase (p70S6k) (4, 5) by interfering with the function of mTOR (6, 7) (also known as FRAP, RAFT1, or RAPT). Although mTOR can phosphorylate both these targets directly *in vitro* (8–10), the mechanism of mTOR regulation of these phosphorylations *in vivo* remains incompletely understood (11).

The p70S6k is activated through a sequential multisite phosphorylation in response to insulin or mitogens *in vivo* (11). In addition, nutrients, especially amino acids, have been shown to regulate the phosphorylation of p70S6k and 4E-BP1 and to be necessary for insulin or mitogen regulation (12–17). The activity of p70S6k *in vivo* is most closely related to the phosphorylation at Thr-412, situated in a hydrophobic motif C-terminal to the canonical catalytic domain (18, 19). The identity of the kinase(s) acting on this site *in vivo* is uncertain; however, this site can be phosphorylated directly by mTOR *in vitro* (9, 10). Recently, site-specific mutagenesis was employed to define a five-amino acid sequence called the TOS (TOR signaling) motif as the minimal functionally important region within this p70S6k noncatalytic N-terminal segment (21). As with N-terminal deletion, mutation of a single Phe within the TOS motif to Ala causes marked inhibition of activity of full-length p70S6k and a loss of sensitivity to rapamycin and amino acid withdrawal in the p70S6k-ΔCT104, lacking C-terminal noncatalytic tail, background. In addition, a TOS motif was identified in the 4E-BPs, wherein mutation of 4E-BP1 Phe-114 to Ala inhibits amino acid- and serum-induced 4E-BP1 phosphorylation.

Raptor is a recently discovered, highly conserved 150-kDa TOR-binding protein that also binds p70S6k and 4E-BPs (22, 23). All raptor homologues (22–24) contain a unique conserved region in their N-terminal half (the RNC domain), followed by three HEAT repeats and seven WD repeats near the C terminus. The binding of TOR to raptor or its *S. cerevisiae* homologue KOG1 (24) is necessary for TOR signaling *in vivo* in *Caenorhabditis elegans* and *S. cerevisiae* (22, 24). Moreover, mTOR-catalyzed phosphorylation of 4E-BP1 *in vitro* is entirely dependent on the presence of raptor, whereas mTOR-catalyzed phosphorylation of p70S6k *in vitro*, although stimulated ~5-fold by the addition of raptor, proceeds in the absence of raptor (22).

Herein we show that the TOS motif is necessary for the binding of p70S6k and 4E-BP1 to raptor. Mutation of the TOS motif abolishes mTOR-catalyzed 4E-BP1 phosphorylation *in vitro* in the presence of raptor and eliminates the raptor-dependent stimulation of mTOR-catalyzed p70S6k phosphorylation. Thus the inhibitory effect of TOS deletion or mutation on 4E-BP1 and p70S6k phosphorylation *in vivo* can be attributed to the inability of these mutants to bind raptor. Moreover we

¹ The abbreviations used are: mTOR, mammalian target of rapamycin; raptor, regulatory associated protein of mTOR; TOS motif, TOR signaling motif; p70S6k, p70 S6 kinase; 4E-BP1, eukaryotic initiation factor 4E-binding protein 1; PVDF, polyvinylidene difluoride; GST, glutathione S-transferase; PDK1, 3-phosphoinositide-dependent protein kinase 1.

suggest that the binding of these mTOR substrates to raptor may also be necessary for their sensitivity to regulation by amino acids and rapamycin.

EXPERIMENTAL PROCEDURES

Reagents and Antibodies—Reagents and antibodies are described previously (10, 12, 25).

cDNAs—The expression vectors of FLAG-tagged wild-type raptor (FLAG-raptor), FLAG-tagged raptor lacking the C-terminal region (FLAG-raptor-ΔCT), GST-fused 4E-BP1 (GST-4E-BP1) (22), GST-fused p70 S6 kinase (GST-p70S6k), GST-fused PDK1 (GST-PDK1), and GST-fused kinase-inactive mutant of p70 S6 kinase lacking the C terminus (GST-p70S6k-KM/ΔCT) (20, 26) were described previously. A mutant of p70 S6 kinase α1 in which Phe-28 was substituted with Ala (p70S6k-F28A) and a mutant of 4E-BP1 in which Phe-114 was substituted with Ala (4E-BP1-F114A) were created by using the QuikChange™ site-directed mutagenesis kit (Stratagene). To make the expression vector of FLAG-tagged raptor lacking the N-terminal region (FLAG-raptor-ΔNT), a cDNA fragment encoding bp 2710–4005 was amplified by PCR using pcDNA1-FLAG-raptor as a template and ligated into pcDNA1-FLAG (22). To make the expression vector of the FLAG-tagged WD repeat domain of raptor (FLAG-raptor-WD), a cDNA fragment encoding bp 3025–4005 was amplified by PCR using pcDNA1-FLAG-raptor as a template and ligated into pcDNA3-FLAG (22). To make pGEX-4E-BP1-F114A, the 4E-BP1-F114A fragment was cut out from pEBG-4E-BP1-F114A and ligated into the pGEX vector.

Transfection—Transient transfection was performed by the lipofection method using LipofectAMINE according to the manufacturer's protocol (Invitrogen).

Cell Culture—HEK293 cells were cultured in Dulbecco's modified Eagle's medium with 10% fetal bovine serum.

GST Pull-down Assay—The GST pull-down assay was performed as described previously (10, 12, 22).

mTOR Kinase Assay—The mTOR kinase assay was performed as described previously (10, 12, 22).

RESULTS AND DISCUSSION

To examine whether the TOS motif in p70S6k is important to the binding of p70S6k to raptor, wild-type GST fusion proteins of full-length p70S6kα1 (GST-p70S6k) or an F28A mutant (GST-p70S6k-F28A) were coexpressed with FLAG-tagged raptor (FLAG-raptor) in HEK293 cells. The F28A mutation of p70S6kα1 corresponds to the F5A mutation of p70S6kα2, a mutation that recapitulates the phenotype of N-terminal deletion (21); thus, like p70S6kΔ2–46 lacking a short segment N terminus (20), hemagglutinin-tagged p70S6kα2-F5A is inactive in HEK293 cells, and its activity can be partially rescued by deletion of the p70S6k C-terminal noncatalytic tail. As expected, we find that GST-p70S6k-F28A is also inactive in HEK293 cells (data not shown). Moreover, although p70S6k binds specifically to coexpressed FLAG-raptor (Fig. 1A, lane 4), p70S6k-F28A does not (Fig. 1A, lane 5). Inasmuch as p70S6k-F28A is inactive in HEK293 cells, we inquired whether p70S6k activity is required for the association between p70S6k and raptor. We find that the ATP site mutant, p70S6k-KM/ΔCT104, which is kinase-inactive and lacks C-terminal 104 residues (20, 26), binds raptor to an extent similar to p70S6k (Fig. 1B, compare lane 4 with lane 6). These results indicate that the recognition of p70S6k by raptor requires an intact p70S6k TOS motif but neither kinase activity nor the carboxyl-terminal tail of p70S6k.

The TOS motif of 4E-BP1 is also required for mTOR signaling to 4E-BP1 *in vivo* (21). We therefore next examined the effects of TOS motif mutation on the ability of 4E-BP1 to bind raptor; GST fusions of wild-type 4E-BP1 (GST-4E-BP1) or an F114A mutant of 4E-BP1 (GST-4E-BP1-F114A) were coexpressed with FLAG-tagged raptor (FLAG-raptor) in HEK293 cells. As observed with p70S6k (Fig. 1A), wild-type 4E-BP1 binds specifically to raptor (Fig. 2, lane 4), whereas 4E-BP1-F114A does not (Fig. 2, lane 5). These results clearly indicate that the TOS motif is critical for the binding of p70S6k and

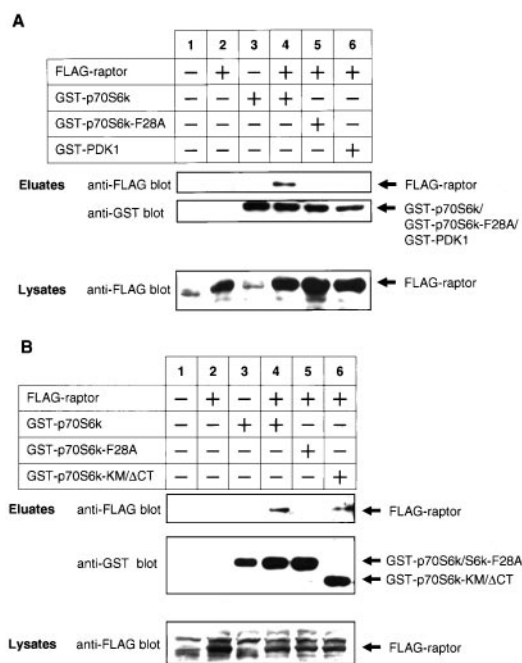


FIG. 1. TOS motif of p70S6k is necessary for binding to raptor. A and B, HEK293 cells are transfected with FLAG-tagged raptor (FLAG-raptor) (lanes 2 and 4–6) together with GST-fused p70 S6 kinase wild-type (GST-p70S6k) (lanes 3 and 4), GST-fused p70 S6 kinase in which Phe-28 is substituted with Ala (GST-p70S6k-F28A) (lane 5), GST-fused PDK1 (GST-PDK1) (lane 6 in A), or GST-fused kinase-inactive mutant of p70 S6 kinase lacking the carboxyl terminus (GST-p70S6k-KM/ΔCT) (lane 6 in B). GST fusion proteins are isolated on GSH-Sepharose, washed, eluted, and transferred onto a PVDF membrane (Eluates) after separation by SDS-PAGE. The upper part of the membrane is immunoblotted with the anti-FLAG antibody (anti-FLAG blot) and the lower part with the anti-GST antibody (anti-GST blot). The cell lysates prepared at the same time are also analyzed by anti-FLAG immunoblot to confirm the expression of FLAG-raptor (Lysates).

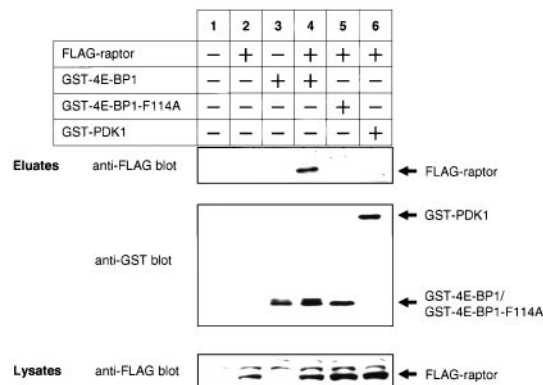


FIG. 2. TOS motif of 4E-BP1 is necessary for binding to raptor. HEK293 cells are transfected with FLAG-raptor (lanes 2 and 4–6) together with GST-fused 4E-BP1 (GST-4E-BP1) (lanes 3 and 4), GST-fused 4E-BP1 in which Phe-114 is substituted with Ala (GST-4E-BP1-F114A) (lane 5), and GST-PDK1 (lane 6). GST fusion proteins are isolated on GST-Sepharose, eluted, separated by SDS-PAGE, and transferred onto a PVDF membrane (Eluates). The upper part of the membrane is immunoblotted with the anti-FLAG antibody (anti-FLAG blot), and the lower part with anti-GST antibody (anti-GST blot). The cell lysate is also analyzed by anti-FLAG immunoblot to confirm the expression of FLAG-raptor (Lysates).

4E-BP1 to raptor and may be a common recognition element by which raptor couples mTOR targets to the mTOR kinase.

To identify the region of raptor that interacts with the TOS motif of p70S6k, we coexpressed several FLAG-tagged raptor fragments with GST-p70S6k or GST-p70S6k-F28A in HEK293 cells. The N-terminal region of raptor (amino acids 1–904,

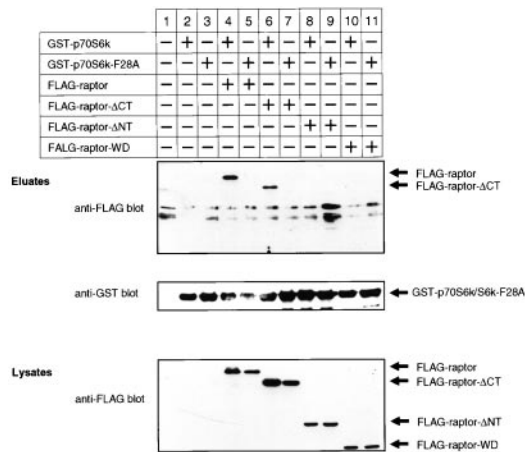


FIG. 3. Amino-terminal portion of raptor is required for binding to the TOS motif in p70S6k. HEK293 cells are transfected with FLAG-tagged raptor (*FLAG-raptor*) (lanes 4 and 5), FLAG-tagged raptor lacking the carboxyl-terminal region (*FLAG-raptor-ΔCT*) (lanes 6 and 7), FLAG-tagged raptor lacking the amino-terminal region (*FLAG-raptor-ΔNT*) (lanes 8 and 9), or FLAG-tagged WD repeat domain of raptor (*FLAG-raptor-WD*) (lanes 10 and 11), together with GST-p70S6k (lanes 2, 4, 6, 8, and 10) or GST-p70S6k-F28A (lanes 3, 5, 7, 9, and 11). Transfected cells are subjected to GST pull-down assay, and the eluates are separated by SDS-PAGE and transferred onto a PVDF membrane (*Eluates*). The membrane is immunoblotted with anti-FLAG antibody (*anti-FLAG blot*) or with anti-GST antibody (*anti-GST blot*). The supernatant prepared at the same time is also analyzed by immunoblot with anti-FLAG antibody to confirm the expression of FLAG-raptor (*Lysates*).

raptor-ΔCT), which contains the unique raptor N-terminal conserved (RNC) region and the HEAT repeats, binds to p70S6k as strongly as does the full-length raptor (Fig. 3, lanes 4 and 6); neither bind to p70S6k-F28A (Fig. 3, lanes 5 and 7). The C-terminal region of raptor (amino acids 904–1335, raptor-ΔNT) and the isolated WD repeat domain of raptor (amino acids 1009–1335, raptor-WD) do not bind to p70S6k (Fig. 3, lanes 8 and 10). These results suggest that the RNC region and/or the HEAT repeats of raptor appear to be involved in the regulation of the TOS motif of p70S6k. The selective binding of p70S6k to the N-terminal portion of raptor contrasts with the requirements for raptor binding to mTOR, which appears to involve multiple sites in raptor (22, 23); thus raptor deleted of its N terminus (236–1335) or C terminus (1–904) fails to bind mTOR. We reported previously that the overexpression of the full-length of raptor or raptor-ΔCT(1–904) in HEK293 cells severely inhibited the kinase activity of coexpressed p70S6k (22). In contrast, the overexpression of raptor-ΔNT(904–1335) or raptor-WD(1009–1335) in HEK293 cells does not significantly inhibit the kinase activity of coexpressed p70S6k (data not shown). Thus, the inhibitory effect of overexpressed full-length raptor and raptor-ΔCT(1–904) may be caused by the ability of these elements to bind coexpressed p70S6k and sequester it from endogenous mTOR, thereby disrupting the ternary complex of mTOR-raptor-p70S6k necessary for effective mTOR-catalyzed p70S6k phosphorylation. Moreover, the ability of p70S6k overexpression to interfere with the phosphorylation of 4E-BP1 is likely to be due to the competition of these elements for a common binding site on raptor.

We had shown previously (22) that the association of raptor with mTOR is absolutely required for the ability of mTOR to catalyze 4E-BP1 phosphorylation *in vitro*. It was not clear, however, whether this reflected a stimulatory effect of raptor on the catalytic activity of mTOR or on the ability of raptor to properly configure or “present” the 4E-BP1 substrate. The finding that point mutation of the substrate TOS motif eliminates the binding of the substrates to raptor enables a test of these

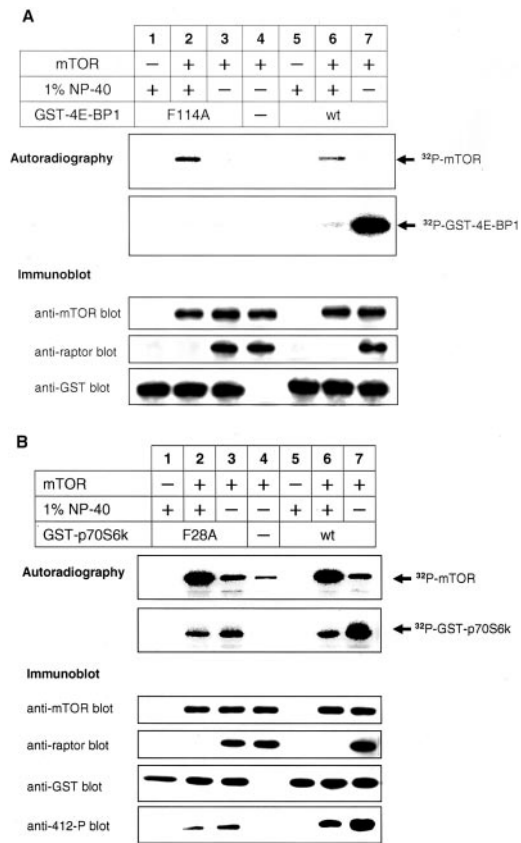


FIG. 4. The effect of TOS motif mutation on the mTOR-catalyzed phosphorylation of 4E-BP1 or p70S6k *in vitro* in the presence or absence of raptor. A, HEK293 cells are lysed without detergent, and immunoprecipitation is performed with the anti-mTOR antibody (lanes 2–4, 6, and 7) or with normal mouse IgG (lanes 1 and 5). Immunoprecipitates are washed with lysis buffer containing 0.5 M NaCl with 1% Nonidet P-40 (lanes 1, 2, 5, and 6) or without 1% Nonidet P-40 (lanes 3, 4, and 7) and subjected to mTOR kinase assay using GST-4E-BP1-F114A (lanes 1–3), vehicle (lane 4), or GST-4E-BP1 (lanes 5–7) as a substrate. The samples are separated by SDS-PAGE, transferred onto a PVDF membrane, and analyzed by autoradiography (*Autoradiography*). They are subsequently immunoblotted with the anti-mTOR antibody (*anti-mTOR blot*), with the anti-raptor antibody (*anti-raptor blot*), and with the anti-GST antibody (*anti-GST blot*). ³²P incorporated into GST-4E-BP1 is quantified by BAS 2500 in arbitrary units: lane 1, 20.6; lane 2, 343; lane 3, 296; lane 4, 30.3; lane 5, 48.4; lane 6, 928; lane 7, 8150. These results are representative of three reproducible experiments. B, HEK293 cells are lysed, immunoprecipitated, and immunoblotted as described in A and then subjected to mTOR kinase assay using GST-p70S6k-F28A (lanes 1–3), vehicle (lane 4), or GST-p70S6k (lanes 5–7) as a substrate. The phosphorylation of threonine 412 of GST-p70S6k and GST-p70S6k-F28A is examined by immunoblotting with the anti-phosphothreonine 412 of p70S6k-specific antibody (*anti-412-P blot*). ³²P incorporated into GST-p70S6k is quantified by BAS 2500 in arbitrary units: lane 1, 200; lane 2, 1580; lane 3, 2060; lane 4, 115; lane 5, 292; lane 6, 1940; lane 7, 7440. These results are representative of three reproducible experiments.

alternatives. Removal of raptor from mTOR by washing with 1% Nonidet P-40 essentially eliminates the ability of mTOR to phosphorylate GST-4E-BP1 *in vitro* (Fig. 4A, compare lane 6 with lane 7), as shown previously (22). In addition, it is evident that mTOR-catalyzed phosphorylation of GST-4E-BP1-F114A is virtually eliminated despite the presence of raptor (Fig. 4A, lane 3). Thus, the ability of 4E-BP1 to bind raptor is indispensable for mTOR-catalyzed 4E-BP1 phosphorylation.

The absolute requirement for raptor is not true for p70S6k (Fig. 4B). As before, endogenous mTOR is immunoprecipitated and washed without or with 1% Nonidet P-40, the latter to remove endogenous raptor. The mTOR kinase activity is assayed using as substrate either recombinant GST-p70S6k or

GST-p70S6k-F28A, each purified from rapamycin-treated HEK293 cells; mTOR-catalyzed phosphorylation is monitored by both ^{32}P incorporation into the recombinant GST fusion protein and by anti-p70S6k Thr(P)-412 immunoreactivity. As shown previously (22), overall mTOR kinase activity toward GST-p70S6k and the specific phosphorylation of Thr(P)-412 (estimated by immunoblot) are enhanced by coimmunoprecipitation of mTOR with raptor (Fig. 4B, lane 7); washing the mTOR immunoprecipitates with detergent so that endogenous raptor is removed substantially reduces the mTOR-catalyzed overall ^{32}P incorporation into GST-p70S6k as well as the anti-Thr(P)-412 immunoreactivity achieved, as compared with that catalyzed by the same mTOR immunoprecipitate that had not been washed with Nonidet P-40 so as to remove coprecipitating endogenous raptor. In contrast, when GST-p70S6k-F28A is employed as substrate for these same mTOR immunoprecipitates, the overall ^{32}P incorporation and phosphorylation of Thr-412 is low and independent of whether or not raptor had been removed by Nonidet P-40 washing. The phosphorylation catalyzed by the mTOR-raptor complex of GST-p70S6k-F28A is only about 20% that of wild-type GST-p70S6k and very close to the phosphorylation of wild-type GST-p70S6k achieved by the raptor-free mTOR immunoprecipitate. Nevertheless, the inability of raptor to alter the residual mTOR-catalyzed phosphorylation of GST-p70S6k-F28A establishes that the stimulatory effects of raptor on the phosphorylation of wild-type p70S6k (and probably 4E-BP1) are not due to a stimulation of intrinsic catalytic activity of the mTOR kinase but entirely to the ability of raptor to bind and present these two substrates in a more effective way.

The continued ability of mTOR to phosphorylate p70S6k-F28A (or wild-type p70S6k in the absence of raptor) is consistent with the earlier demonstration that mTOR can phosphorylate prokaryotic recombinant fragments of p70S6k that lack entirely the N-terminal region containing the TOS motif on a variety of sites, including Thr-412 (9). The present demonstration of a persistent TOS- and raptor-independent component of mTOR-catalyzed p70S6k phosphorylation *in vitro* raises the possibility that this activity may be responsible for the insulin-responsive but rapamycin- and amino acid-insensitive phosphorylation *in vivo* of the various N-terminal mutant p70S6ks (20, 21). Thus the F28A mutation of the TOS motif, like N-terminal deletion, makes p70S6k Thr-412 phosphorylation and thus catalytic activity very low; deletion of the C terminus partially restores Thr-412 phosphorylation, which is now insulin-responsive but insensitive to rapamycin and amino acid sufficiency (12, 19, 21). The insensitivity to rapamycin and amino acid sufficiency were interpreted initially to indicate that the persistent insulin-responsive Thr-412 phosphorylation could not be due to an insulin-induced increase in mTOR-catalyzed Thr-412 phosphorylation (12). Nevertheless, the present demonstration that mTOR-catalyzed phosphorylation of GST-p70S6k-F28A *in vitro* persists, although at a greatly reduced efficiency as compared with wild-type GST-p70S6k, suggests that such a reaction may account for the low but persistent *in vivo* activity of the p70S6k $\Delta 2-46/\Delta\text{CT}104$ variant. The p70 variants that are unable to bind to raptor may be very poorly phosphorylated by mTOR *in vivo*, but the persistent modest ability of mTOR to catalyze this modification is facilitated by deletion of the p70S6k C-terminal tail, as is seen with PDK1-catalyzed phosphorylation of the p70S6k activation loop (26). The insulin stimulation of these variants *in vivo* may reflect disinhibition from the TSC1-TSC2 complex (27, 28). Finally, insensitivity to amino acids and rapamycin suggests that the association of p70S6k with raptor is necessary for p70

regulation to be responsive to these inputs. Thus, the rapamycin-FKBP12 complex may interfere with mTOR-catalyzed p70 phosphorylation only when p70S6k is complexed with raptor. This hypothesis implies that the model of action of the rapamycin-FKBP12 complex *in vivo* at moderately inhibitory concentrations of rapamycin (e.g. 2–20 nM) is not through suppression of the mTOR catalytic activity but by interference with the effective interaction between mTOR and its substrates. Such a proposal has been offered previously, based in part on the nearly hundredfold higher concentrations of rapamycin (in the presence of excess FKBP12) required for inhibition of mTOR kinase activity *in vitro* (8, 10).

Evaluating this hypothesis will require much additional insight into the mechanism by which the mTOR-raptor complex interacts with its substrates, as well as an understanding of the contributions of mLst8 (24) and the TSC1-TSC2 (27, 28) complex to the signaling functions and kinase activity of mTOR. Nevertheless, the present results establish one critical mechanism by which the protein raptor participates in coupling mTOR to its cellular substrates.

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REFERENCES

- Schmelzle, T., and Hall, M. N. (2000) *Cell* **103**, 253–262
- Lin, T. A., Kong, X., Saltiel, A. R., Blackshear, P. J., and Lawrence, J. C. Jr. (1995) *J. Biol. Chem.* **270**, 18531–18538
- von Manteuffel, S. R., Gingras, A. C., Ming, X. F., Sonenberg, N., and Thomas, G. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 4076–4080
- Chung, J., Kuo, C. J., Crabtree, G. R., and Blenis, J. (1992) *Cell* **69**, 1227–1236
- Price, D., Grove, J. R., Calvo, V., Avruch, J., and Bierer, B. E. (1992) *Science* **257**, 973–977
- Brown, E. J., Beal, P. A., Keith, C. T., Chen, J., Shin, T. B., and Schreiber, S. L. (1995) *Nature* **377**, 441–446
- Hara, K., Yonezawa, K., Kozlowski, M. T., Sugimoto, T., Andrabi, K., Weng, Q. P., Kasuga, M., Nishimoto, I., and Avruch, J. (1997) *J. Biol. Chem.* **272**, 26457–26463
- Brunn, G. J., Hudson, C. C., Sekulic, A., Williams, J. M., Hosoi, H., Houghton, P. J., Lawrence, J. C., Jr., and Abraham, R. T. (1997) *Science* **277**, 99–101
- Burnett, P. E., Barrow, R. K., Cohen, N. A., Snyder, S. H., and Sabatini, D. M. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 1432–1437
- Isotani, S., Hara, K., Tokunaga, C., Inoue, H., Avruch, J., and Yonezawa, K. (1999) *J. Biol. Chem.* **274**, 34493–34498
- Avruch, J., Belham, C., Weng, Q. P., Hara, K., and Yonezawa, K. (2001) in *Progress in Molecular and Subcellular Biology* (Rhoads, R. E., ed) pp. 115–154, Springer-Verlag Berlin, Berlin
- Hara, K., Yonezawa, K., Weng, Q. P., Kozlowski, M. T., Belham, C., and Avruch, J. (1998) *J. Biol. Chem.* **273**, 14484–14494
- Wang, X., Campbell, L. E., Miller, C. M., and Proud, C. G. (1998) *Biochem. J.* **334**, 261–267
- Patti, M. E., Brambilla, E., Luzi, L., Landaker, E. J., and Kahn, C. R. (1998) *J. Clin. Invest.* **101**, 1519–1529
- Fox, H. L., Kimball, S. R., Jefferson, L. S., and Lynch, C. J. (1998) *Am. J. Physiol.* **274**, C206–C213
- Shigemitsu, K., Tsujishita, Y., Hara, K., Nanahoshi, M., Avruch, J., and Yonezawa, K. (1999) *J. Biol. Chem.* **274**, 1058–1065
- Xu, G., Kwon, G., Marshall, C. A., Lin, T. A., Lawrence, J. C., Jr., and McDaniel, M. L. (1998) *J. Biol. Chem.* **273**, 28178–28184
- Pearson, R. B., Dennis, P. B., Han, J. W., Williamson, N. A., Kozma, S. C., Wettenhall, R. E., and Thomas, G. (1995) *EMBO J.* **14**, 5279–5287
- Weng, Q. P., Kozlowski, M., Belham, C., Zhang, A., Comb, M. J., and Avruch, J. (1998) *J. Biol. Chem.* **273**, 16621–16629
- Weng, Q. P., Andrabi, K., Kozlowski, M. T., Grove, J. R., and Avruch, J. (1995) *Mol. Cell. Biol.* **15**, 2333–2340
- Schalm, S. S., and Blenis, J. (2002) *Curr. Biol.* **12**, 632–639
- Hara, K., Maruki, Y., Long, X., Yoshino, K., Oshiro, N., Hidayat, S., Tokunaga, C., Avruch, J., and Yonezawa, K. (2002) *Cell* **110**, 177–189
- Kim, D. H., Sarbassov, D. D., Ali, S. M., King, J. E., Latek, R. R., Erdjument-Bromage, H., Tempst, P., and Sabatini, D. M. (2002) *Cell* **110**, 163–175
- Loewith, R., Jacinto, E., Wulschleger, S., Lorberg, A., Crespo, J. L., Bonenfant, D., Oppliger, W., Jenoe, P., and Hall, M. N. (2002) *Mol. Cell* **10**, 457–468
- Nishizuma, T., Hara, K., Tsujishita, Y., Kaneko, K., Shii, K., and Yonezawa, K. (1998) *Biochem. Biophys. Res. Commun.* **252**, 440–444
- Alessi, D. R., Kozlowski, M. T., Weng, Q. P., Morrice, N., and Avruch, J. (1998) *Curr. Biol.* **8**, 69–81
- Kwiatkowski, D. J., Zhang, H., Bandura, J. L., Heiberger, K. M., Glogauer, M., el-Hashemite, N., and Onda, H. (2002) *Human Mol. Genet.* **11**, 525–534
- Manning, B. D., Tee, A. R., Logsdon, M. M., Blenis, J., and Cantley, L. C. (2002) *Mol. Cell* **10**, 151–162

The Mammalian Target of Rapamycin (mTOR) Partner, Raptor, Binds the mTOR Substrates p70 S6 Kinase and 4E-BP1 through Their TOR Signaling (TOS) Motif

Hiroki Nojima, Chiharu Tokunaga, Satoshi Eguchi, Noriko Oshiro, Sujuti Hidayat, Ken-ichi Yoshino, Kenta Hara, Noriaki Tanaka, Joseph Avruch and Kazuyoshi Yonezawa

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Additions and Corrections

Vol. 278 (2003) 15461–15464

The mammalian target of rapamycin (mTOR) partner, raptor, binds the mTOR substrates, p70 S6 kinase and 4E-BP1, through their TOR signaling (TOS) motif.

Hiroki Nojima, Chiharu Tokunaga, Satoshi Eguchi, Noriko Oshiro, Sujuti Hidayat, Ken-ichi Yoshino, Kenta Hara, Noriaki Tanaka, Joseph Avruch, and Kazuyoshi Yonezawa

Page 15461, Abstract, line 9 should read: Herein we demonstrate that raptor binds to p70S6k and 4E-BP1 through their respective TOS (conserved TOR signaling) motifs, a short conserved segment previously shown to be required for amino acid- and mTOR-dependent regulation of these mTOR substrates *in vivo*. The phrase “a short conserved segment previously shown” was inadvertently omitted.

Vol. 278 (2003) 18330–18335

Kinetics control preferential heterodimer formation of platelet-derived growth factor from unfolded A- and B-chains.

Carsten Müller, Susanne Richter, and Ursula Rinas

Page 18332, Equation 5: This equation was printed incorrectly. The correct equation is shown below:

$$k = A \exp\left(\frac{\Delta S^\ddagger}{R}\right) \exp\left\{-\left(\frac{\Delta H^\ddagger}{R} \frac{1}{T}\right)\right\} \quad (\text{Eq. 5})$$

This does not affect the results of the paper.

Vol. 278 (2003) 12703–12709

Characterization of a functional bacterial homologue of sodium-dependent neurotransmitter transporters.

Andreas Androutsellis-Theotokis, Naomi R. Goldberg, Kenji Ueda, Teruhiko Beppu, Matthew L. Beckman, Shonit Das, Jonathan A. Javitch, and Gary Rudnick

The grant support for Matthew L. Beckman was inadvertently omitted.

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We suggest that subscribers photocopy these corrections and insert the photocopies at the appropriate places where the article to be corrected originally appeared. Authors are urged to introduce these corrections into any reprints they distribute. Secondary (abstract) services are urged to carry notice of these corrections as prominently as they carried the original abstracts.