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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 p70S6kΔCT104, lacking C-terminal non-catalytic 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 domain 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 vivo 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 vitro 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, 22).

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-ΔKM/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-ΔN), 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 pcdNA1-FLAG (22). To make pEGX-4E-BP1-F114A, the 4E-BP1-F114A fragment was cut out from pEGX-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 F28A 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/H9251, whereas 4E-BP1-ΔN-terminal region of raptor (amino acids 1–904, 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,
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 9). 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 the N-terminal region of raptor (22), and with the anti-GST antibody (anti-GST blot) or with anti-GST antibody (anti-GST blot). The supernatant prepared at the same time is also analyzed by immunoblot with anti-GST antibody to confirm the expression of FLAG-raptor (Lysates).

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 (Autoradiography). The membrane is immunoblotted with anti-GST antibody (anti-GST blot) or with anti-GST antibody (anti-GST blot). The supernatant prepared at the same time is also analyzed by immunoblot with anti-GST antibody to confirm the expression of FLAG-raptor (Lysates).

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) and GST-p70S6k (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-GST antibody (anti-GST blot), with the anti-mTOR antibody (anti-mTOR blot), and with the anti-FLAG antibody (anti-FLAG 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 only when p70S6k is complexed with raptor. Nevertheless, the present results establish one critical mechanism by which the protein raptor participates in coupling mTOR to its cellular substrates.

**REFERENCES**

1. Schneizle, T., and Hall, M. N. (2000) *Cell* **103**, 253–262
2. Lin, T. A., Kong, X., Saltiel, A. R., Blackshear, P. J., and Lawrence, J. C. Jr. (1995) *J. Biol. Chem.* **270**, 18531–18538
3. von Manteuffel, S. R., Gingras, A. C., Ming, X. F., Sonenberg, N., and Thomas, G. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 5787–5800
4. Chung, J., Kuo, C. J., Crabtree, G. R., and Blenis, J. (1992) *Cell* **70**, 1227–1236
5. Price, D., Grove, J. R., Calvo, V., Avruch, J., and Bierer, B. E. (1993) *Science* **267**, 973–977
6. Brown, R. J., Beal, P. A., Keith, C. T., Chen, J., Shin, T. B., and Schrier, S. L. (1995) *Nature* **377**, 441–446
7. Hara, K., Yonezawa, K., Koslowki, M. T., Sugimoto, T., Andrahi, K., Weng, Q. P., Kasuga, M., Nishimoto, I., and Avruch, J. (1997) *J. Biol. Chem.* **272**, 26457–26465
8. Brun, G. J., Hudson, C. C., Sekulic, A., Williams, J. M., Hossi, H., Houghton, P. J., Lawrence, J. C. Jr., and Abraham, R. T. (1997) *Science* **277**, 99–101
9. 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
10. Isotani, S., Hara, K., Tokunaga, C., Inoue, H., Avruch, J., and Yonezawa, K. (1999) *J. Biol. Chem.* **274**, 34493–34498
11. 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
12. Hara, K., Yonezawa, K., Weng, Q. P., Koslowki, M. T., Belham, C., and Avruch, J. (1999) *J. Biol. Chem.* **274**, 14484–14494
13. Wang, X., Campbell, L. E., Miller, C. M., and Proud, C. G. (1998) *Biochem. J.* **334**, 261–267
14. Patti, M. E., Brambilla, E., Luzi, L., Landaker, E. J., and Kahn, C. R. (1998) *J. Clin. Invest.* **101**, 1519–1529
15. Fox, H. L., Kimball, S. R., Jefferson, L. S., and Lynch, C. J. (1998) *Am. J. Physiol.* **274**, C206–C213
16. Shigemitsu, K., Tsujishita, Y., Hara, K., Nanahoshi, M., Avruch, J., and Yonezawa, K. (1999) *J. Biol. Chem.* **274**, 1058–1065
17. Xu, G., Xiong, G., Marshall, C. A., Lin, T. A., Lawrence, J. C. Jr., and McDaniel, M. L. (1998) *J. Biol. Chem.* **273**, 20178–20184
18. Pearson, R. B., Dennis, P. B., Han, J. W., Williamson, N. A., Kozuma, S. C., and Onda, H. (2002) *Human Mol. Genet.* **11**, 525–534
19. Alesse, D. R., Koslowki, M. T., Weng, Q. P., Morrice, N., and Avruch, J. (1998) *Curr. Biol.* **8**, 69–81
20. Kwiatkowski, D. J., Zhang, H., Bandura, J. L., Heiberger, K. M., Glogauer, M., el-Hashemite, N., and Onda, H. (2002) *Mol. Cell* **10**, 151–162
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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:

$$k = A \exp \left( \frac{\Delta S^R}{R} \right) \exp \left( \frac{\Delta H^F}{RT} \right)$$

(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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