

## Structure of S6 Kinase 1 Determines whether Raptor-mTOR or Rictor-mTOR Phosphorylates Its Hydrophobic Motif Site\* ♦

Received for publication, March 21, 2005,  
and in revised form, April 4, 2005  
Published, JBC Papers in Press, April 4, 2005,  
DOI 10.1074/jbc.C500125200

Siraj M. Ali and David M. Sabatini‡

From the Whitehead Institute for Biomedical Research and Massachusetts Institute of Technology, Department of Biology, Cambridge, Massachusetts 02142

The mTOR protein kinase is the target of the immunosuppressive and anti-cancer drug rapamycin and is increasingly recognized as a key regulator of cell growth in mammals. S6 kinase 1 (S6K1) is the best characterized effector of mTOR, and its regulation serves as a model for mTOR signaling. Nutrients and growth factors activate S6K1 by inducing the phosphorylation of threonine 389 in the hydrophobic motif of S6K1. As phosphorylation of Thr<sup>389</sup> is rapamycin sensitive and mTOR can phosphorylate the same site *in vitro*, it has been suggested that mTOR is the physiological Thr<sup>389</sup> kinase. This proposal is not supported, however, by the existence of mutants of S6K1 that are phosphorylated *in vivo* on Thr<sup>389</sup> in a rapamycin-resistant fashion. Here, we demonstrate that the raptor-mTOR complex phosphorylates the rapamycin-sensitive forms of S6K1, while the distinct rictor-mTOR complex phosphorylates the rapamycin-resistant mutants of S6K1. Phosphorylation of Thr<sup>389</sup> by rictor-mTOR is independent of the TOR signaling motif and depends on removal of the carboxyl terminal domain of S6K1. Because many members of the AGC family of kinases lack an analogous domain, rictor-mTOR may phosphorylate the hydrophobic motifs of other kinases.

The large protein kinase mTOR regulates mammalian cell growth through a complicated and poorly understood signaling pathway. Within cells mTOR exists in at least two distinct protein complexes, one containing mTOR, GβL, and raptor (1–4) and the other mTOR, GβL, and rictor (5, 6). When bound to its intracellular receptor FKBP12, the immunosuppressant and anti-cancer compound rapamycin interacts with the rap-

tor-mTOR complex and inhibits its kinase activity (7–9). The best characterized effector of raptor-mTOR is the translational regulator S6 kinase 1 (S6K1)<sup>1</sup> and *in vitro* raptor-mTOR directly phosphorylates S6K1 on a key residue (1), threonine 389 (Thr<sup>389</sup>) within the hydrophobic motif COOH-terminal to the kinase domain (8) (Fig. 1A). Consistent with its capacity to inhibit raptor-mTOR function *in vitro*, rapamycin treatment of cells triggers the rapid dephosphorylation of Thr<sup>389</sup> and inactivation of S6K1 (10–12).

Despite the evidence supporting mTOR as the *bona fide* Thr<sup>389</sup> kinase, this notion is still controversial because of the unexplained behavior of certain mutants of S6K1 that are phosphorylated on Thr<sup>389</sup> in a rapamycin-resistant fashion (13, 14). To understand whether mTOR participates in the phosphorylation of these mutants we undertook, in lieu of rapamycin treatment, a loss of function approach to inhibit mTOR function. Using RNAi to suppress the expression of mTOR and its associated proteins we find that mTOR is the Thr<sup>389</sup> kinase of wild-type and rapamycin-resistant S6K1. However, distinct mTOR complexes recognize the different forms of S6K1, and this recognition depends on the structure of S6K1.

### EXPERIMENTAL PROCEDURES

**Materials**—Reagents were obtained from the following sources: protein G-Sepharose from Pierce; glutathione-Sepharose from Amersham Biosciences; mTOR, TSC2, S6K1, and GST antibodies as well as horseradish peroxidase-labeled anti-mouse, anti-goat, and anti-rabbit secondary antibodies from Santa Cruz Biotechnology; raptor, phospho-Thr<sup>389</sup>, and phospho-Ser<sup>411</sup> S6K1 antibodies from Cell Signaling; HA monoclonal antibody from Covance; myc monoclonal antibody from Oncogene Research Products; Dulbecco's modified Eagle's medium from Invitrogen; and rapamycin and LY294002 from Calbiochem. The rictor antibody has been described previously (5).

**Immunoblots, Immunoprecipitations, and Kinase Assays**—3 × 10<sup>6</sup> HEK293T cells growing in 10-cm dishes were rinsed once with cold phosphate-buffered saline and lysed on ice for 20 min in 1 ml of ice-cold lysis buffer (40 mM Hepes, pH 7.5, 120 mM NaCl, 1 mM EDTA, 10 mM pyrophosphate, 10 mM glycerophosphate, 50 mM NaF, and EDTA-free protease inhibitors (Roche Applied Science)) containing either 0.3% CHAPS or 1.0% Triton. After centrifugation at 13,000 × g for 10 min, 4 μg of the indicated antibodies were added to the cleared supernatant and incubated with rotation for 90 min. 20 μl of a 50% slurry of protein G-Sepharose was then added and the incubation continued for 1 h. Captured immunoprecipitates were washed four times with lysis buffer (5). Samples were resolved by SDS-PAGE and proteins transferred to polyvinylidene difluoride and visualized by immunoblotting as described (5). *In vitro* mTOR kinase assays were performed as described (15).

**Generation of Stable Cell Lines**—cDNAs for rat S6K1, F5A S6K1, ΔCT S6K1, and F5AΔCT S6K1 were prepared by PCR mutagenesis and subcloned into the MSCV Puro vector (16). For these myc-tagged constructs the cytoplasmic p70 version of S6K1 was used. Moloney retroviruses were generated and HEK293T cells infected and selected with puromycin at 2 μg/ml.

**In Vitro Kinase Substrates**—cDNAs for rat p85 S6K1 and its mutants were subcloned into HA-GST PRK5 (8). The p85 version of S6K1 was used for the *in vitro* kinase assay substrates because it was expressed at higher levels than the p70 version and this facilitated its purification. Plasmids were transfected into HEK293T cells and HA-GST fusion proteins purified as described (8).

**Lentiviral shRNA Cloning and Lentivirus Infection**—Sequences of

\* This work was supported by National Institutes of Health Grants AI47389 and CA103866 (to D. M. S.) and fellowships from the Pew Charitable Trust and Rita Allen Foundation (to D. M. S.) and the Poitras Family and the Howard Hughes Medical Institute (to S. M. A.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

♦ This article was selected as a Paper of the Week.

‡ To whom correspondence should be addressed: Whitehead Inst. for Biomedical Research and Massachusetts Inst. of Technology, Dept. of Biology, Nine Cambridge Center, Cambridge, MA, 02142. Tel.: 617-258-6407; Fax: 617-258-5213; E-mail: sabatini@wi.mit.edu.

<sup>1</sup> The abbreviations used are: S6K1, S6 kinase 1; RNAi, RNA interference; HA, hemagglutinin; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid; GST, glutathione *S*-transferase; shRNA, short hairpin RNA; TOS, TOR signaling; EGFR, epidermal growth factor receptor.

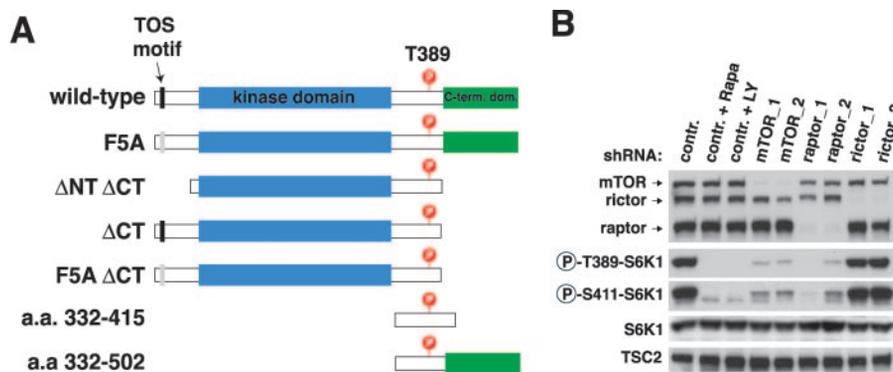


FIG. 1. **mTOR and raptor but not rictor are necessary for Thr<sup>389</sup> phosphorylation of wild-type S6K1.** *A*, domain structure, TOS motif location, and phosphorylation sites in S6K1 mutants and fragments used in this study. *B*, shRNA-mediated knockdowns of mTOR and raptor, but not rictor, inhibit phosphorylation of S6K1 on Thr<sup>389</sup>. HEK293T cells were infected with lentiviruses expressing a control shRNA (*contr.*) or shRNAs targeting the mRNAs of the indicated proteins. Cellular lysates were prepared 4 days post infection and analyzed by immunoblotting. Cells were treated with 20 nM rapamycin (*Rapa*) or 25  $\mu$ M LY294002 (*LY*) for 20 min before lysis where indicated.

lentiviral shRNAs have been described (15). Plasmids were propagated in and purified from Stbl2 bacterial cells (Invitrogen) and co-transfected together with the Delta VPR CMV VSVG plasmids into actively growing HEK293T using FuGENE (Roche Applied Science) as described (17). Virus-containing supernatants were collected at 36 and 60 h after transfection and concentrated by ultracentrifugation for 1.5 h at 23,000 rpm in an SW28 rotor at 4  $^{\circ}$ C. Pellets were resuspended overnight at 4  $^{\circ}$ C in 1/600th of the original volume. Cells were infected twice in the presence of 6  $\mu$ g/ml protamine sulfate and analyzed on the 4th day after infection. Cells were lysed and analyzed by immunoblotting as described as above. Equal amounts of protein were separated by SDS-PAGE except for samples from HEK293T cells stably expressing S6K1 mutants that were normalized to amounts of S6K1 mutants.

#### RESULTS AND DISCUSSION

To determine whether mTOR is essential for the phosphorylation of rapamycin-resistant mutants of S6K1, we inhibited the expression of mTOR and its associated proteins with RNAi mediated by lentivirally transduced shRNAs (15, 17). To control for potential off target effects of the shRNAs we used two distinct shRNAs to target the mRNA of each protein. Reductions in the expression of mTOR or raptor, but not rictor, strongly inhibit the phosphorylation of Thr<sup>389</sup> and Ser<sup>411</sup> of wild-type S6K1 (Fig. 1*B*). As expected these phosphorylations are also sensitive to rapamycin and to LY294002, an inhibitor of kinases, like mTOR, that have a phosphatidylinositol 3-kinase-like kinase domain (18). These results are consistent with raptor-mTOR being the *in vivo* regulator and likely hydrophobic motif site kinase for S6K1 and indicate that rictor-mTOR is not involved in S6K1 signaling.

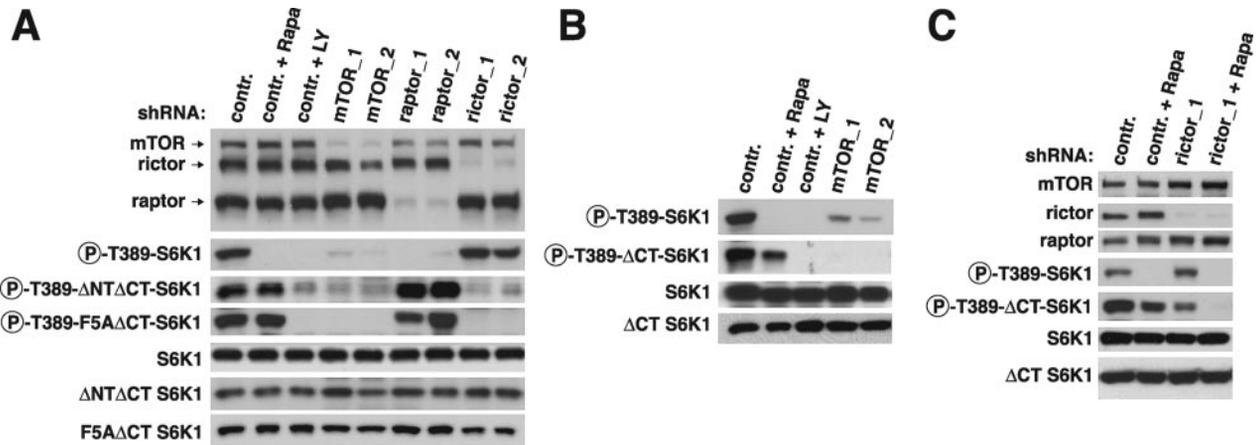
Having validated our RNAi reagents we asked whether the raptor-mTOR complex regulates Thr<sup>389</sup> phosphorylation of the rapamycin-resistant mutants of S6K1. To address this question we employed three established mutants of S6K1 (Fig. 1*A*):  $\Delta$ NT  $\Delta$ CT S6K1, which has truncations at the NH<sub>2</sub> and COOH termini (13, 14); F5A  $\Delta$ CT S6K1, which shares the same COOH-terminal truncation as  $\Delta$ NT  $\Delta$ CT S6K1 but has an inactivating mutation in the TOR signaling (TOS) motif in lieu of the NH<sub>2</sub>-terminal truncation (19); and  $\Delta$ CT S6K1 mutant, which only has the COOH-terminal truncation (13, 14). Thr<sup>389</sup> phosphorylation of the  $\Delta$ NT  $\Delta$ CT and F5A  $\Delta$ CT mutants is completely resistant to rapamycin (13, 14, 19, 20) while that of  $\Delta$ CT is only partially resistant (13, 14, 20). The Blenis group has previously shown that the  $\Delta$ NT  $\Delta$ CT and F5A  $\Delta$ CT S6K1 mutants are functionally equivalent as both lack the NH<sub>2</sub>-terminal TOS motif critical for recognition by the raptor-mTOR complex (19). We created lines of HEK293T cells stably expressing myc-tagged version of each mutant. Decreases in mTOR expression inhibit Thr<sup>389</sup> phosphorylation of both the  $\Delta$ NT  $\Delta$ CT and F5A  $\Delta$ CT S6K1 mutants, but surprisingly,

knockdowns of raptor do not (Fig. 2*A*). Instead, reductions in rictor expression eliminate the phosphorylation of both mutants (Fig. 2*A*), suggesting an important role of the rictor-mTOR complex in the phosphorylation of the prototypical rapamycin-resistant mutants of S6K1. As a rictor knockdown does not affect the phosphorylation of wild-type S6K1 (Fig. 1*B*), rictor-mediated regulation of S6K1 must require deletion of the region COOH-terminal to the kinase domain and/or destruction of the TOS motif.

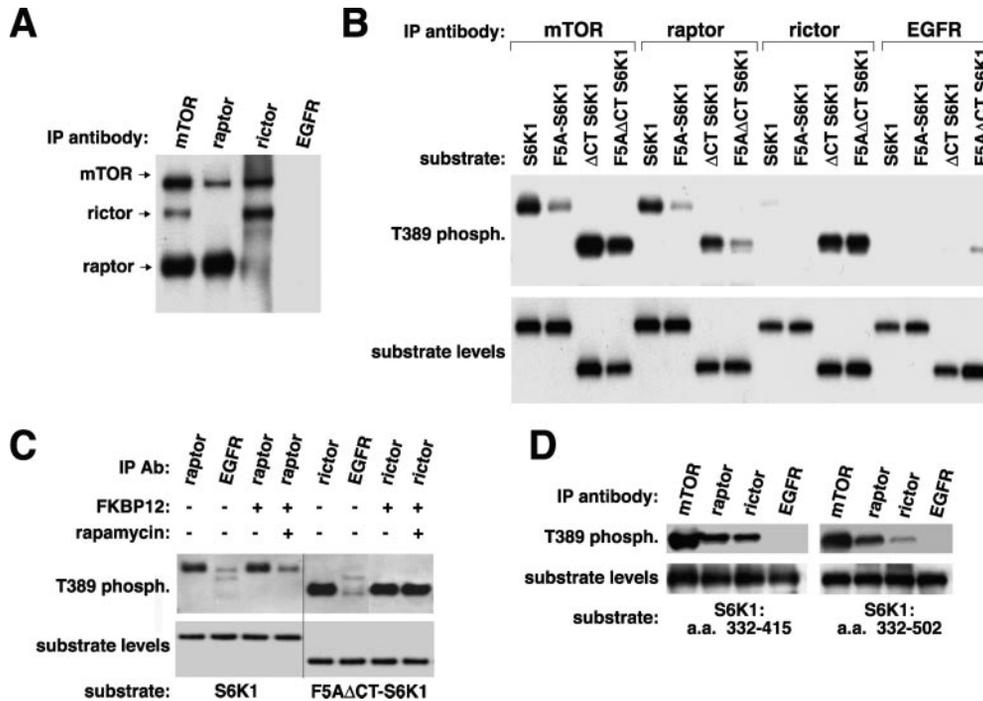
To clarify this issue we examined the role of mTOR, raptor, and rictor in the phosphorylation of  $\Delta$ CT S6K1, the partially rapamycin-resistant mutant that is missing the carboxyl-terminal region but has an intact TOS motif. Knockdown of mTOR strongly inhibits Thr<sup>389</sup> phosphorylation of  $\Delta$ CT S6K1 (Fig. 2*B*), but knockdowns of raptor or rictor have variable and weak effects on its phosphorylation (data not shown). This result suggests that perhaps both the raptor-mTOR and rictor-mTOR complexes regulate  $\Delta$ CT S6K1 and that the presence of either is sufficient for Thr<sup>389</sup> phosphorylation. To test this possibility we first reduced the expression of rictor to inhibit rictor-mTOR and then treated cells with 20 nM rapamycin for 20 min to inhibit raptor-mTOR. Neither the rictor knockdown nor rapamycin treatment has strong effects on the Thr<sup>389</sup> phosphorylation of  $\Delta$ CT S6K1 (Fig. 2*C*). The combination of both, however, eliminates Thr<sup>389</sup> phosphorylation (Fig. 2*C*), consistent with raptor-mTOR and rictor-mTOR redundantly mediating the phosphorylation of  $\Delta$ CT S6K1. These findings indicate that deletion of the COOH-terminal region of S6K1 rather than inactivation of the TOS motif is necessary for the rictor-mTOR complex to phosphorylate S6K1. Moreover, our results support the notion that the TOS motif is necessary for raptor-mTOR to regulate S6K1 (19) and also indicate that the COOH-terminal region of S6K1 does not interfere with raptor-mTOR function.

We used *in vitro* kinase assays to test if the raptor-mTOR and rictor-mTOR complexes have the substrate preferences predicted by our experiments in tissue culture cells. For this work we purified HA-GST-tagged versions of S6K1 and its mutants expressed in mammalian cells (8). We prepared four forms of S6K1 for use as substrates (Fig. 1*A*): wild-type S6K1, a TOS-deficient S6K1 (F5A S6K1) (19), the rapamycin-resistant mutant (F5A  $\Delta$ CT S6K1) (19), and the partially rapamycin-resistant mutant ( $\Delta$ CT S6K1) (13, 14). As a source of mTOR complexes we prepared immunoprecipitates from HEK293T cells with antibodies specific to mTOR, raptor, or rictor (15).

The mTOR immunoprecipitates contain both the raptor-mTOR and rictor-mTOR complexes (Fig. 3*A*) and phosphorylate wild-type S6K1 as well as both COOH-terminally truncated mutants ( $\Delta$ CT S6K1 and F5A  $\Delta$ CT S6K1) (Fig. 3*B*). The mTOR



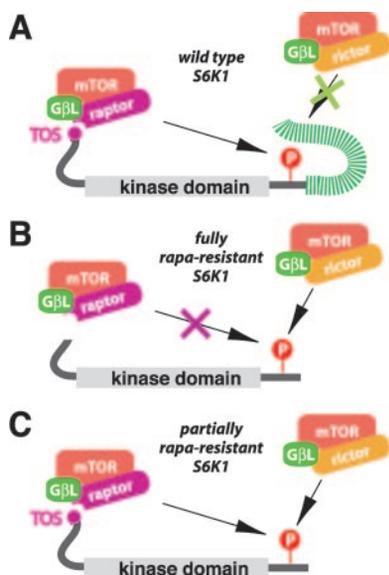
**FIG. 2. mTOR and rictor are necessary for Thr<sup>389</sup> phosphorylation of the rapamycin (Rapa)-resistant S6K1 mutants.** A, shRNA-mediated knockdowns of mTOR and rictor, but not raptor, inhibit Thr<sup>389</sup> phosphorylation of the ΔNT ΔCT and F5A ΔCT mutants of S6K1. HEK293T cells stably expressing myc-ΔNT ΔCT S6K1 or myc-F5AΔCT S6K1 were infected with lentiviruses expressing shRNAs targeting the mRNAs of the indicated proteins and analyzed as described in the legend to Fig. 1B. *contr.*, control; *LY*, LY294002. B, Thr<sup>389</sup> phosphorylation of ΔCT S6K1 is sensitive to an mTOR knockdown and to LY294002 treatment (*LY*). HEK293T cells stably expressing ΔCT S6K1 were infected with lentiviruses expressing a control (*contr.*) or mTOR-directed shRNA or treated with the indicated compounds and analyzed as above. C, rapamycin treatment combined with a rictor knockdown completely inhibits Thr<sup>389</sup> phosphorylation of ΔCT S6K1. HEK293T cells stably expressing ΔCT S6K1 were infected with lentiviruses expressing a control or rictor-directed shRNA, treated with or without 20 nM rapamycin for 20 min, and analyzed as above. *contr.*, control.



**FIG. 3. Rictor-mTOR phosphorylates the rapamycin-resistant S6K1s *in vitro*.** A, immunoblot analysis for indicated proteins of immunoprecipitates (*IP*) prepared from HEK293T cells with antibodies specific to mTOR, raptor, rictor, or EGFR. B, *in vitro* kinase assays using mTOR, raptor, rictor, or EGFR immunoprecipitates (*IP*) as source of mTOR-containing complexes and indicated HA-GST-S6K1 variants as substrates. Kinase assays were performed as described (15) and analyzed by immunoblotting for phosphorylation on Thr<sup>389</sup> using a phospho-Thr<sup>389</sup> S6K1 antibody and for substrate levels using an HA antibody. HA-GST-S6K1 substrates were expressed in and purified from mammalian cells. C, rapamycin inhibits raptor-mTOR, but not rictor-mTOR, kinase activity *in vitro*. Raptor, rictor, or EGFR immunoprecipitates (*IP*) were prepared and used in kinase assays with wild-type S6K1 or F5A ΔCT S6K1 as substrates. *Ab*, antibody. D, *in vitro* kinase assays using mTOR, raptor, rictor, or EGFR immunoprecipitates (*IP*) and the indicated bacterially produced fragments of S6K1 as substrates. Kinase assays were performed as described (15) and analyzed by immunoblotting for phosphorylation on Thr<sup>389</sup> using a phospho-Thr<sup>389</sup> S6K1 antibody and for substrate levels using a GST antibody. Where indicated reactions contained 20 nM GST-FKBP12 and excess rapamycin at 500 nM.

immunoprecipitates only marginally phosphorylate the TOS deficient full-length F5A S6K1 mutant (Fig. 3B), consistent with the complete inactivity of this mutant when expressed in cells (19, 21). To determine which mTOR complex phosphorylates which substrate we repeated the kinase assays using just the raptor-mTOR or rictor-mTOR complexes (Fig. 3B). The raptor-mTOR complex phosphorylates wild-type S6K1 and ΔCT S6K1 but neither of the TOS-deficient mutants. This

result clearly indicates that raptor-mTOR requires a TOS motif for efficient phosphorylation of full-length or truncated S6K1 but is unfazed by the presence of the region COOH-terminal to the kinase domain. On the other hand, the rictor-mTOR complex phosphorylates the COOH-terminal truncation mutants irrespective of an intact TOS motif but does not act on wild-type or F5A S6K1. These *in vitro* results recapitulate our *in vivo* findings and lead to the following conclusions: 1) the



**FIG. 4. Models depicting how raptor-mTOR and rictor-mTOR phosphorylate the wild-type and rapamycin-resistant versions of S6K1.** *A*, raptor-mTOR phosphorylation of S6K1 on Thr<sup>389</sup> requires the binding of raptor to the TOS motif of S6K1. Rictor-mTOR cannot phosphorylate wild-type S6K1 because of the presence of the carboxyl terminal region. *B*, in the  $\Delta$ NT  $\Delta$ CT rapamycin-resistant S6K1 mutant, deletion of the carboxyl-terminal region of S6K1 permits rictor-mTOR to phosphorylate Thr<sup>389</sup>, while inactivation of the TOS motif prevents raptor-mTOR phosphorylation of S6K1. *C*, in the  $\Delta$ NT  $\Delta$ CT partially rapamycin-resistant S6K1 mutant, deletion of the carboxyl-terminal region and retention of the TOS motif allow both rictor-mTOR and raptor-mTOR to phosphorylate S6K1 on Thr<sup>389</sup>.

mTOR-raptor complex requires an intact TOS motif to phosphorylate S6K1 but is indifferent to the presence of the region COOH-terminal to the kinase domain; 2) the rictor-mTOR complex does not require a TOS motif but cannot phosphorylate an S6K1 having the COOH-terminal region. From these conclusions we can explain the behavior of the S6K1 mutants within cells. Elimination of the TOS motif by mutation or NH<sub>2</sub>-terminal truncation creates a “dead” S6K1 (19) because it is no longer recognized by raptor-mTOR and the intact COOH-terminal region prevents phosphorylation by rictor-mTOR. Subsequent deletion of the COOH-terminal region from a TOS-deficient S6K1 creates an S6K1 that is exclusively a rictor-mTOR substrate and is thus completely rapamycin-resistant. Removal of only the COOH-terminal region creates a partially rapamycin-resistant S6K1 because both raptor-mTOR and rictor-mTOR can phosphorylate it. As expected, *in vitro*, raptor-mTOR phosphorylation of full-length S6K1 is rapamycin-sensitive, while rictor-mTOR phosphorylation of F5A  $\Delta$ CT S6K1 is rapamycin-resistant (Fig. 3C).

Our *in vitro* results reinforce the proposal that raptor-mTOR requires a TOS motif to phosphorylate full-length or COOH-terminally truncated S6K1. We next asked whether raptor-mTOR requires a TOS motif to phosphorylate the bacterially produced fragments of S6K1 we used as mTOR substrates (8) before we knew that mTOR exists in two distinct complexes. Even though neither fragment contains a TOS motif, the raptor complex robustly phosphorylates a fragment of S6K1 consisting of amino acids 332–502 as well as a shorter fragment of amino acids 332–415 (Fig. 3D). This result suggests that in addition to targeting S6K1 to raptor-mTOR, the TOS motif may have an additional role in opening up full-length S6K1 to make Thr<sup>389</sup> accessible to mTOR. Alternatively, these S6K1 fragments may be small enough to not require a TOS motif to enter

the catalytic site of the raptor-mTOR complex. Interestingly, rictor-mTOR also phosphorylates both S6K1 fragments, although the presence of the intact COOH-terminal region dampens rictor-mTOR activity (Fig. 3D), a result consistent with the studies using the full-length proteins.

The proposal that mTOR is the *in vivo* Thr<sup>389</sup> kinase of S6K1 (8) has been difficult to reconcile with the rapamycin-resistant behavior of certain S6K1 mutants (13, 14, 20). We demonstrate that deletion of the COOH-terminal region of S6K1 unexpectedly creates a version of S6K1 that is phosphorylated by the rapamycin-insensitive rictor-mTOR complex. Thus, mTOR is likely the Thr<sup>389</sup> kinase for all forms of S6K1, but depending on the structure of S6K1, mTOR requires different partner proteins to function. mTOR needs raptor to phosphorylate S6K1s with intact TOS motifs and rictor to phosphorylate S6K1s missing the COOH-terminal region. The behavior of the fully rapamycin-resistant mutants of S6K1 is explained by inactivation of the TOS motif and loss of the COOH-terminal region, modifications that inhibit and permit phosphorylation by raptor-mTOR and rictor-mTOR, respectively (see models in Fig. 4). S6K1 is distinct from most AGC family kinase members because of the carboxyl terminal extension beyond its hydrophobic motif. Interestingly, rictor-mTOR also phosphorylates the hydrophobic motif site of Akt/PKB (15), whose COOH-terminal region resembles the truncated S6K1 mutants. We would not be surprised then if rictor-mTOR also regulates other members of the AGC family of kinases having COOH-terminal regions similar to Akt/PKB and truncated S6K1.

*Acknowledgment*—We thank Dos D. Sarbassov for insightful discussions.

#### REFERENCES

- 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
- Kim, D. H., Sarbassov, D. D., Ali, S. M., Latek, R. R., Guntur, K. V., Erdjument-Bromage, H., Tempst, P., and Sabatini, D. M. (2003) *Mol. Cell* **11**, 895–904
- Hara, K., Maruki, Y., Long, X., Yoshino, K., Oshiro, N., Hidayat, S., Tokunaga, C., Avruch, J., and Yonezawa, K. (2002) *Cell* **110**, 177–189
- Loewith, R., Jacinto, E., Wullschlegel, S., Lorberg, A., Crespo, J. L., Bonenfant, D., Oppliger, W., Jenoe, P., and Hall, M. N. (2002) *Mol. Cell* **10**, 457–468
- Sarbassov, D. D., Ali, S. M., Kim, D. H., Guertin, D. A., Latek, R. R., Erdjument-Bromage, H., Tempst, P., and Sabatini, D. M. (2004) *Curr. Biol.* **14**, 1296–1302
- Jacinto, E., Loewith, R., Schmidt, A., Lin, S., Ruegg, M. A., Hall, A., and Hall, M. N. (2004) *Nat. Cell Biol.* **6**, 1122–1128
- 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
- Oshiro, N., Yoshino, K., Hidayat, S., Tokunaga, C., Hara, K., Eguchi, S., Avruch, J., and Yonezawa, K. (2004) *Genes Cells* **9**, 359–366
- Chung, J., Kuo, C. J., Crabtree, G. R., and Blenis, J. (1992) *Cell* **69**, 1227–1236
- Price, D. J., Grove, J. R., Calvo, V., Avruch, J., and Bierer, B. E. (1992) *Science* **257**, 973–977
- Kuo, C. J., Chung, J., Fiorentino, D. F., Flanagan, W. M., Blenis, J., and Crabtree, G. R. (1992) *Nature* **358**, 70–73
- Cheatham, L., Monfar, M., Chou, M. M., and Blenis, J. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92**, 11696–11700
- Weng, Q. P., Andrabi, K., Kozlowski, M. T., Grove, J. R., and Avruch, J. (1995) *Mol. Cell. Biol.* **15**, 2333–2340
- Sarbassov, D. D., Guertin, D. A., Ali, S. M., and Sabatini, D. M. (2005) *Science* **307**, 1098–1101
- Grez, M., Akgun, E., Hilberg, F., and Ostertag, W. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 9202–9206
- Stewart, S. A., Dykxhoorn, D. M., Palliser, D., Mizuno, H., Yu, E. Y., An, D. S., Sabatini, D. M., Chen, I. S., Hahn, W. C., Sharp, P. A., Weinberg, R. A., and Novina, C. D. (2003) *RNA (N. Y.)* **9**, 493–501
- Brunn, G. J., Williams, J., Sabers, C., Wiederrecht, G., Lawrence, J. C., Jr., and Abraham, R. T. (1996) *EMBO J.* **15**, 5256–5267
- Schalm, S. S., and Blenis, J. (2002) *Curr. Biol.* **12**, 632–639
- Dennis, P. B., Pullen, N., Kozma, S. C., and Thomas, G. (1996) *Mol. Cell. Biol.* **16**, 6242–6251
- Nojima, H., Tokunaga, C., Eguchi, S., Oshiro, N., Hidayat, S., Yoshino, K., Hara, K., Tanaka, N., Avruch, J., and Yonezawa, K. (2003) *J. Biol. Chem.* **278**, 15461–15464