Target of Rapamycin (TOR)-signaling and RAIP Motifs Play Distinct Roles in the Mammalian TOR-dependent Phosphorylation of Initiation Factor 4E-binding Protein 1*

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The translational repressor protein eIF4E-binding protein 1 (4E-BP1, also termed PHAS-I) is regulated by phosphorylation through the rapamycin-sensitive mTOR (mammalian target of rapamycin) pathway. Recent studies have identified two regulatory motifs in 4E-BP1, an mTOR-signaling (TOS) motif in the C terminus of 4E-BP1 and an RAIP motif (named after its sequence) in the N terminus. Other recent work has shown that the protein raptor binds to mTOR and 4E-BP1. We show that raptor binds to full-length 4E-BP1 or a C-terminal fragment containing the TOS motif but not to an N-terminal fragment containing the RAIP motif. Mutation of several residues within the TOS motif abrogates binding to raptor, indicating that the TOS motif is required for this interaction. 4E-BP1 undergoes phosphorylation at multiple sites in intact cells. The effects of removal or mutation of the RAIP and TOS motifs differ. The RAIP motif is absolutely required for phosphorylation of sites in the N and C termini of 4E-BP1, whereas the TOS motif primarily affects phosphorylation of Ser-64/65, Thr-69/70, and also the rapamycin-insensitive site Ser-101. Phosphorylation of N-terminal sites that are dependent upon the RAIP motif is sensitive to rapamycin. The RAIP motif thus promotes the mTOR-dependent phosphorylation of multiple sites in 4E-BP1 independently of the 4E-BP1/raptor interaction.

Initiation Factor 4E-binding Protein 1* plays a key role in mRNA translation in mammalian cells and in its regulation (1). eIF4E binds the 5'-cap structure of the mRNA, which contains the 7-methyl-GTP (m7GTP) moiety. It also interacts with the scaffold eIF4G and thereby recruits 40 S ribosomal subunits to the mRNA (2). A large body of evidence shows that eIF4E plays key roles in cell proliferation (1) and cell survival/apoptosis (3). For example, overexpression of eIF4E can transform cells (4, 5), and many human tumor cells express high levels of this protein (6). Nuclear eIF4E seems to be important in the export of certain mRNAs to the cytoplasm such as that for cyclin D1 (7), which is important in cell cycle progression.

The transforming abilities of eIF4E may be linked to this effect (8, 9).

The availability of eIF4E for binding to eIF4G is regulated by its interaction with heat-stable proteins termed eIF4E-binding proteins (4E-BPs). 4E-BP1 is by far the best understood of these. Phosphorylation of 4E-BP1 leads to its release from eIF4E, allowing eIF4E to form initiation complexes with eIF4G (1). Expression of 4E-BP1 reverses the transforming ability of increased levels of eIF4E (10). Phosphorylation of 4E-BP1 induced by insulin or other agents is blocked by the immunosuppressant rapamycin, a specific inhibitor of the mammalian target of rapamycin (mTOR) (11). 4E-BP1 undergoes phosphorylation at up to seven sites in intact cells; phosphorylation is complex, hierarchical, and, at some sites, is required for the subsequent modification of others (12–15).

Regulatory motifs have been identified in the N and C termini of 4E-BP1. The N terminus contains the so-called “RAIP” motif (from the single letter code for its amino acid sequence; Ref. 16). Mutation of this motif results in a low basal level of phosphorylation of 4E-BP1 that is barely affected by insulin. The extreme C terminus contains a TOR-signaling (TOS) motif that is similar to a sequence in the N terminus of the S6 kinases (S6Ks; see below) and which is required for its regulation (17). Mutation or deletion of this motif impairs the insulin-stimulated phosphorylation of 4E-BP1 (15, 17, 18).

Signaling through mTOR also regulates the phosphorylation of other proteins involved in controlling mRNA translation, such as the ribosomal protein S6 kinases (S6K1/2; Ref. 19). Across the Eukaryotae, TOR homologs are also involved in controlling a wide range of cellular functions (20–22). Signaling through mTOR is regulated by amino acid availability (23), and effectors of mTOR signaling, such as the S6Ks and 4E-BP1, regulate animal cell size (24–26). Furthermore, mTOR signaling has recently been implicated in certain human cancers (27–29). Therefore, there is currently a high level of interest in mTOR signaling.

Recent studies have identified a novel component of the mTOR pathway, a scaffold protein termed “raptor” for which homologs exist in other eukaryotes (30–32). Raptor binds to mTOR and 4E-BP1 and may act to promote signaling from mTOR to 4E-BP1, although the actual function of the raptor/4E-BP1 interaction is unclear. Here, we confirm that the TOS motif of 4E-BP1 is required for binding to raptor but find no evidence that the RAIP motif interacts with this scaffold protein. Despite this, mutation of the RAIP motif has more drastic effects on the mTOR-dependent phosphorylation of 4E-BP1 in living cells than does mutation of the TOS motif. The RAIP motif thus seems to mediate insulin and mTOR-dependent inputs into 4E-BP1 independently of an interaction with raptor.
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MATERIALS AND METHODS

Vectors—Expression vectors for HisMyc-tagged versions of 4E-BP1 have been described earlier (15, 16). Vectors for glutathione S-transferase (GST-N-BP1 (encoding residues 1–24 of rat 4E-BP1 fused to GST) and GST-C-BP1 (last 20 amino acids of rat 4E-BP1 fused to GST) were created by PCR using primers containing restriction sites for BamHI and EcoRI and were inserted using these sites into pGEX3X. The F113A and E114A,D116A mutants of these fusion proteins were created by PCR and cloned into pGEX3X. The F113A mutant of rat 4E-BP1 was generated by PCR. The mutated DNA fragment was cut with BamHI and HindIII and ligated into pcDNA3.1His/Myc (16).

The F114A and E115A,D117A mutants of human 4E-BP1 were created by PCR using the human cDNA for wild-type 4E-BP1 as template. This step was performed overnight at 4 °C. Further development of the blots was done exactly as for standard Western blots (33). In some cases, HisMyc-tagged 4E-BP1 was isolated from cell lysates by binding to Ni-nitrilotriacetic acid-agarose (Qiagen) beads (33). In some cases, His/Myc-tagged 4E-BP1 was isolated from cell lysates to Ni-nitrilotriacetic acid-agarose (Qiagen) beads prior to analysis by SDS-PAGE and Western blotting, as described previously (15). The sources of the antisera have also been described previously (15).

RESULTS

Raptor Binds to the C Terminus of 4E-BP1 but Not to the N Terminus Containing the RAIP Motif—Raptor has been described previously as interacting with both mTOR and 4E-BP1, as assessed in pull-down experiments (29, 30). In trying to use such approaches to study the interaction between 4E-BP1 and raptor, we experienced substantial difficulties of non-specificity. In view of these difficulties, we tested two alternative approaches to investigating the interaction between 4E-BP1 and raptor. These involve simple dot blot and far Western techniques which have been used previously, e.g. to study the binding of 4E-BP1 to eIF4E (34).

To confirm that raptor binds to 4E-BP1 using such methods, we performed a dot blot using either 4E-BP1, expressed as a GST fusion protein in bacteria, or GST itself as a negative control. Samples were applied to a membrane that was then blocked and subsequently probed with extract from HEK293 cells expressing raptor as a Myc-tagged fusion. Any raptor associated with the 4E-BP1 or GST was detected using anti-Myc antibody.

A clear signal was seen for GST-4E-BP1 but not for GST (Fig. 1A). No signal was seen when blots were probed with extracts from cells not expressing Myc-raptor (showing that anti-Myc does not bind to 4E-BP1). Thus, the dot blot technique can be used to detect an interaction between 4E-BP1 and raptor. A very faint signal was seen when eIF4E was applied to the membrane, and this was then probed with cell extract containing Myc-raptor. However, a signal of similar strength was seen on the negative control membrane probed with control extract, indicating a weak interaction of anti-Myc with eIF4E. Thus, raptor does not bind detectably to eIF4E itself, at least in this type of analysis.

To assess whether the regions of 4E-BP1 that contain the RAIP or TOS motifs of 4E-BP1 can bind to raptor, we expressed the N and C termini of rat 4E-BP1 as GST-fusion proteins in Escherichia coli. These fusions contained either the first 24 (GST-N-BP1) or the final 20 (GST-C-BP1) amino acids of 4E-BP1 fused to GST. Residues adjacent to the motifs were included in these fusions in case they were also required for intermolecular interactions. We anticipated that, because the RAIP motif is required for phosphorylation of all the mTOR-regulated sites in 4E-BP1 (16), raptor might interact with the N-terminal fragment. Therefore, we also made a variant N-terminal fusion protein in which the RAIP motif was altered to AAAA, which abrogates phosphorylation of 4E-BP1 within intact cells (16). In the dot blot analyses, we found that raptor bound to full-length 4E-BP1 and GST-C-BP1. However, despite repeated attempts, we were consistently unable to detect any binding of raptor to the N-terminal fragment (Fig. 1B).

Our data suggest that raptor binds the C terminus of 4E-BP1 but cannot interact with its N-terminal region. Other recent studies also suggest that the TOS motif is the critical region of 4E-BP1 for this interaction (18, 35).

However, our data contrast with other findings, suggesting that both the TOS and RAIP motifs are involved in the binding of raptor to 4E-BP1 (36). A potentially important point is that our experiment involved studying the binding of raptor to fragment of 4E-BP1 made in E. coli. Thus, it was possible that the interaction of raptor with the RAIP motif requires a modification of 4E-BP1 that does not take place in bacteria. Therefore, we transfected HEK293 cells with vectors encoding GST-N-BP1, GST-C-BP1, or GST itself. Samples of the resulting cell lysates were mixed with glutathione-Sepharose, and the bound material was resolved by SDS-PAGE and transferred to Immobilon, where it was probed using cell lysate containing Myc-raptor. Raptor bound to GST-C-BP1 but not GST-N-BP1 or GST in this type of “far Western” assay (Fig. 1C), and the interaction was not affected by pretreatment of the cells with...
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We also wished to study the effects of mutations in the TOs motif of the human protein upon its phosphorylation within cells. Reasons for this included our recent findings (i) that removal of the last six residues (including the TOs motif) from human 4E-BP1 affects its phosphorylation at multiple sites, and (ii) that phosphorylation of Ser-65 in human 4E-BP1 requires a novel "constitutive" phosphorylation site (Ser-101; Ref. 15). Rat 4E-BP1 also contains a serine at this position. The F114A mutation had effects on the phosphorylation of the human 4E-BP1 similar to those seen for F113A mutation in the rat protein. Again, insulin can still increase the phosphorylation of the N-terminal threonyl sites in the F114A mutant. To further test the role of the TOs motif, we also altered the acidic residues within it to Ala (E115A,D117A). Unexpectedly, this double mutation had much less effect on the phosphorylation of any of the four sites studied in human 4E-BP1 than did the F113A mutation (Fig. 3B). Although their basal levels of phosphorylation were decreased, insulin can still markedly enhance the phosphorylation of all of them. Comparison with the loading (anti-Myc) control reveals that the E115A,D117A mutation does impair the insulin-induced phosphorylation of Thr-70, a site that is believed to be particularly important in regulating the binding of 4E-BP1 to eIF4E (12, 13). The small effect of the E115A,D117A mutation on the phosphorylation of 4E-BP1 is surprising, given that this mutation abolishes raptor binding (Fig. 2B). In another study (18), the I → A mutation at the extreme C terminus of 4E-BP1 almost abolished binding of 4E-BP1 to raptor but had only a modest effect on phosphorylation of Ser-65, the only site studied by Schalm et al. (18). Thus far, the phenylalanine seems to be the most important residue in the TOs motif of 4E-BP1. Our data contrast with the effects of mutating the two acidic residues in the TOs motif of 4E-BP1. Our data contrast with the effects of mutating the two acidic residues in the TOs motif of 4E-BP1, which completely prevented its activation (17).

To study the effects of the mutations in the TOs motif on the regulation by insulin of the binding of 4E-BP1 to eIF4E, we transfected cells with vectors encoding wild-type 4E-BP1 or the F113A or E115A,D117A mutants. After serum starvation of the cells, and in some cases insulin treatment, the cells were lysed, and the resulting samples were subjected to affinity chromatography on m7GTP-Sepharose, which retains eIF4E and associated proteins. The bound material was analyzed by SDS-PAGE and Western blotting. As shown in Fig. 3C, insulin treatment caused the release of the wild-type 4E-BP1, as expected. In the case of the F113A mutant, which fails to undergo phosphorylation at Ser-65 and Thr-70 in response to insulin, no release at all was seen in response to insulin (Fig. 3C). For the E115A,D117A mutant, when expressed at very low levels, some partial release was sometimes observed, but generally little effect of insulin was seen. This result is despite the fact that insulin is able to induce phosphorylation of all the sites studied here (Fig. 3B). However, as noted above, the extent of phosphorylation of Thr-70 was markedly decreased relative to the wild-type protein. This finding likely explains why release is poor, because this site plays a key role in this event (12, 13). The continued presence of a proportion of the mutant 4E-BP1 that is not phosphorylated at this site probably provides a sufficiently large pool of 4E-BP1 in a form that can still bind to eIF4E.

Disruption of the TOs Motif in 4E-BP1 Interferes with Phosphorylation of Ser-101, a Rapamycin-insensitive Site—A major effect of the F114A mutation is on the insulin-induced phosphorylation of Ser-65. Because this is dependent on the presence...
After lysis, samples containing equal amounts of total protein were subjected to affinity chromatography on m\(^{-}\)GTP-Sepharose. The bound material was analyzed by SDS-PAGE and Western blotting with anti-Myc and anti-eIF4E. The positions of these proteins are indicated. D, HEK293 cells were transfected with vectors for human 4E-BP1 (with His/Myc tags) in which either Ser-65 (S65A) or Ser-65 and Phe-114 (S65A,F114A) were changed to Ala. Cells were starved of serum overnight and, where indicated, were treated with insulin (100 nM for 80 min) with or without pretreatment with rapamycin (100 nM for 30 min). Lysates were prepared and then analyzed by SDS-PAGE and Western blotting using the indicated antisera.

**FIG. 3.** Effect of mutation of the TOS and RAIP motifs on the phosphorylation of 4E-BP1 at specific sites. HEK293 cells were transfected with vectors for wild-type rat (A) or human (B) 4E-BP1 (each with His/Myc tags) variants in which the RAIP motif was altered to AAAA or in which Phe-113 (rat), Phe-114, Glu-115, and Asp-117 (E115A,D117A) human) were changed to alanine. Cells were starved of serum overnight and were treated with insulin where indicated (100 nM for 60 min). Lysates were prepared and then analyzed by SDS-PAGE and Western blotting using the indicated antisera. A, in the panel showing the anti-phospho-Ser-64 blot (Anti-S64[P]), the band for the endogenous 4E-BP1, to avoid interference from phosphorylation at Ser-65 and Thr-70, which act as priming sites for phosphorylation of Ser-65 to Thr-70 (14) (not indicated). The effect of the TOS motif on phosphorylation of Ser-65 and Thr-70 are not indicated here, as they may be indirect, i.e., mediated through the loss of phosphorylation of the N-terminal threonines, which appears to be required for phosphorylation of Ser-65 to Thr-70 (14) (not indicated). The effect of the TOS motif on phosphorylation of Ser-65 may be a secondary effect of its influence on phosphorylation of Ser-101 and Thr-70, which act as priming sites (indicated by broken arrows). Potential binding partners for the RAIP motif are unknown (?), as are the identities of the physiologically important kinases acting at the sites in the C-terminal half of 4E-BP1.

**FIG. 4.** Phosphorylation of 4E-BP1 harboring a mutation in the TOS motif is sensitive to rapamycin. A, HEK293 cells were transfected with vectors for wild-type rat 4E-BP1 (WT) or the F113A. Cells were starved of serum overnight and, where indicated, were treated with insulin (100 nM for 60 min) with or without pretreatment with rapamycin (100 nM for 30 min). Lysates were prepared and then analyzed by SDS-PAGE and Western blotting using the indicated antisera.

**FIG. 4.** Phosphorylation of 4E-BP1 harboring a mutation in the TOS motif is sensitive to rapamycin. A, HEK293 cells were transfected with vectors for wild-type rat 4E-BP1 (WT) or the F113A. Cells were starved of serum overnight and, where indicated, were treated with insulin (100 nM for 60 min) with or without pretreatment with rapamycin (100 nM for 30 min). Lysates were prepared and then analyzed by SDS-PAGE and Western blotting using the indicated antisera. B, scheme interpreting current data relating to regulation of the phosphorylation of 4E-BP1. The RAIP and TOS motifs are shown. The eIF4E-binding motif is shown in the black rectangle with white spots. Numbering is based on the human sequence (diagram not to scale). Arrows denote roles of specific motifs in phosphorylation of individual sites. The TOS motif that binds raptor affects phosphorylation of Ser-65, Ser-101, and Thr-70, whereas the RAIP motif plays a role in the phosphorylation of all the rapamycin-sensitive sites, although it does not directly bind to raptor. Effects of the RAIP motif on the phosphorylation of Ser-65 and Thr-70 are not indicated here, as they may be indirect, i.e., mediated through the loss of phosphorylation of the N-terminal threonines, which appears to be required for phosphorylation of Ser-65 to Thr-70 (14) (not indicated). The effect of the TOS motif on phosphorylation of Ser-65 may be a secondary effect of its influence on phosphorylation of Ser-101 and Thr-70, which act as priming sites (indicated by broken arrows). Potential binding partners for the RAIP motif are unknown (?), as are the identities of the physiologically important kinases acting at the sites in the C-terminal half of 4E-BP1.
motif apparently also prevents phosphorylation at the N-terminal threonines as well. Given that the TOR motif binds the mTOR partner raptor, it was important to assess (i) the extent to which phosphorylation of all these sites is actually dependent upon mTOR, and (ii) whether rapamycin affected the residual phosphorylation observed in mutants lacking a functional TOS motif. In wild-type rat 4E-BP1, the phosphorylation of all of these sites is sensitive to rapamycin, which eliminates or severely impairs the increase seen in response to insulin (all sites tested) and reduces the level of any basal phosphorylation (Thr-36/45) (Fig. 4A). As shown above and in Fig. 4A, insulin can still increase phosphorylation of Thr-69 and Thr-36/45 in the F113A mutant, and this remains sensitive to rapamycin. Very similar data were obtained for the corresponding human proteins (wild type and F114A, data not shown). Thus, mTOR can still increase phosphorylation of Thr-69 and Thr-36/45 (Thr-36/45 sites tested) and reduces the level of any basal phosphorylation that cannot bind raptor, indicating the operation of raptor-independent mechanisms that contribute to its regulation.

**Discussion**

Our data confirm that, as recently reported (18, 35, 36), the TOR motif in 4E-BP1 is essential for its interaction with raptor, but contrary to another recent report (36), we find no evidence for an interaction between raptor and the N-terminal region of 4E-BP1 containing the RAIP motif. Mutating the TOR motif in full-length 4E-BP1 abolishes its ability to bind raptor (18, 35, 36), again indicating that the RAIP motif is insufficient to mediate binding to raptor (because if the still-intact RAIP motif could also bind raptor, binding would have been seen). The RAIP motif, therefore, apparently regulates 4E-BP1 independently of an interaction with raptor. Consistent with this, mutation of the RAIP and TOS motifs has distinct effects on the phosphorylation of 4E-BP1 in cells. Loss of the TOR motif primarily affects the C-terminal sites, whereas mutation of the RAIP motif also has a drastic effect on the N-terminal threonyl sites. Because phosphorylation of 4E-BP1 shows a marked hierarchy, it is possible that the effects of the RAIP motif on phosphorylation of Ser-64/65 and Thr-70/70 are indirect, e.g. because of the loss of phosphorylation of the N-terminal sites.

The present data provide an important advance over two recent reports (35, 36). We have studied the roles of the TOR and RAIP motifs on 4E-BP1 phosphorylation in intact cells, whereas other workers only examined their importance for phosphorylation of 4E-BP1 by mTOR in vitro. Their data and those reported in Ref. 18 showed that disruption of the TOR motif greatly decreased the phosphorylation of 4E-BP1 by mTOR in vitro. Our data for 4E-BP1 phosphorylation in intact cells show that the loss of the TOR motif has relatively little effect on phosphorylation of the N-terminal threonyl sites. Interestingly, these are the sites that are primarily phosphorylated by mTOR in vitro (37, 38). In contrast, Ser-64/65, which is hardly phosphorylated by mTOR at all in vitro, is the most sensitive to disruption of the TOR motif within cells. These data must cast doubt on a simple model in which raptor, via the TOR motif, recruits mTOR to phosphorylate 4E-BP1 directly. Our findings for the TOR motif confirm and extend those of Schalm et al. (18), which appeared when this paper was in preparation but do not include data on the role of the RAIP motif.

The F113A mutation completely abrogates the ability of insulin to induce release of 4E-BP1 from eIF4E (Fig. 3C). The E115A,D117A mutation also almost completely blocked this effect. This seems surprising because this mutant can still undergo insulin-induced phosphorylation at many sites. As discussed above, this effect likely reflects the fact that the E115A,D117A mutation does significantly impair the phosphorylation of Thr-70, a site critical for the release of 4E-BP1 from eIF4E (12, 13). Thus, the site-by-site analysis of the phosphorylation of 4E-BP1 using the phosphospecific antisera is actually more informative than the m7GTP-Sepharose pull-down experiment in terms of analyzing in detail the effects of point mutations on the regulation of 4E-BP1.

Phosphorylation of Thr-36/45 (Thr-37/46) is substantially blocked by rapamycin, and their residual phosphorylation in the TOR mutant (e.g. Fig. 4A, F113A) remains sensitive to rapamycin. Similarly, although loss or mutation of the TOR motif reduces the insulin-induced phosphorylation of Thr-69/70, it does not abolish it completely, and the remaining phosphorylation is still blocked by rapamycin (Fig. 4A and Ref. 15). This indicates that mTOR makes a contribution to their phosphorylation that does not require an intact TOR motif and may instead be mediated by means of the RAIP motif. On the other hand, phosphorylation of Ser-64/65 is completely dependent upon the TOR motif. Our data suggest that this likely reflects the greatly decreased levels of phosphorylation of Ser-101 and Thr-70 in the F114A mutant.

These data thus indicate that insulin/mTOR signaling to 4E-BP1 is not mediated solely by means of the TOR motif or, presumably, via raptor. Given the drastic effects of the loss of the RAIP motif, it seems that it plays an important but different role in the mTOR-dependent inputs that regulate several sites in 4E-BP1 (see Fig. 4B). However, the S6Ks lack a recognizable RAIP motif, suggesting that it is not essential for mTOR signaling per se and may perform a function specific to 4E-BP1/2 (4E-BP3 also lacks a RAIP motif; Ref. 16).

A clear priority now is to identify how the RAIP motif regulates 4E-BP1 phosphorylation. It may, for example, interact with (unidentified) scaffold proteins other than raptor. It is likely that phosphorylation of the other sites in 4E-BP1 (e.g. in human 4E-BP1, Ser-65, Thr-70, and Ser-101) is mediated by so far unidentified kinases, the action of which on 4E-BP1 requires phosphorylation of priming sites and/or the TOR motif. Such kinases may conceivably be part of the multicomponent mTOR complex.

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**References**

1. Gingras, A.-C., Raught, B., and Sonenberg, N. (1999) *Annu. Rev. Biochem.* **68**, 913–963.
2. Raught, B., Gingras, A.-C., and Sonenberg, N. (2000) in *Translation Control of Gene Expression* (Sonenberg, N., Hershey, J. W. B., and Mathews, M. B., eds) pp. 245–293, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
3. Clemens, M. J. (2001) *J. Cell. Mol. Med.* **5**, 221–239.
4. De Benedetti, A., and Roods, R. E. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 8212–8216.
5. Lazzar-Karatzas, A., Montine, K. S., and Sonenberg, N. (1990) *Nature* **345**, 544–547.
6. De Benedetti, A., and Harris, A. L. (1999) *Int. J. Biochem. Cell Biol.* **31**, 59–72.
7. Rousseau, D., Kaspar, R., Rosenwald, I., Gehrke, L., and Sonenberg, N. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 1065–1070.
8. Hashemolhosseini, S., Nagamine, Y., Morley, S. J., Desrivieres, S., Merecp, L., and Ferrari, S. (1998) *J. Biol. Chem.* **273**, 14424–14429.
9. Lai, H. K., and Borden, K. L. (2003) *Oncogene* **19**, 1623–1634.
10. Rousseau, D., Gingras, A. C., Pause, A., and Sonenberg, N. (1996) *Oncogene* **13**, 2415–2420.
11. Gingras, A.-C., Raught, B., and Sonenberg, N. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98**, 143–147.
12. Mothe-Satney, I., Yang, D., Fadden, P., Haystead, T. A. J., and Lawrence, J. C. (2000) *Mol. Cell. Biol.* **20**, 3558–3567.
13. Gingras, A.-C., Gygi, S. P., Raught, B., Polakiewicz, R. D., Abraham, R. T., Hoeckstra, M. F., Aebbersold, R., and Sonenberg, N. (1999) *Genes Dev.* **13**, 1422–1437.
14. Schalm, S. S., Fingar, D. C., Sabatini, D. M., and Blenis, J. (2003) *Mol. Cell. Biol.* **23**, 1544–1557.
15. Urban, J. C. (2003) *Mol. Cell. Biol.* **23**, 1544–1557.
16. Tee, A. R., and Proud, C. G. (2002) *Mol. Cell. Biol.* **22**, 1674–1683.
17. Schalm, S. S., and Blenis, J. (2002) *Curr. Biol.* **12**, 632–638.
18. Schalm, S. S., Fingar, D. C., Sabatini, D. M., and Blenis, J. (2003) *Curr. Biol.* **13**, 797–806.
19. Avruch, J., Belham, C., Weng, Q., Harra, K., and Yonemaya, K. (2001) *Progress in Molecular Biology and Translational Science*. 0.001

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Mol. Subcell. Biol. 26, 115–154

20. Abraham, R. T. (2002) Cell 111, 9–12
21. Raught, B., Gingras, A.-C., and Sonenberg, N. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 7037–7044
22. Rohde, J., Heitman, J., and Cardenas, M. E. (2001) J. Biol. Chem. 276, 9583–9586
23. Kimball, S. R. (2001) Mol. Biol. Cell 12, 1725–1731
24. Shima, H., Pende, M., Chen, Y., Fumagalli, S., Thomas, G., and Kozma, S. C. (1998) EMBO J. 17, 6649–6659
25. Montagne, J., Stewart, M. J., Stocker, H., Hafen, E., Kozma, S. C., and Thomas, G. (1999) Science 285, 2126–2129
26. Fingar, D. C., Salama, S., Tsou, C., Harlow, E., and Blenis, J. (2002) Genes Dev. 16, 1472–1487
27. Asli, M., Blazek, E., and Vogt, P. K. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 136–141
28. Neshat, M. S., Mellinghoff, I. K., Tran, C., Stiles, B., Thomas, G., Petersen, R., Frost, P., Gibbons, J. J., Wu, H., and Sawyers, C. L. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 10314–10319
29. Hara, K., Maruki, Y., Long, X., Yoshino, K., Oshiro, N., Hidayat, S., Tokunaga, C., Avruch, J., and Yonezawa, K. (2002) Cell 110, 177–189
30. 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
31. Laosith, R., Jacinto, E., Wulfschlegl, S., Lorberg, A., Crespo, J. L., Benenfant, D., Opplung, W., Jenoe, P., and Hall, M. N. (2002) Mol. Cell 10, 457–468
32. Tee, A. R., and Proud, C. G. (2000) Oncogene 19, 3021–3031
33. Wang, X., Janmaat, M., Beugnet, A., Paulin, F. E. M., and Proud, C. G. (2000) Biochem. J. 367, 475–481
34. Pause, A., Belsham, G. J., Gingras, A.-C., Donzé, O., Lin, T. A., Lawrence, J. C., and Sonenberg, N. (1994) Nature 371, 762–767
35. 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
36. Choi, K. M., McMahon, L. P., and Lawrence, J. C. (2003) J. Biol. Chem. 278, 19667–19673
37. Yang, D., Brun, G. J., and Lawrence, J. C. (1999) FERS Lett. 453, 387–390
38. 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