Phospholipase D Mediates Nutrient Input to Mammalian Target of Rapamycin Complex 1 (mTORC1)*

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The mammalian target of rapamycin (mTOR) is a critical sensor of nutritional sufficiency. Although much is known about the regulation of mTOR in response to growth factors, much less is known about the regulation of mTOR in response to nutrients. Amino acids have no impact on the signals that regulate Rheb, a GTPase required for the activation of mTOR complex 1 (mTORC1). Phospholipase D (PLD) generates a metabolite, phosphatidic acid, that facilitates association between mTOR and the mTORC1 co-factor Raptor. We report here that elevated PLD activity in human cancer cells is dependent on both amino acids and glucose and that amino acid- and glucose-induced increases in mTORC1 activity are dependent on PLD. Amino acid- and glucose-induced PLD activity and mTORC1 activity were also dependent on the GTPases RalA and ARF6 and the type III phosphatidylinositol-3-kinase hVps34. Thus, a key stimulatory event for mTORC1 activation in response to nutrients is the generation of phosphatidic acid by PLD.

The mammalian target of rapamycin (mTOR) is a critical regulator of cell growth and cell cycle progression. mTOR is activated in response to both growth factors and nutrients (1). mTOR exists as two complexes, mTORC1 and mTORC2. Although both mTORC1 and mTORC2 are responsive to growth factors, mTORC1 is believed to be the primary sensor of nutrient and energy sufficiency (2–4). mTORC1 is activated in response to insulin and other peptide hormones that activate type I phosphatidylinositol (PI) 3-kinases (PI3Ks) that generate PI 3,4,5-trisphosphate. The activation of mTORC1 via PI3K involves the Akt-mediated suppression of the tuberous sclerosis complex (TSC), which consists of TSC1 and TSC2. TSC1/2 functions as a GTPase-activating protein for Rheb (Ras homologue enriched in brain), a GTPase that directly interacts with mTOR constitutively active in the absence of nutrients. Significantly, RalA is constitutively associated with phospholipase D1 (PLD1) (13, 14). PLD1 generates the lipid second messenger phosphatidic acid (PA), which is required for the activity of both mTORC1 and mTORC2 (15–17). Although RalA does not activate PLD1 directly, it stimulates the association of PLD1 with ADP-ribosylation factor (ARF) family GTPases, which do increase the activity of PLD1 (18). Significantly, ARF6 has been implicated in the response of mTORC1 to glucose (19). An important recent study also linked Rheb and PLD1. Chen and colleagues (20) reported that Rheb, like ARF and Rho family GTPases, interacts with and activates PLD1. This study suggested the possibility that the activation of Rheb in response to growth factors can induce PLD activity and the generation of the PA critical for mTORC1 activation.

Tamanoi and colleagues (21) recently identified two mTOR mutants from human cancers with point mutations that made mTOR constitutively active in the absence of nutrients. Significantly, one of these nutrient-insensitive mTOR mutants was resistant to inhibition by 1-BtOH while retaining sensitivity to rapamycin. 1-Butanol (1-BtOH) suppresses the production of PA by PLD and is used to implicate PLD activity (17). This finding indicated that a nutrient-insensitive mTOR mutant has lost its PA requirement. The insensitivity of this mutant to both nutrients and PA is consistent with the hypothesis that nutritional input into mTORC1 is mediated by PLD.

Another link between amino acids and mTOR is the class III PI3K, hVps34 (22–25). hVps34 generates PI 3-phosphate (PI-3-P) instead of the PI 3,4,5-trisphosphate generated by type I PI3Ks (26). It is not known how hVps34 contributes to the activation of mTORC1, but PI-3-P serves to recruit proteins with PX domains (26). In this regard, it is of interest that both PLD1

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2 The abbreviations used are: mTOR, mammalian target of rapamycin; mTORC1 and mTORC2, mTOR complex 1 and 2, respectively; ARF, ADP-ribosylation factor; BtOH, butanol; PA, phosphatidic acid; PI, phosphatidylinositol; PI-3-P, PI-3-phosphate; PLD, phospholipase D; TSC, tuberous sclerosis complex.
and PLD2 have PX domains (27, 28) that could be influenced by hVps34 activity. Thus, a common denominator for several factors previously implicated in the regulation of nutrient-dependent mTORC1 activity is PLD.

In addition to being a central regulator of the cell response to nutrients and energy, mTOR has emerged as a central player in cancer (29). mTOR signaling is dysregulated in what may be virtually all human cancers and has been widely implicated in cancer cell survival signals (29, 30). There are many mutations in the signals that mediate growth factor signaling to mTOR, such as PI3K, Akt, and the TSC (31). However, these growth factor-signaling intermediates that lead to elevated mTORC1 activity are not affected by nutrients (4). PLD, like mTORC1, has also been implicated in cancer cell survival signals (32). We report here that elevated PLD activity in human cancer cells is dependent on the presence of nutrients that stimulate mTORC1. The study suggests that PLD-generated PA represents a critical signal for nutrient sensing by mTORC1.

**EXPERIMENTAL PROCEDURES**

**Cells, Cell Culture Conditions, and Transfection**—The MDA-MB-231, T24, and Calu-1 cells used in this study were obtained from the American Type Culture Collection. All cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal bovine serum.

**Materials**—Reagents were obtained from the following sources. Antibodies against ARF1, ARF6, Rheb, and hVps15 were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA); antibodies against S6 kinase, phosphorylated S6 kinase (Thr389), hVps34, RalA, and actin were obtained from Cell Signaling. siRNAs targeting RalA (M-009235-00-0005), RhoB (M-009692-02-0005), ARF1 (M-011580-01-0005), ARF6 (M-004008-01-0005), hVps34 (M-005250-00-0005), and hVps15 (M-005025-02-0005) were obtained from Dharmacon. Insulin, 1-BtOH, and t-BtOH were from Sigma; wortmannin was from Calbiochem. DMEM (D5796) and DMEM lacking Arg, Leu, and Lys (D9443) were obtained from Sigma. 50X minimum essential medium amino acid solution without l-Glu (Sigma M5550) was used for stimulation by amino acids. For stimulation, the amino acid solution was added to a 2X concentration. Glucose-free DMEM was from Invitrogen (11966-025). PLD inhibitors for PLD1 (Compound 14, VU0379595) (1R,2R)-N-[(S)-1-[(4-[5-bromo-2-oxo-2,3-dihydro-1H-benzo(d)imidazol-1-yl]piperidin-1-yl)propan-2-yl]-2-phe- nylcyclopropancarboxamide) and PLD2 (Compound 22a, VU0364739) (N-(2-((3-fluorophenyl)-4-oxo-1,3,8-triazaspiro[4,5]decan-8-yl)ethyl)-2-naphthamide) were provided by Dr. H. Alex Brown (Vanderbilt University) and have been described elsewhere (33, 34).

siRNA—Cells were plated on 6-well plates at 30% confluence in medium containing 10% serum. After 1 day, cells were transfected with siRNA at 80 nM concentration using Lipofectamine RNAiMAX (Invitrogen) according to manufacturer’s instructions. After 24 h, the medium was changed to fresh medium containing 10% serum, and 2 days later, cells were lysed and analyzed by Western blot.

**Western Blot Analysis**—Extraction of proteins from cultured cells and Western blot analysis of extracted proteins was performed using the ECL system (Thermo Scientific) as described previously (17). Relative levels of p70S6K phosphorylation were determined by measuring the intensity of the appropriate band in the autoradiograph with ImageJ software.

**PLD Activity**—PLD activity was determined by the transphosphatidylation reaction in the presence of 0.8% 1-BtOH as described previously (17). Cells in 60-mm culture dishes were labeled with [3H]myristic acid (3.0 µCi) for 4–6 h, and 1-BtOH (0.8%) was added 20 min before lipids were collected. Lipids were extracted and characterized by thin layer chromatography as described previously (17). Relative levels of PLD activity were then determined by measuring the intensity of the corresponding phosphatidyl-1-BtOH band in the autoradiograph with ImageJ software or by scraping the thin layer chromatography plates and scintillation counting of the phosphatidyl-1-BtOH band.
RESULTS

PLD Activity in Human Cancer Cells Is Dependent on Amino Acids and Glucose—We reported previously that the PLD activity is elevated in several human cancer cell lines, especially those harboring Ras mutations (35–37). The PA generated by PLD is required for the activation of mTORC1 (32). mTORC1 is a sensor of nutritional sufficiency and is active in the presence of amino acids and glucose (1–4). We therefore examined whether the elevated PLD activity observed in human cancer cell lines was dependent on the presence of essential amino acids and glucose. MDA-MB-231, T24, and Calu-1 cells all display elevated PLD activity, especially when deprived of serum (35, 36). All three of these cell lines harbor Ras mutations, which contribute to the elevated PLD activity (36, 37). The PLD activity in the MDA-MB-231, T24, and Calu-1 cells was evaluated in the presence and absence of essential amino acids (A) or glucose (B). As shown, the level of PLD activity in all three cell lines was substantially reduced when the medium lacked either amino acids or glucose. The nutrient-dependent PLD activity in all three cell lines was partially sensitive to selective inhibitors of both PLD1 (compound 14) and PLD2 (compound 22a), indicating that both PLD1 and PLD2 are likely to be involved.

Amino Acid- and Glucose-induced Increases in mTORC1 Activity Are Dependent on PLD Activity—The ability of amino acids to activate mTORC1 has been widely reported (3). Chen and colleagues (20) recently reported that serum-induced increases in mTORC1 activity were dependent on PLD activity. MDA-MB-231, T24, and Calu-1 cells were plated in 6-well plates at 50% confluence and then placed in medium containing 10% serum overnight. The cells were then shifted to medium lacking serum for 24 h. The cells were then shifted to medium lacking either essential amino acids (A) or glucose (B), 4 h later, amino acids or glucose were added back in the presence of either 1-BtOH or t-BtOH, as indicated. Cells were harvested 30 min later, and p70S6K phosphorylation at Thr389 (P-p70S6K) was examined by Western blot. The data shown are representative experiments repeated at least two times. The levels of phospho-p70S6K relative to the level of p70S6K were determined by densitometer and normalized to the level observed in the absence of amino acids (A) or glucose (B) and are provided above each blot. The data shown are for representative experiments repeated at least two times. C, T24 cells were prepared and treated as in A and B. Amino acids or glucose were added as indicated in the presence of the indicated concentration of the PLD inhibitors 14 and 22A. Phospho-p70S6K was examined 30 min later as in A and B. Shown is a representative experiment as well as a graphic representation for two independent experiments with error bars representing S.D. values.
phosphorylation of the mTORC1 site on p70 S6 kinase (p70S6K) (Thr389) was dependent on the presence of amino acids. Chen’s group (20) also demonstrated that serum-induced PLD activity was dependent on amino acids. These data are consistent with an amino acid and PLD dependence for serum-induced mTORC1 activation. We therefore examined whether amino acid-stimulated p70S6K phosphorylation in MDA-MB-231, T24, and Calu-1 cells was dependent on PLD activity. Cells were shifted to medium lacking serum overnight, at which time the cells were then harvested, and the PLD activity was determined as in Fig. 1A. Cells were first transfected with Rheb or scrambled siRNA as in A. After overnight incubation in medium containing 5% serum, the cells were placed in medium containing 0% serum for 4 h lacking essential amino acids or glucose. Where indicated, amino acids or glucose were added back for 30 min, and p70S6K phosphorylation was determined by Western blot using an antibody specific for Thr389. The levels of p70S6K protein, Rheb, and actin were also determined by Western blot. C, T24 cells were treated as in B with Rheb or scrambled siRNA and then put in medium lacking glucose for 4 h. Glucose was then added where indicated, and the levels of phosphorylated p70S6K, p70S6K, Rheb, and actin were determined 30 min later by Western blot analysis. The data shown are representative experiments repeated at least two times. The levels of phospho-p70S6K (P-p70S6K) relative to p70S6K were determined as in Fig. 2B.

We also examined the PLD dependence for the activation of mTORC1 by glucose. MDA-MB-231, T24, and Calu-1 cells were placed in medium lacking serum overnight and then shifted to medium lacking both serum and glucose. Four hours later, glucose was added along with either 1-BtOH or t-BtOH, and the phosphorylation of p70S6K was evaluated. As shown in Fig. 2B, the induction of p70S6K phosphorylation was suppressed by 1-BtOH but not by t-BtOH. We also examined the effect of the PLD inhibitors on the amino acid- and glucose-induced increases in p70S6K phosphorylation. As shown in Fig. 2C, the combination of Compounds 14 and 22a suppressed the induction of p70S6K phosphorylation by both amino acids and glucose in the T24 cells. Neither of the inhibitors alone had a significantly stronger impact of p70S6K phosphorylation (data not shown), making it likely that both PLD1 and PLD2 are involved. These data further implicate PLD activity for the induction of mTORC1 with either glucose or amino acids.

Elevated PLD Activity and Nutrient-dependent mTORC1 Activity in Human Cancer Cells Is Dependent on Rheb—Chen and colleagues (20, 38) recently reported that serum-induced PLD activity was at least partially dependent on Rheb and that Rheb could be co-immunoprecipitated with PLD1. We therefore examined whether the amino acid-dependent PLD activity
in human cancer cells was dependent on Rheb. As shown in Fig. 3A, suppression of Rheb expression with siRNA suppressed the amino acid-dependent PLD activity in MDA-MB-231, T24, and Calu-1 cells. Consistent with previous reports (2), knockdown of Rheb also suppressed the amino acid-induced phosphorylation of p70s6k phosphorylation at the mTORC1 Thr389 site (Fig. 3B). We also examined the impact of suppressing Rheb expression on the stimulation of mTORC1 with glucose in the T24 cells. As shown in Fig. 3C, the glucose-induced increase in p70s6k phosphorylation was also dependent on Rheb. These data demonstrate that nutrient-dependent PLD activity is dependent on Rheb and that the nutrient induction of mTORC1 is also dependent on Rheb.

**Amino Acids Do Not Impact on TSC**—The amino acid induction of mTORC1 is dependent on Rheb and therefore also dependent on the suppression of TSC1/2, which suppresses Rheb by stimulating the hydrolysis of GTP to GDP (4). We therefore investigated the impact of amino acids on the phosphorylation state of TSC2, which is both activated and suppressed by phosphorylation at different sites (39). Akt phosphorylates TSC2 at Thr1462, which leads to the suppression of the GTPase activity of Rheb (39). As shown in Fig. 4A, amino acids had no effect on the phosphorylation state of TSC2 at Thr1462 in either the MDA-MB-231 or T24 cells. Suppression of PA generation by PLD with 1-BtOH also had no effect on the phosphorylation of TSC2 at this site (Fig. 4A). In contrast, insulin was able to increase phosphorylation at this site (Fig. 4B). TSC2 is phosphorylated by Akt, which is activated by PI3K (39). Importantly, suppression of PI3K with wortmannin was able to suppress TSC2 phosphorylation at Thr1462, and this led to reduced phosphorylation of p70s6k at Thr389. Collectively, the data in Figs. 3 and 4 indicate that although signaling through TSC and Rheb is required for the amino acid induction of PLD and mTORC1, this signaling pathway is not impacted by amino acids. These findings are consistent with previous reports indicating that nutrient signaling to mTOR does not up-regulate the TSC-Rheb pathway (4).

**Amino Acid and Glucose-dependent PLD Activity Is Dependent on RalA**—The lack of an impact of amino acids on TSC1/2 suggest that the amino acid-stimulated increase may be acting through the regulation of PLD and does not involve changes in the activity of Rheb. The amino acid-stimulated increase in mTORC1 activity was reported to be dependent on RalA (12). The RalA dependence for the amino acid-dependent activation of mTORC1 is particularly intriguing in that RalA is constitutively associated with PLD1 (13, 14) and is required for the recruitment of activating ARF family GTPases to PLD1 (18). RalA was knocked down with siRNA, and the impact on amino acid-dependent PLD activity was evaluated in the MDA-MB-231 and T24 cells. As shown in Fig. 5A, the amino acid-dependent PLD activity in both cell lines was dependent on RalA. We next examined the effect of RalA knockdown on the induction of p70s6k phosphorylation by amino acids. As shown in Fig. 5B, RalA knockdown suppressed the amino acid induction of p70s6k phosphorylation at the mTORC1 site at Thr389 in both the MDA-MB-231 and T24 cells. The induction of p70s6k phosphorylation by glucose was similarly suppressed by RalA knockdown in the T24 cells (Fig. 5C). In contrast, the serum induction of p70s6k phosphorylation was not impacted by RalA knockdown in these cells (Fig. 5C). These data are consistent with RalA being a key target of nutrients for the activation of mTORC1 and further implicate PLD1, which interacts directly with RalA as a conduit to mTORC1 activation.

**Amino Acid and Glucose-dependent PLD Activity Is Dependent on ARF6**—We previously reported that RalA promotes the activation of PLD1 by recruitment of ARF family GTPases into a RalA-PLD1 complex (14, 18). We also demonstrated that H-Ras-induced PLD activity was dependent on ARF6 (40). ARF6 has also been implicated in the glucose stimulation of insulin secretion (19), indicating that ARF6 is activated in response to nutrients. Thus, the involvement of RalA, in the amino acid- and glucose-dependent PLD activity shown in Fig. 5 suggests the possible involvement of ARF GTpases, which are direct activators of PLD1 (41). As reported previously for the MDA-MB-231 cells (37), knockdown of either ARF1 or ARF6 with siRNA suppressed the nutrient-dependent PLD activity in the T24 cells (Fig. 6A). As shown in Fig. 6B, ARF6 knockdown suppressed the induction of p70s6k phosphorylation by both amino acids and by glucose in the T24 cells. As with RalA, the serum induction of p70s6k phosphorylation was not impacted by ARF6 knockdown. In contrast with ARF6, knockdown of ARF1 did not affect the stimulation of p70s6k phosphorylation by amino acids, glucose, or serum (Fig. 6C). These data are consistent with ARF6 being a key target of nutrients.
for the activation of mTORC1 and further implicate PLD1, which interacts directly with RalA as a conduit to mTORC1 activation.

*hVps34 Is Required for Amino Acid-dependent PLD Activity*—
hVps34 has been implicated in amino acid-stimulated activation of mTORC1 (22–25). hVps34 is intriguing in that it is a type III PI3K kinase that generates PI-3-P, which interacts with PX domains, and significantly, both PLD1 and PLD2 have a PX domain that is critical for activity (27, 28). We therefore examined whether hVps34 was required for the amino acid-dependent PLD activity in the T24 cells. As shown in Fig. 7A, siRNA knockdown of hVps34 suppressed the amino acid-dependent PLD activity. We also examined the effect of suppressing hVps15 (formerly known as P150) on PLD activity in the T24 cells. hVPS15 regulates the activity of hVps34 and has also been implicated in nutrient sensing (42). As shown in Fig. 7A, hVPS15 also suppressed the PLD activity in these cells. These data are consistent with our previous finding that the PI3K inhibitor LY294002 strongly inhibited PLD activity in the T24 and Calu-1 cells (36). We next examined whether the knockdown of hVps34 suppressed the induction of p70S6K phosphorylation by serum, amino acids, and glucose. We were unable to detect significant effects upon the phosphorylation of p70S6K in the T24 cells (data not shown). However, because the hVps34 has been shown previously to be required for amino acid-induced increases in mTORC1 activity (22–25), we examined the effect of suppressing the hVps15. As shown in Fig. 7B, suppression of hVps15 expression suppressed both the
amino acid and glucose induction of p70S6K phosphorylation in T24 cells. It is not clear at this point why the knockdown of hVps15 was more effective than knockdown of hVps34, but the effect of hVps15 and hVps34 siRNAs on the amino acid- and glucose-dependent PLD activity suggests that the signals generated by hVps34 that lead to mTORC1 go through PLD.

DISCUSSION

In this report, we have provided evidence that nutrient stimulation of mTORC1 is dependent on PLD activity. Whereas growth factor stimulation of mTORC1 is largely regulated through the PI3K/Akt/TSC/Rheb pathway, this signaling pathway is not impacted by nutrients (4). Thus, the finding that nutrients are feeding into PLD reveals a novel mechanism for activating mTORC1. Although nutrients do not impact on Rheb GTP loading (5–7), Rheb is clearly required for the nutrient-dependent increase in mTORC1. Significantly, Rheb was required for the nutrient-dependent PLD activity, which is consistent with a report from the Chen group (20) that has shown that Rheb interacts with and contributes to the activation of PLD1. The activation of nutrient-dependent mTORC1 activity was also dependent on the GTPases RalA and ARF6. Both of
these GTPases have been implicated in both response to nutrients (12, 19, 43) and stimulation of PLD activity (13, 14, 18, 40). RalA is constitutively associated with PLD1 but does not activate PLD1 by itself. RalA contributes to the activation of PLD1 by recruiting ARF6, which does activate PLD1 activity, into a RalA-ARF6-PLD1 complex (18). Although it is still not clear from data provided here or elsewhere how the presence of nutrients activates RalA and ARF6, the data provided here indicate that a key target of RalA and ARF6 for the stimulation of mTORC1 is PLD1.

The involvement of RalA and ARF6 in the nutrient-dependent increases in PLD and mTORC1 activity implicates PLD1, which is constitutively associated with RalA (14). However, PLD2 has also been implicated in the regulation of mTORC1. Exogenously expressed PLD2 was shown to increase p70S6K phosphorylation in MCF7 cells (44). It was also reported that PLD2 forms a functional complex with mTOR and Raptor, and this interaction was essential for mitogen stimulation of mTORC1 (45). More recently, dominant negative mutants of both PLD1 and PLD2 were able to suppress the activation of mTORC1 (17). Data provided here reveal that the nutrient-dependent PLD activity in the cells used in this study were sensitive to PLD inhibitors specific for both PLD1 and PLD2. Thus, it would appear that both PLD1 and PLD2 are involved in the nutrient-dependent activation of mTORC1. In this regard, it is of interest that hVps34 is also probably required for nutrient-dependent PLD activity and mTORC1 activation. hVps34 stimulates the production of PI-3-P, which serves to recruit proteins with PX domains (26), and both PLD1 and PLD2 contain PX domains (27, 28). Thus, the apparent involvement of PLD2 as well as PLD1 may involve the activation of hVps34.

Interestingly, Neufeld and colleagues (46) reported that in Drosophila, although Vps34 promotes autophagy and endocytosis, it does not stimulate TOR signaling. However, as reviewed by Sun and Chen (38), Drosophila TOR is not likely to be regulated by PA and PLD. This is because Drosophila TOR lacks the Arg at 2109 that is critical for PA binding to mTOR (15, 47). Moreover, an RNAi screen that included PLD did not lead to a phenotype indicative of any involvement in the activation of TOR (48). Also of interest in this regard is a study with Drosophila that implicated ARF GTPases in the regulation of nutrient input into Drosophila TOR. In this study, ARF1 rather than ARF6 was implicated (43), further distinguishing the regulation of TOR in Drosophila and in mammals, where PLD activity and PA have been implicated.

Sabatini and colleagues (9–11) recently reported that mTORC1 is targeted to lysosomal membranes by Rag GTPases in response to amino acids. They went on to show that constitutive targeting of mTORC1 to the lysosomal surface was sufficient to render the mTORC1 pathway amino acid-insensitive and independent of Rag (10), leading them to propose that Rag-mediated translocation of mTORC1 to lysosomal membranes...

FIGURE 7. hVps34 is required for amino acid-dependent PLD activity. A, T24 cells were plated at 30% confluence overnight. The cells were transfected with hVps34, hVps15, or scrambled control siRNA as indicated. 24 h later, the cells were treated with fresh medium containing 10% serum for an additional 24 h, at which time the cells were shifted to medium containing 0.5% serum for an additional 24 h. The cells were then harvested, and the relative PLD activity was determined as described under “Experimental Procedures.” Values were normalized to the control scrambled siRNAs, which were given a value of 100%. B, T24 cells were treated with either hVps15 or scrambled siRNA and then put in medium lacking serum (all lanes) and either amino acids or glucose as indicated for 4 h. Serum, amino acids, and glucose were then added where indicated, and the levels of p70S6K phosphorylation, hVps15, and actin were determined 30 min later by Western blot analysis. The levels of phospho-p70S6K (P-p70S6K) relative to p70S6K were determined as in Fig. 2. Shown is a representative experiment. Also shown is a graphic representation of three independent experiments with error bars representing S.D. values.

PLD Dependence of Nutrient-dependent mTOR Activation
is the key event in amino acid signaling to mTORC1. It was hypothesized that mTORC1 was activated by simply bringing mTORC1 to where GTP-bound Rhb was localized, accounting for the activation of mTORC1 in response to amino acids. Our data are consistent with this model in that there is agreement that Rhb is critical for the activation of mTORC1 in response to amino acids. However, our study also takes into account previous studies linking the nutrient response of mTORC1 to Rag GTPases to lysosomal membranes where nutrients stimulate the activity of mTORC1 via the activation of PLD1 and possibly PLD2 by the activation of RalA, ARF6, and hVps34. Interestingly, Rhb is required for PLD activity, revealing cross-talk between the two inputs into mTORC1 activation. Elevated PLD activity generates the PA necessary for the association between mTOR and Raptor, which is essential for mTORC1 activity.

ularly, rapamycin causes apoptosis when these cells are deprived of serum (49). Therefore, the finding reported here that the PLD activity in these cells is dependent on nutrient input suggests additional strategies for reversing the survival signals in cancer cells by targeting the nutrient sources and signals generated by nutrients. Consistent with this hypothesis, Blenis and colleagues (50) have reported recently that TSC2 null cells, which have hyperactive mTORC1 signals, are uniquely sensitive to deprivation of the nutrients glucose and glutamine.

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