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Phosphatidic Acid and Lipid Sensing by mTOR

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Abstract

Mammalian target of rapamycin (mTOR) has been implicated as a sensor of nutrient sufficiency for dividing cells and is activated by essential amino acids and glucose. However, cells also require lipids for membrane biosynthesis. A central metabolite in the synthesis of membrane phospholipids is phosphatidic acid (PA), which is required for the stability and activity of mTOR complexes. While PA is commonly generated by the phospholipase D-catalyzed hydrolysis of phosphatidylcholine, PA is also generated by diacylglycerol kinases and lysophosphatidic acid acyltransferases, which are at the center of phospholipid biosynthesis. It is proposed that the responsiveness of mTOR/TOR to PA evolved as a means for sensing lipid precursors for membrane biosynthesis prior to doubling the mass of a cell and dividing.

Keywords

mTOR; phosphatidic acid; DG kinase; LPAAT; phospholipase D

Lipid sensing by TOR

A critical need for cell growth is the presence of the raw materials needed for doubling the mass of a cell during cell division. Target of rapamycin (TOR) is an evolutionarily conserved sensor of essential nutrients needed for the synthesis of biological molecules (1, 2). TOR has long been known to respond to amino acids, glucose, and energy (3, 4). However, over the last decade, the mammalian TOR (mTOR) has been shown to require PA for the stability of mTOR complexes and their activity (5–8). A rationale for the involvement of PA has been missing, and hence the role of PA in regulating mTOR has been controversial (9). Of significance, PA is at the center of membrane glycero-phospholipid synthesis. Based on this central position of PA generation, it is speculated that the PA requirement for mTOR and evolutionally more primitive TOR proteins represents a means for TOR to sense the presence of sufficient lipid precursors for membrane biosynthesis, cell growth and proliferation. Phospholipase D (PLD), which is activated in response to a variety of extra-cellular stimuli also generates PA and represents an alternative mechanism for generating PA needed for mTOR activation (6, 7). Importantly, PA generation via PLD is commonly elevated in human cancers where active mTOR provides signals that promote cancer cell survival (10). In this perspective, it is proposed that in addition to amino acids, glucose and energy status, TOR responds to PA as an indicator of lipid sufficiency in

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dividing cells. It is also proposed that the generation of PA by PLD represents a means for TOR activation in response to extracellular signals, in multicellular organisms.

Nutrient sensing by mTOR and cell cycle progression

G1 cell cycle progression has been divided into two parts G1-pm (post-mitotic) and G1-ps (pre-S) separated by a growth factor dependent restriction point (11). During G1-pm, the cell determines whether there are instructions to divide – growth factors. During G1-ps, the cell determines whether the resources needed for doubling the mass of the cells are available (12). There are likely several checkpoints in late G1 that sense the presence of sufficient nutrients for cell growth. mTOR is a key sensor of nutrients and is sensitive to the presence of amino acids, glucose, ATP, and insulin (1–4). mTOR also regulates passage through late G1, and significantly, rapamycin treatment leads to the accumulation of small cells with G1 DNA content (13, 14). Given the critical role that mTOR plays in promoting G1 cell cycle progression, it is no surprise that it has been suggested that signals that regulate mTOR are the most commonly dysregulated signals in cancer (15, 16). Although activating gain-of-function mTOR mutations have been reported in human cancers (17), more commonly, there are mutations in genes that encode proteins that regulate mTOR activity (9). The most common mutations that result in elevated mTOR activity are to phosphatidylinositol (PI)-3-kinase (PI3K), which phosphorylates PI-4,5-bisphosphate at position 3 of the inositol ring to generate PI-3,4,5-trisphosphate; and loss-of-function mutations for PTEN, which dephosphorylates PI-3,4,5-trisphosphate at the 3 position. These mutations result in elevated signaling through Akt, the tuberous sclerosis complex (TSC), and Rheb – leading to the activation of mTOR complex1 (mTORC1) (Figure 1). This pathway is activated physiologically by insulin and insulin-like growth factor (9). Insulin also leads to an increase in glucose transporters on the plasma membrane and increased uptake of glucose (18). Increased uptake of glucose is a hallmark of cancer cells that was first observed by Otto Warburg more than 80 years ago (19). Importantly, mTOR is required for much of the metabolic reprogramming that takes place in cancer cells where oxidative phosphorylation is reduced even in the presence of O_2 (9, 20). Thus, mTOR not only responds to the presence of nutrients and energy, it also promotes metabolic reprogramming to promote cell growth by enhancing greater utilization of glucose for generating the biological molecules needed for a cell to double its mass upon cell division.

Regulation of mTOR by phosphatidic acid

While much is known about the regulation of mTOR via the PI3K/Akt/TSC/Rheb pathway, mTOR is also dependent on PA, which interacts with mTOR in a manner that is competitive with rapamycin (5, 21) and is required for the stability of both mTORC1 and mTORC2 complexes (8). mTORC1 is much more sensitive than mTORC2 to the levels of PA, which stabilizes mTOR, and to rapamycin, which disrupts the mTOR complexes (8). Consequently, it is mTORC1 that is more likely than mTORC2 to be responsive to changes in PA levels. The PA most commonly associated with mTOR regulation is generated by the hydrolysis of phosphatidylcholine by PLD (7, 10). However, recent reports have revealed that knockout of both PLD1 and PLD2 yields viable mice (22, 23). In contrast, mTOR knockouts are embryonic lethal (24, 25). Thus, if PA is essential for mTOR activity, then PA from other sources must be used.

Multiple sources of PA

PA synthesis is at the center of membrane phospholipid and triglyceride synthesis (26–29) and therefore represents a potential indicator of the capability for generating the membrane phospholipids needed for doubling cell mass. The PA targeted for membrane biogenesis is most commonly synthesized from glycerol 3-phosphate (G3P) and newly synthesized fatty

acids (FAs). G3P gets acylated twice by distinct acyltransferases – the last step being catalyzed by a lysophosphatidic acid acyltransferase (LPAAT) (Figure 2a). Interestingly, this pathway is dependent on the glycolytic pathway intermediate dihydroxyacetone phosphate (DHAP), which gets reduced to G3P and is then acylated to generate PA. The Acyl-CoA needed for the acylation of G3P comes from both dietary FAs and newly synthesized palmitic acid, which is catalyzed by fatty acid synthase (FAS). Thus, the generation of PA via the LPAAT pathway involves nutrient input from glucose, dietary FAs, and *de novo* FA synthesis. PA can be converted by PA phosphatase to diacylglycerol (DG), which can be acylated to form triglycerides for fat storage. DG is also an intermediate for the synthesis of a subset of membrane glycerol-phospholipids. In the reverse process, PA can be generated from stored triglycerides by deacylation to DG, which can be either fed directly into membrane phospholipid biosynthesis or be phosphorylated by a DG kinase to generate PA (Figure 2a). Thus, the central position of PA in phospholipid metabolism makes PA an ideal indicator of lipid sufficiency to proceed with membrane biogenesis in a dividing cell. Importantly, LPAAT and DG kinase-θ, which generate PA, have been shown to stimulate mTOR (30, 31), although there are also reports that DG kinases can suppress mTOR (32, 33), which will be addressed below. Thus, there is a connection between the enzymes that generate the PA critical for phospholipid and membrane biosynthesis and the activation of mTOR. Intriguingly, suppression of LPAAT suppressed mTOR activity and disrupted survival and proliferative signals in several cancer cell lines (34).

An alternative pathway for growth factor induced PLD-induced PA production is via a phospholipase C (PLC)-mediated production of DG followed by the conversion of DG to PA by DG kinase as described previously (29). Like PLD, PLC is commonly activated by growth factors and could account for ability of PLD null mice to survive. It will therefore be of interest to determine whether in the absence of PLD, there is a compensatory increase in the level of PA generated by PLC and DG kinase in response to growth factors.

In addition to the known DG kinases (29), it was recently reported that ER-localized PKR-like ER kinase (PERK), a kinase that responds to ER stress, has an intrinsic DG kinase activity (35). Importantly, the PA produced in response to PERK stimulated both mTORC1 and mTORC2. ER stress or the unfolded protein response (UPR) that occurs on the ER induces different responses depending on nutrient availability (36). The outcome can be apoptosis under nutritional stress, or a homeostatic response that restores ER function. Thus, the ability of PERK to generate PA and stimulate Akt phosphorylation at Ser473 – a site phosporylated by mTORC2 – may be part of the UPR that leads to restoration of ER function. The stimulation of mTOR by the UPR and PERK would promote the uptake of glucose and the generation of anabolic intermediates needed to alleviate ER stress. Interestingly, loss of either TSC1 or TSC2, which leads to hyperactive mTOR, also triggers ER stress and the UPR (37) – indicating that hyperactive mTOR leads to the activation of PERK and generates the PA to support increased mTOR activity.

Altered metabolism in proliferating cells leads to increased utilization of metabolites for anabolic needs and cell growth – including PA production

When a cell commits to dividing there is a “metabolic transformation” that takes place whereby there is a shift from catabolic metabolism that favors the mitochondrial production of ATP via the electron transport chain to anabolic metabolism that favors the production of NADPH, which is used for the synthesis of biological molecules – especially FAs (38, 39). Glucose metabolism is highly impacted in proliferating cells, most significantly through increased glucose transport (40). Interestingly, dividing cells express an embryonic form of the enzyme pyruvate kinase M2 (PKM2) that catalyzes the last step of glycolysis – the conversion of phosphoenolpyruvate (PEP) to pyruvate (41). PKM2 is inefficient in

converting PEP to pyruvate and is suppressed further by growth factor-induced tyrosine phosphorylation (42). The reduced PKM2 activity combined with increased glucose uptake results in the increase of glycolytic intermediates (43). These glycolytic intermediates are shunted off into pathways for the synthesis of nucleotides and amino acids (Figure 2a). Glucose-6-phosphate (G6P) can be converted to ribose via the pentose phosphate shunt, and 3-phosphoglycerate converted to serine and other amino acids via phosphoglycerate dehydrogenase. This last pathway, which leads to serine synthesis, is required for certain breast cancers (44). While these two shunts have been discussed in recent reviews (15, 43), there is another critical shunt leading to the synthesis of membrane phospholipids involving the conversion of DHAP to G3P. Interestingly, triose phosphate isomerase, the glycolytic enzyme that converts DHAP to glyceraldehyde-3-P is suppressed by PEP – the substrate of PKM2 (45). Thus PKM2, by slowing the conversion of PEP to pyruvate, further enhances the accumulation of DHAP during glycolysis and the production of G3P – the substrate for the acyl-transferases that generate PA (Figure 2b). Enhancement of G3P levels by suppression of both pyruvate kinase and triose phosphate isomerase activity strongly indicates that generating G3P, a precursor of PA, is critical in dividing and cancerous cells.

Does mTOR sense dietary essential FAs?

FA synthesis generates palmitic acid – a 16 carbon saturated FA, which can be incorporated into membrane lipids via acyl-transferases (see Figure 2). In mammalian cells, palmitic acid can be elongated, but mammals are limited in their ability to desaturate FAs between C-10 and the methyl terminal end. Hence, they need exogenously supplied linoleic and linolenic acids, 18 carbon poly-unsaturated FAs with 2 and 3 double bonds respectively that have double bonds at the omega 3 and 6 positions. These FAs are needed for the synthesis of critical poly-unsaturated FAs such as arachidonic acid (46, 47). Hence, linoleic and linolenic acids are considered “essential fatty acids” – analogous to the essential amino acids not synthesized by mammalian cells. In this regard, it is of interest that PA with two saturated palmitates actually inhibits mTORC2 (48), whereas 1-palmitoyl, 2-steroyl-PA is stimulatory for both mTORC1 and mTORC2 (8, 49). These data suggest that PA with some degree of unsaturation is required for a functional interaction with mTOR. Or alternatively, that di-palmitoyl-PA, with two saturated FAs is targeted for energy storage, and thusly, is dephosphorylated and acylated to triacylglycerol. This is shown schematically in Figure 3a, where there are two outcomes from FA and PA synthesis: 1) in storage mode, newly synthesized palmitate is converted into triglycerides for energy storage; and 2) in proliferation mode, there is the generation of longer chain FAs with some degree of unsaturation that are incorporated into PA targeted for membrane phospholipids. It is PA with at least one FA containing an unsaturated FA that interacts with mTOR to promote complex stability and activity. Whether there is a mechanism for mTOR to distinguish PA species with poly-unsaturated FAs derived from the essential linoleic and linolenic acids remains to be determined. However, there does appear to be a means for distinguishing PA with only saturated palmitate, which inhibits mTORC2, from PA with two saturated FAs (48). This effect may explain the observation alluded to above that under some conditions, DG kinase can suppress mTOR (32, 33). If the DG species being phosphorylated contains two saturated FAs, then it would inhibit rather than stimulate mTOR. mTOR responds to essential amino acids specifically (50, 51) and it will therefore be of interest to determine if mTOR can sense essential FAs and whether this involves PA.

Conservation of the PA requirement of TOR

Jie Chen and colleagues established that positively charged Arg^{2109} in the rapamycin-binding domain of mTOR was critical for interaction with PA (5). This interaction was subsequently verified in an NMR structural study (52). Significantly, this site is highly

conserved evolutionarily – with an Arg or Lys at this site in a wide variety of species from yeast to humans (53). The only exception to a positively charged amino acid at position 2109 is Drosophila, where there is Gln instead (Figure 3b). This piece of evidence along with the finding that PLD knockout in Drosophila did not impact on TOR signaling (54), led Sun and Chen (7) to suggest that the regulation of mTOR by PLD might be restricted to mammals. However, while there is a Gln instead of an Arg or Lys at position 2109, a conserved positive charge exists at the adjacent amino acid at 2110. Moreover, although Gln does not carry a positive charge, it does have amide hydrogens capable of forming hydrogen-bonds with electrons on the phosphate oxygens of PA. Thus, while PLD may not be required to generate PA for TOR activation in Drosophila, it is still quite possible that there is a PA requirement for Drosophila TOR that is met by LPAAT, DG kinase, or a PLC/DG kinase mechanism. However, there is indirect evidence that PA may regulate TOR in Dictyostelium. Both over expression of PLD and exogenously supplied PA suppresses the starvation response in Dyctyostelium (55), and starvation in Dictyostelium inhibits 4E–BP1 phosphorylation (56). Thus, PA suppresses the starvation response that involves inhibition of phosphorylation of an mTORC1 substrate. Thus, it is highly likely that PA is regulating mTOR in an evolutionarily primitive organism. These studies not only implicate PA as an activator of TOR in a primitive organism, they also implicate PLD as activator of TOR at an early stage of evolution. Since the sequence in the PA-binding region of TOR is so highly conserved, there is a compelling evidence that PA is critical for TOR in distant species including yeast, Dictyostelium, and C. elegans, – all of which have the highly conserved PA-binding region of TOR with two positively charged amino acids at position 2109 and 2110, as well as several other conserved amino acids flanking this site (Figure 3b). The critical amino acids for binding of Raf to PA have also been mapped and while there are differences between the Raf and TOR sequences, the two consecutive positively charged amino acids are conserved (57).

PA generated for membrane lipid biosynthesis versus PLD

During proliferation, there is substantial membrane phospholipid synthesis going on, and as a consequence, there is significant PA production that is targeted for membrane phospholipid biosynthesis. PLD-generated PA, which is derived from a membrane phospholipid, is therefore not involved in cell growth. However, mTOR is not stimulated solely by nutrients; it is also stimulated by a variety of growth factors – perhaps most significantly by insulin and insulin-like growth factor1 (9). Importantly, these and other growth factors also activate PLD (10), and the stimulation of mTORC2 by insulin is dependent on PLD-generated PA (8). Thus, it is tempting to speculate that growth factor-induced PLD activity evolved as a means to promote TOR complex stabilization in the absence of anabolic PA synthesis by DG kinase and LPAAT. Growth factor signals to PLD activation involve the Ras-related protein RalA (58), which is constitutively associated with PLD1 (59). Of significance, RalA has also been implicated in amino acid-induced mTOR activation (60). Thus, the generation of PA by PLD has apparently been integrated into nutrient-induced mTOR activation. Consistent with this hypothesis, it was recently reported that the activation of mTOR with both amino acids and glucose is dependent on PLD (50, 51, 61). Thus, at least in mammalian cells, nutrient-induced signals that stimulate mTOR have utilized PLD-derived PA – indicating that PLD-generated PA contributes to nutrient sensing by mTORC1.

Phosphatidic acid and cancer

PLD activity is commonly elevated in human cancer and human cancer cells (10). Importantly, elevated PLD activity is essential for the survival of cancer cells (62–69). Cancer cells are particularly sensitive to suppression of PLD and mTOR activity in the

absence of serum (14). Intriguingly, there is a rapid increase in PLD activity in most human cancer cells, when serum is withdrawn (67–69). Thus, cancer cells may have co-opted PLD in order to generate PA to keep mTOR intact in the absence of exogenously supplied lipids. This would indicate that PLD could be a good therapeutic target for what is likely a large number of human cancers, that have elevated PLD activity. Interestingly, the plant compound honokiol, which suppresses PLD activity in cancer cells, only suppresses the PLD that is activated in response to the loss of serum lipids (69). Honokiol is highly toxic to cancer cells deprived of serum, while having minimal effects on normal cells (69, 70). Since PLD knockout mice are viable (22, 23), targeting PLD as an anti-cancer therapeutic approach might be less toxic than other approaches that target mTOR. There are currently several drugs that target both PLD1 and PLD2 specifically, and more are in development (71–73). Thus, it may be possible to target a non-essential cellular function to treat human cancers by inhibiting the elevated PLD activity observed in human cancers.

Concluding remarks

While the generation of PA from PLD seems critical for mTOR activity in cancer cells, and perhaps under other stressful conditions, clearly PLD is not essential for development. However, mTOR is essential and requires PA for maintaining the stability of the mTOR complexes. Thus, there must be other means for generating the PA needed to stabilize mTOR complexes and for mTOR kinase activity. Four mechanisms for doing this are described: 1) the acylation of G3P; 2) the phosphorylation of DG generated from triglycerides; 3) the phosphorylation of DG generated from PLC; and lastly 4) the generation of PA from DG by PERK in response to ER stress and the UPR. The first two mechanisms are central to membrane phospholipid synthesis and it is proposed that the PA requirement of TOR complexes represents a means for TOR to sense sufficient PA for membrane biosynthesis, cell growth, and division. The ability to generate PA by phospholipases, PLD and PLC, likely evolved as a means for multi-cellular organisms to generate PA in response to inter-cellular signals, such as insulin, that stimulate mTOR. Of significance, PLD activity, which is commonly activated by growth factors and insulin, is elevated in many human cancers and promotes survival in an mTOR-dependent manner. A significant role for elevated PA levels in cancer cells generated by DG kinase and LPAAT activity has not been observed. However, it is still possible that interfering with PA and membrane lipid synthesis could have an impact on the survival of cancer cells. Targeting FA synthesis, which generates FAs that contribute to the synthesis of PA, has been proposed as a promising clinical target for several human cancers (74). TOR functions as a nutrient sensor and is responsive to amino acids and glucose that are important for the synthesis of proteins and many other biological molecules needed for cel growth. Since PA is central to the metabolic pathways that generate membrane phospholipids, there is a strong rationale for why TOR is dependent on PA – namely to sense the presence of sufficient phospholipids for cell growth. The strong conservation of the PA-binding domain in TOR – from yeast to mammals – suggests that the PA requirement for TOR was a very early evolutionary event.

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Outstanding Questions

- Is there a PA requirement for TOR proteins from more primitive species?
- When did organisms start generating PA for mTOR activation via PLD? Was this an adaptation to multicellularity?
- Are there compensatory increases in alternative pathways for PA production in PLD null cells?
- Are there differences in the PA species generated via different enzymatic pathways and do different PA species impact differentially on mTORC1 and mTORC2?
- Does mTOR have an ability to respond to dietary “essential fatty acids”?
- To what extent do serum lipids contribute to mTOR activation?
- Are there lipid sensitive cell cycle checkpoints? And if so, are they dysregulated in human cancers?
- Can metabolic pathways leading to PA generation be targeted in human cancers?

Highights

- Phosphatidic acid (PA) is a central metabolite in membrane biosynthesis
- TOR/mTOR is a sensor of amino acids and glucose needed for cell growth
- PA is a critical regulator of mTOR and likely more primitive TOR species as well
- The PA requirement for TOR may represent a means for the sensing of lipids, which are also required for cell growth

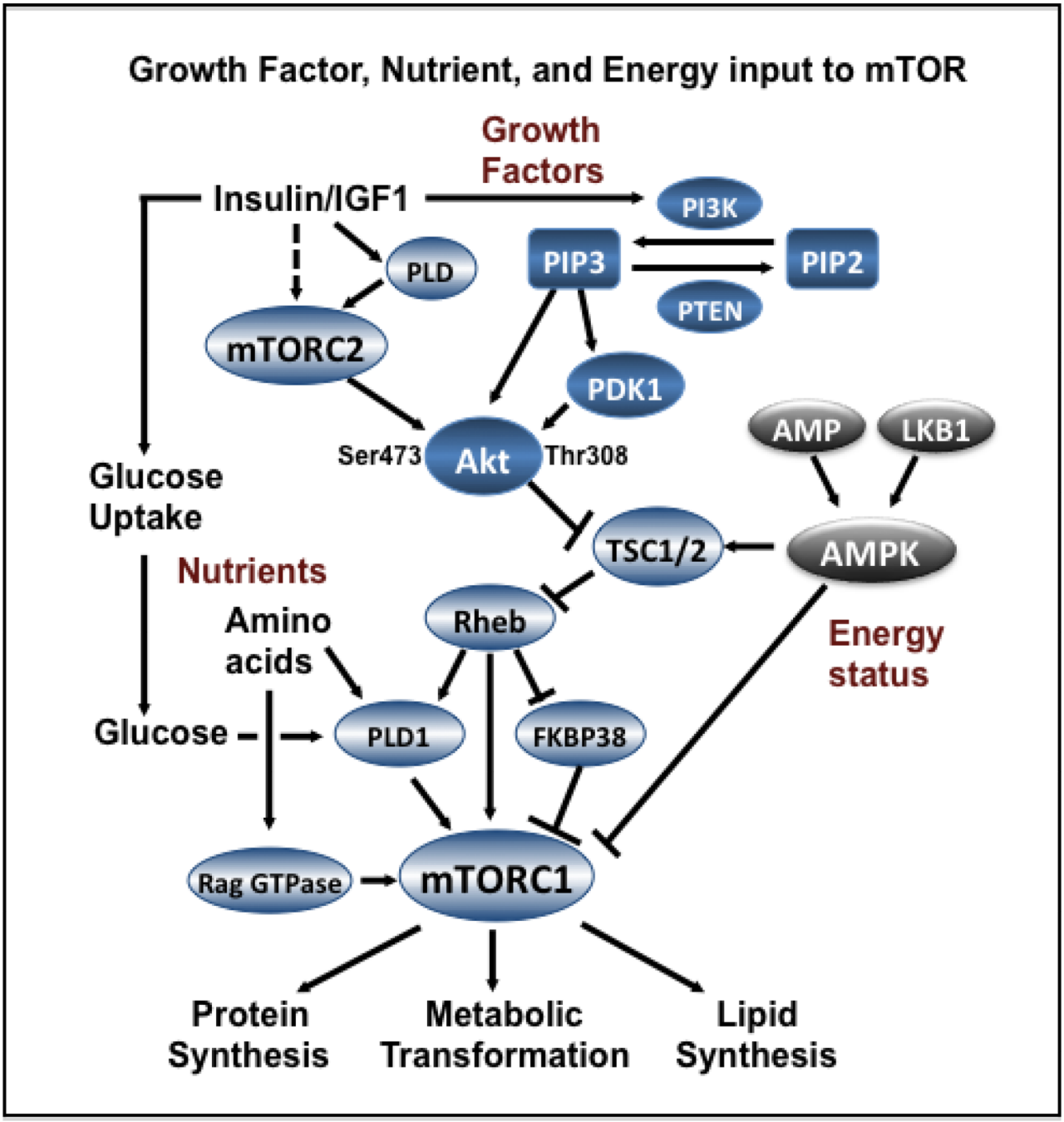

Figure 1. Nutrient signals to mTOR

Regulation of mTOR has many inputs. The PI3K input involves the generation of PIP3 from PIP2, which recruits and activates phosphoinositide-dependent kinase 1 (PDK1), which then phosphorylates Akt at Thr308. Subsequently, Akt phosphorylates and suppresses the GAP activity of the tuberous sclerosis complex (TSC) consisting of TSC1 and TSC2 (TSC1/2). Suppression of TSC1/2 results in elevated activation of the GTPase Rheb, which leads to a complex activation of mTORC1 via the activation of PLD1 and suppression of FKBP38 whereby elevated PLD activity generates the PA necessary for the formation of mTORC1 complex, and FKBP38 dissociates from mTORC1(49). This pathway is also impacted by AMPK, which in combination with the tumor suppressor LKB1 activates TSC1/2, which

suppresses Rheb and thus mTOR – under conditions where ATP levels are low and AMP levels are high. Akt is also phosphorylated by mTORC2 at Ser473 in response to insulin and insulin-like growth factor 1 (IGF1), in a PLD-dependent manner. Phosphorylation at this site has been correlated with altered substrate specificity and kinase activity for Akt. Insulin also increases the level of glucose transporters and increased uptake of glucose (18). A common theme in this complex signaling network leading to mTORC1 activation is that it is highly sensitive to the presence of glucose and amino acids – nutrients needed for cell growth.

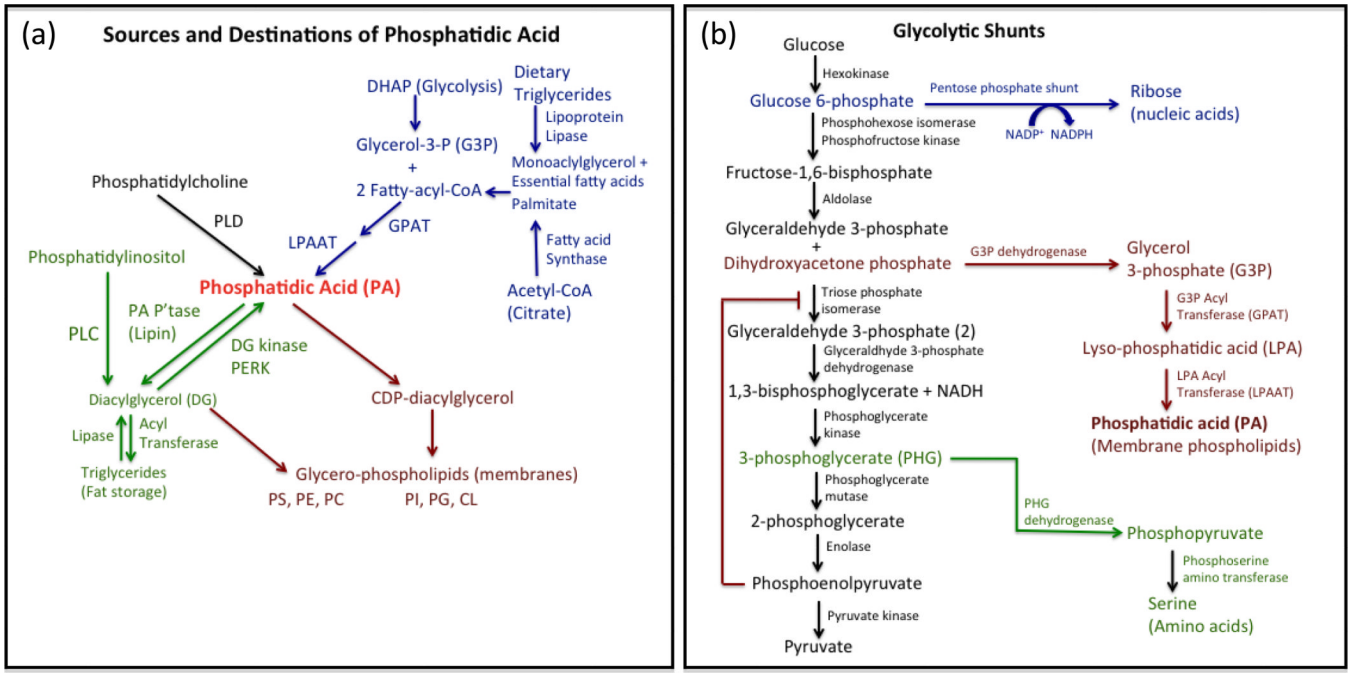

Figure 2. Phosphatidic acid metabolism

(a) PA can be generated by three major mechanisms – first by the *de novo* synthesis pathway that involves the acylation of G3P by GPAT and LPAAT (Blue). G3P is generated by the reduction of the glycolytic intermediate DHAP. The FAs that acylate G3P can be synthesized by FAS and then elongated and mono-desaturated in mammalian cells. However, dietary essential FAs are required in mammalian cells for the synthesis of poly-unsaturated FAs needed for the acylation of membrane phospholipids. The second pathway involves the phosphorylation of DG by DG kinases (Green). The DG required for this pathway must come from either deacylated triglyceride or PLC-generated DG derived primarily from phosphatidylinositol-4,5-trisphosphate. Thus, DG kinase can generate PA in response to growth factor induced PLC, or from stored lipids via triglyceride lipases. The third pathway involves the hydrolysis of phosphatidylcholine by PLD (Black). This pathway is not likely involved in the generation of PA for membrane biosynthesis since the PA is derived from a membrane phospholipid. The same is true for the PLC pathway that hydrolyzes phosphatidylinositol to generate DG. These pathways likely represent growth factor-dependent stimulation of PA production that occurs in the absence of membrane biosynthesis and is restricted to multicellular organisms. As indicated, PA is a substrate for the synthesis of phosphatidylinositol (PI), phsophatidylglycerol (PG) and cardiolipin (CL). DG generated by PA phosphatase (PA P'tase) is the substrate for the synthesis phosphatidylserine (PS), phosphatidylethanolamine (PE), and phosphatidylcholine (PC) (Red). **(b)** PA is generated from the glycolytic intermediate DHAP. Glycolysis represents the conversion of the 6-carbon glucose to two molecules of the 3-carbon pyruvate. The last step is catalyzed by pyruvate kinase (PK), which in dividing cells involves an embryonic isoform known as PKM2, which has a slower catalytic rate (41) that can be made even slower by tyrosine phosphorylation (42). The outcome of the reduced pyruvate kinase activity coupled with increased glucose uptake is the accumulation of glycolytic intermediates (43). The elevated level of glycolytic intermediates is the conversion to molecules for anabolic synthesis of biological molecules that are needed to double the mass of a dividing cell. The most understood utilization of a glycolytic intermediate is the pentose phosphate shunt, which generates ribose that can be utilized in the synthesis of nucleic acids (Blue). The pentose phosphate shunt also leads to the generation of NADPH that can be

utilized in anabolic reactions – especially FA synthesis. Amino acids, most notably serine, are generated from 3-phosphoglycerate (PHG) via the conversion to phosphopyruvate by PHG dehydrogenase and then to serine by phosphoserine amino transferase (Green). Glycerol-3-phosphate, the substrate for the acyltransferases that generate PA, is generated from DHAP by reduction to G3P by G3P dehydrogenase (Red). Of interest, the enzyme that converts DHAP to glyceraldhyde-3-phosphate, triose phosphate isomerase, is suppressed by phosphoenolpyruvate (45).

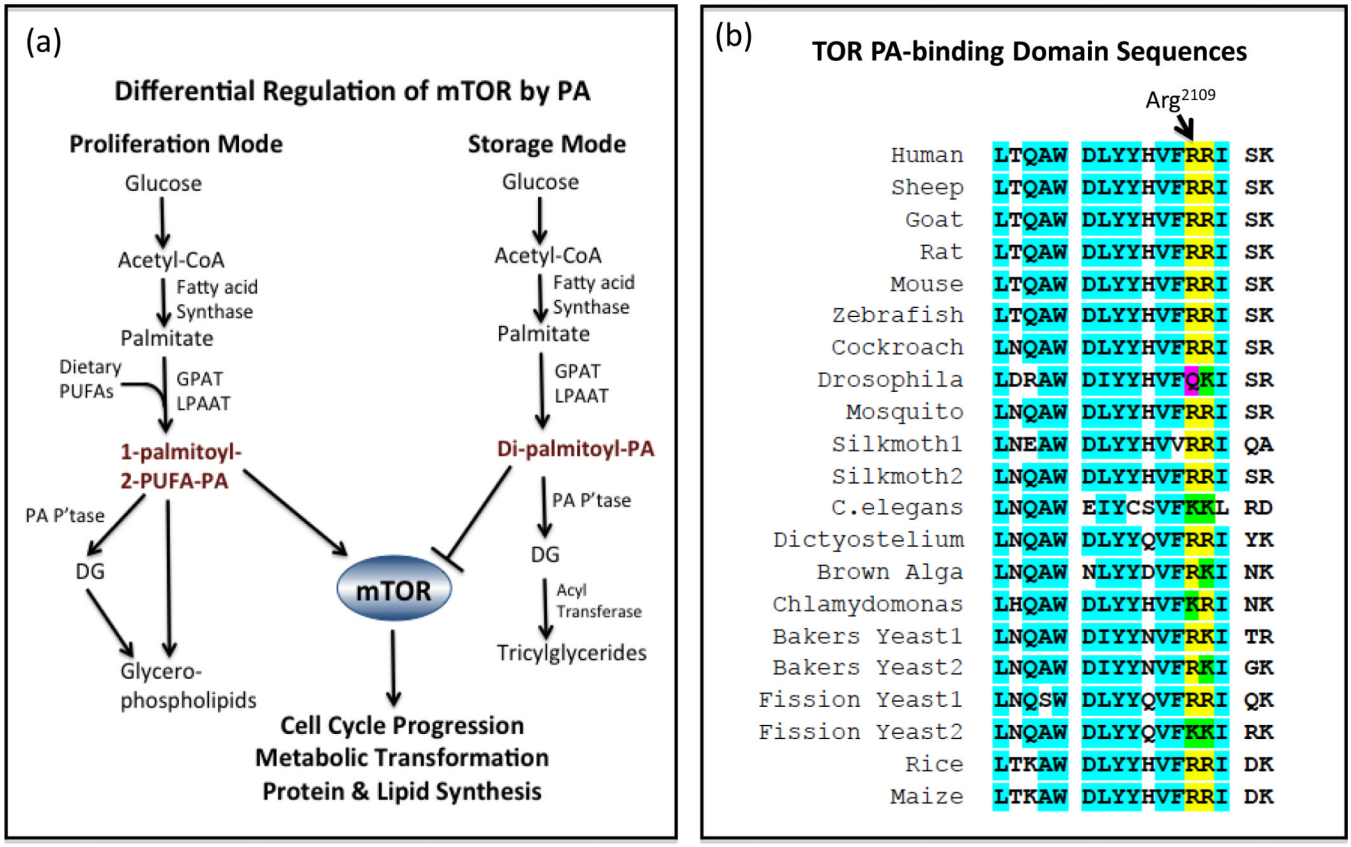

Figure 3. Regulation of TOR by phosphatidic acid

(a) There are two major destinations for newly synthesized PA – membrane phospholipids when cells are in a proliferative mode, and triglycerides when cells are in a storage mode. In storage mode it is postulated that there is more newly synthesized saturated palmitic acid used for triglyceride synthesis; whereas in proliferation mode, there are more elongated and desaturated FAs utilized to maintain membrane fluidity and provide poly-unsaturated FAs (PUFAs) for eicosanoid synthesis. PA with two saturated FAs was reported to suppress mTORC2 (48). Thus, there appears to a mechanism whereby mTOR can distinguish between PA directed towards membrane biosynthesis and PA directed to triglyceride storage. **(b)** The PA-binding domain of TOR is within the region of TOR that also binds rapamycin (5, 52). This sequence contains a critical Arg residue at position 2109 (human sequence), critical for PA binding (5). There is also a conserved positively charged amino acid at the adjacent position at 2110, and highly conserved regions flanking these two positive charges all the way from yeast to humans. Sequence number is relative to Arg 2109 of the human sequence. Many of these sequences were assembled previously (53).