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The emerging role of mTORC1 signalling in placental nutrient-sensing

Thomas Jansson¹, Irving LMH Aye¹, and Deborah CI Goberdhan²

¹Center for Pregnancy and Newborn Research, Department of OB/GYN, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900, USA

²University of Oxford, Department of Physiology, Anatomy and Genetics, South Parks Road, Oxford, OX1 3QX, UK

Abstract

Nutrient-sensing signalling pathways regulate cell metabolism and growth in response to altered nutrient levels and growth factor signalling. Because trophoblast cell metabolism and associated signalling influence fetal nutrient availability, trophoblast nutrient sensors may have a unique role in regulating fetal growth. We review data in support of a role for mammalian target of rapamycin complex 1 (mTORC1) in placental nutrient-sensing. Placental insulin/IGF-I signalling and fetal levels of oxygen, glucose and amino acids (AAs) are altered in pregnancy complications such as intrauterine growth restriction, and all these factors are well-established upstream regulators of mTORC1. Furthermore, mTORC1 is a positive regulator of placental AA transporters, suggesting that trophoblast mTORC1 modulates AA transfer across the placenta. In addition, placental mTORC1 signalling is also known to be modulated in pregnancy complications associated with altered fetal growth and in animal models in which maternal nutrient availability has been altered experimentally. Recently, significant progress has been made in identifying the molecular mechanisms by which mTORC1 senses AAs, a process requiring shuttling of mTOR to late endosomal and lysosomal compartments (LELs). We recently identified members of the proton-assisted amino acid transporter (PAT/SLC36) family as critical components of the AA-sensing system or ‘nutrisome’ that regulates mTORC1 on LEL membranes, placing AA transporters and their subcellular regulation both upstream and downstream of mTORC1-driven processes. We propose a model in which placental mTORC1 signalling constitutes a critical link between maternal nutrient availability and fetal growth, thereby influencing the long-term health of the fetus.

Keywords

mammalian target of rapamycin; fetus; trophoblast; fetal growth; maternal-fetal exchange; proton-assisted amino acid transporters; amino acids

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Corresponding author: Thomas Jansson, Center for Pregnancy and Newborn Research, Department of Obstetrics and Gynecology, University of Texas Health Science Center San Antonio, Mail Code 7836, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900, Telephone: 210 567 7043, Fax: 210 567 1001.

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1. Introduction

In single cell organisms, such as yeast, nutrient-sensing signalling pathways up-regulate nutrient uptake and metabolism in response to increased levels of intracellular nutrients or their metabolites. Conversely, when subjected to nutrient deprivation these nutrient sensors cause down-regulation of membrane nutrient transporter expression and inhibition of cell metabolism, providing a direct link between nutrient availability and cell growth (1, 2). In contrast, in mammalian cells nutrient uptake, metabolism and growth have long been believed to be controlled primarily by growth factors. However, in the past 10–15 years it has become increasingly clear that mammalian cells have an array of additional nutrient-sensing signalling pathways, such as AMP-activated protein kinase (AMPK), mammalian target of rapamycin complex 1 (mTORC1) and the hexosamine signalling pathway, which regulate cell metabolism in response to altered nutrient levels. In general, these nutrient sensors promote an anabolic phenotype when nutrient availability is abundant and a catabolic phenotype in times of nutrient deprivation. Mammalian nutrient-sensing pathways operate both independently of, and in concert with, growth factor signalling. While these nutrient-sensing signalling pathways are relatively well characterized, in many cases the molecular identity of the actual sensor remains elusive (1).

In the human term placenta only two cell layers separate the maternal and the fetal circulations, the syncytiotrophoblast cells, which are in direct contact with maternal blood, and the fetal capillary endothelium. The syncytiotrophoblast, the transporting epithelium and the primary endocrine cell of the human placenta, plays a critical role in determining fetal growth and development, mediated by a multitude of functions including nutrient transport to the fetus and orchestrating the maternal physiological adaptation to pregnancy. Thus, nutrient-sensing in the trophoblast is unique in that it not only directly regulates trophoblast metabolism and growth but may also influence the development and long-term health of the fetus. In this review, we initially provide a brief overview of trophoblast signalling pathways, which may function as placental nutrient sensors. Subsequently, we focus on mTORC1 because there is significant evidence for a role for this signalling pathway in placental nutrient-sensing. First, we argue that mTORC1 is regulated by a large number of upstream signals, which are likely to be altered in association to common pregnancy complications. Second, we discuss the evidence that mTORC1 is a powerful positive regulator of key placental nutrient transporters. Third, we review data demonstrating that placental mTORC1 activity changes in relation to maternal nutrient availability. Finally, we discuss exciting new data on the molecular mechanisms of AA-sensing, its impact on the mTORC1 signalling pathway and the possibility that placental mTORC1 signalling directly influences fetal metabolism.

2. Placental nutrient sensors

Because an extensive discussion of all potential placental nutrient sensors is beyond the scope of this brief review, we will focus on some important examples. In most instances, the activity of nutrient-sensing pathways in the placenta has been assessed in placental homogenates whereas studies in cultured trophoblast cells have so far been uncommon. The **amino acid response (AAR) signal transduction pathway** is activated by limitation or imbalance of essential amino acids (EAAs) (3). In these situations, there is an increase of uncharged tRNA species, which bind to general control non-derepressible 2 (GCN2) kinase. As a result, the translation initiation factor eIF2 α is phosphorylated, which leads to inhibition of global translation, but increased translation of activating transcription factor (ATF) 4. ATF4 increases the expression of a small group of target genes involved in transport, metabolism, and oxidative stress (3). The placental AAR pathway has been reported to be activated in a rat model of maternal protein restriction (4). Furthermore,

eIF2 α phosphorylation is increased in IUGR placentas (5)3 however, whether this is mediated by GCN2 kinase or by activation of protein kinase-like endoplasmic reticulum kinase (PERK) in the unfolded protein response (UPR) pathway (5) is unknown.

AMPK is well known as the global energy sensor of the cell (6). In response to rising AMP levels, which signal a depletion of ATP, AMPK maintains cellular energy balance by switching off ATP-consuming anabolic pathways including protein synthesis and switching on ATP-generating catabolic pathways such as increasing glucose uptake and fatty acid oxidation, mediated by phosphorylation of downstream targets. Placental AMPK phosphorylation has been shown to be increased by mid-gestation in sheep subjected to 50% calorie restriction (7) and decreased in placentomes of over-nourished, obese pregnant ewes (8). In contrast, placental AMPK activity was unaltered in response to maternal protein restriction in the rat (9), consistent with the possibility that a low protein diet does not cause extensive placental energy deprivation.

Glycogen synthase-3 (GSK-3) is inhibited by insulin/IGF-I/Akt signalling (10). In addition, GSK-3 has recently been shown to function as a glucose sensor (11). Consistent with this concept, GSK-3 inhibition in response to increased glucose metabolism leads to stabilization of antiapoptotic proteins of the Bcl-2 family, which inhibit apoptosis. Interestingly, placental GSK-3 is activated in pregnancies complicated by intrauterine growth restriction (IUGR) (5), but whether these changes are the result of impaired insulin/IGF-I signalling (5, 12, 13) or caused by altered placental glucose availability remain to be established. The nutrient-sensing **hexosamine signalling pathway** modulates the levels of O-linked N-acetylglucosamine (O-GlcNAc) on key targets, which influences cellular signalling, protein turnover and gene expression (14). The activity of this signalling pathway is dependent on the availability of nutrients such as glucose, glutamine and acetyl-CoA, and on enzymes that add (O-GlcNAc transferase) and remove (O-GlcNAcase) O-GlcNAc, respectively. The hexosamine signalling pathway is believed to be a versatile cellular regulator modulating numerous cellular signalling cascades influencing growth, metabolism and cellular stress. Preliminary studies have demonstrated that the hexosamine signalling pathway is active in first trimester human placenta and influences hormone production and IGF signalling (15).

3. mTORC1 signalling

mTOR is a ubiquitously expressed serine/threonine kinase that exists as two complexes with distinct regulation and function (16, 17). One key difference between the two complexes is the association of mTOR with the accessory protein raptor in mTOR Complex 1 (mTORC1) and with rictor in mTORC2. mTORC2 phosphorylates Akt at Serine-473, protein kinase C α (PKC α) and serum/glucocorticoid regulated kinase 1 (SGK1) and is not believed to be directly involved in nutrient-sensing (18, 19). In contrast, mTORC1 is located in the center of a nutrient-sensing network that controls cellular metabolism and growth (16, 17). mTORC1 is stimulated by a wide array of upstream signals including AAs, glucose, oxygen, cytokines, growth factors and energy levels, many of which impinge on the Ras-related small G-protein Rheb and/or tuberous sclerosis complex 2 (TSC2), which lie directly upstream of mTORC1. When Rheb is present in its GTP-bound form it activates mTORC1. The heterodimeric complex of TSC1 and TSC2 stimulates the conversion of Rheb to its GDP-bound form thereby inhibiting mTORC1 (20). Growth factors, such as insulin and IGF-I, and cytokines activate mTORC1 by inhibiting TSC2. Thus, mTORC1 activity is controlled directly by cellular nutrient and energy levels and indirectly by systemic nutrient availability and metabolism mediated by hormones and growth factors (Fig 1).

mTORC1 is a master regulator of the translational machinery, and has been believed to activate protein synthesis via several downstream targets including ribosomal S6 kinases

(S6K1 and S6K2), eukaryotic initiation factor 4E binding proteins (4E-BP1 and 4E-BP2) and eukaryotic initiation factor 4G (eIF4G) (21, 22). Recent high-resolution transcriptome-scale ribosome profiling following mTOR inhibition, however, suggests that mTORC1 regulates mRNA translation largely mediated by the 4E-BPs (23) (Fig 1). It should be noted that mTORC1 is not the sole regulator of 4E-BP1, which also can be phosphorylated by extracellular signal-regulated kinase 1/2 (ERK1/2) (24). Whereas mTORC1 has a modest influence on the translation of all mRNAs, this signalling pathway has a marked effect on the translation of mRNAs with a 5' tract of oligopyrimidines (5'-TOP), which predominantly are mRNAs encoding for cytoplasmic ribosomal proteins and other proteins involved in protein synthesis (23). Thus, mTORC1 signalling has a general effect on protein synthesis, affecting overall translation rates. In contrast, the translation of subgroups of specific mRNAs containing motifs such as internal ribosome entry site elements (IRES) and the CCAAT-enhancer binding protein-activating transcription factor (C/EBP-ATF) responsive element (CARE) are regulated in response to nutrient deprivation in a mTORC1 independent manner (21, 25). The mTORC1 signalling pathway also affects the transcription of genes involved in AA, lipid and nucleotide metabolism, protein synthesis, and immune modulation (26, 27). This effect may, in part, be mediated by increased translation of the transcription factor hypoxia-inducible factor-1 α (HIF-1 α) (28).

There is an extensive interaction between nutrient-sensing pathways, ensuring coordinated responses (Fig 1). For example, in response to energy depletion AMPK inhibits mTORC1 indirectly by phosphorylation of TSC2 and directly by phosphorylation of raptor (29). GCN2 signalling also modulates mTORC1 because phosphorylation of eIF2 α by GCN2 in response to decreasing levels of charged tRNAs inhibits mTORC1 signalling through the induction of regulated in development and DNA damage response 1 (REDD1), thereby coordinating the responses in the two pathways (30, 31). GSK-3 inhibits the mTORC1 pathway by phosphorylating TSC2 in a manner dependent on AMPK-priming phosphorylation (32). Furthermore, emerging evidence suggests that the hexosamine signalling pathway influence the translational machinery at many different levels (33). Of these nutrient sensing pathways some, such as AMPK, AAR/GCN2 and GSK-3 signalling, respond to limited numbers of stimuli, whereas others, in particular mTORC1 and the hexosamine signalling pathway, have a broader range of upstream regulators. It is possible that mTORC1 may be of particular importance for placental nutrient sensing because this signalling pathway (1) has an extraordinary versatility to sense local nutrient, energy and oxygen levels, (2) has the ability to monitor whole organism nutrition via growth factor signalling and (3) receives extensive information on the activity of other nutrient sensing pathways.

4. Evidence supporting a role for mTORC1 in placental nutrient-sensing

mTOR is highly expressed in the syncytiotrophoblast of the human placenta (34) and mTORC1 in cultured primary human trophoblast cells is regulated by glucose and AA concentrations as well as by growth factor signalling (35). Placental insulin/IGF-I signalling is inhibited in IUGR (5, 12, 13) and the IUGR fetus is often hypoxemic and hypoglycaemic and has low circulating levels of EAAs (36, 37), consistent with the possibility that nutrient and oxygen levels are also low in the trophoblast. It is predicted that these changes would inhibit placental mTORC1 activity in IUGR. Indeed, the majority of cases of idiopathic normotensive IUGR have been reported to have placental hypoxic-ischemic histological lesions (38) consistent with placental hypoxia. In hypoxia, mitochondrial respiration is impaired, leading to low ATP levels and activation of AMPK, which inhibits mTORC1. Furthermore, hypoxia also affects mTORC1 in AMPK-independent ways by inducing the expression of REDD1, which inhibits mTORC1 by promoting the assembly of TSC1–TSC2 (Fig 1) (39). On the other hand, we speculate that diabetes with suboptimal control may be

associated with increased placental ATP and nutrient levels, which would tend to activate mTORC1 signalling. Thus, local levels of nutrients and growth factors, well-established stimuli of mTORC1, are almost certainly altered in the placenta in association with common pregnancy complications such as IUGR.

Nutrient-sensing signalling pathways regulate cell function and growth in response to changes in local and systemic nutrient availability. Nutrient transport is a primary function of the syncytiotrophoblast and nutrient uptake by this cell not only determines placental growth but also has a major influence on fetal nutrient availability and growth. Using primary villous explants and cultured primary human trophoblast cells we reported that mTORC1 is a positive regulator of the System A and System L AA transporters, which are critical for the transport of EAAs to the fetus (34, 40). Unexpectedly, mTORC1 regulation of AA transporters occurs predominantly at the post translational level by affecting the plasma membrane trafficking of specific transporter isoforms and preliminary data suggest that Nedd4-2 regulated ubiquitination may be involved (41, 42). Specifically, inhibition of placental mTORC1 decreases while mTORC1 activation increases the cell surface abundance of AA transporters in the trophoblast. It is therefore possible that trophoblast mTORC1 signalling links maternal nutrient availability to fetal growth by modulating the flux of AAs across the placenta.

Placental mTORC1 signalling is altered in pregnancy complications associated with abnormal fetal growth and in animal models where maternal nutrient availability has been altered experimentally. In human IUGR, placental mTORC1 activity is inhibited (5, 34) and preliminary studies indicate an activation of placental mTORC1 signalling in association with maternal obesity (43, 44). Furthermore, placental mTORC1 activity has been reported to be decreased in hyperthermia-induced IUGR in the sheep (45), in response to a maternal low protein diet in the rat (9) and maternal calorie restriction in the baboon (46). It is well established that circulating adiponectin is inversely correlated to BMI, thereby representing an important nutritional cue carrying information on the maternal nutritional status. Interestingly, maternal adiponectin inhibits placental mTORC1 signalling, which may represent an endocrine link between maternal adipose tissue, placental function and fetal growth (47). Collectively, this emerging evidence is consistent with an important role for mTORC1 in placental nutrient sensing. Therefore, understanding the mechanisms by which this sensing occurs may help identify novel approaches to modulate placental function in pregnancy complications.

5. The nature of the intracellular amino acid sensing mechanism and the role of PAT1

The level of extracellular AAs is one of the key inputs affecting mTORC1 activity. mTORC1 is particularly sensitive to changes in the levels of specific AAs, such as leucine (48). Indeed the leucine-bound leucyl-tRNA synthetase has recently been shown to act as a leucine sensor for mTORC1 activation, although as discussed below, this does not provide the only mechanism by which AAs are sensed and may act more as a permissive signal under normal cellular condition (49, 50). Our understanding of the sensing mechanisms that link overall levels of extracellular AAs to mTORC1 activity has advanced rapidly in recent years, driven by a combination of studies in human cell lines and *in vivo* analysis in the fruit *Drosophila melanogaster* fly. Several candidate mediators of the AA signal have been reported (reviewed in (51)).

The identification of the proton-assisted amino acid transporter (PAT/SLC36) family in a genetic screen in flies for cell growth regulators (52) was particularly unexpected, because PAT1 had previously been highlighted as a lysosomal AA transporters (LYAAT1; reviewed

in (53)). This finding was the forerunner for a series of studies in cultured human cells that identified the late endosomal and lysosomal compartments (LELs) as the site of mTORC1 regulation. Experiments in human embryonic kidney-293 (HEK-293) cells have shown that PAT1 and PAT4, the two ubiquitously expressed PATs, which are located on intracellular membranes in these cells, are required for AA-dependent mTORC1-mediated signalling and cell proliferation (54). Stimulation of HEK-293 cells with AAs shuttles mTOR to LELs and this event is required for activation of the mTORC1 signalling cascade (55). Studies from the Sabatini group have highlighted a critical role for components of a multiprotein complex located on LELs that interacts with mTOR under AA-stimulated conditions and is required to assemble active mTORC1. This includes a heterodimeric pair of Rag GTPases, which in mammals consists of RagA or RagB associated with RagC or RagD (55) and which in *Drosophila* has been shown to promote growth (56). In addition, a trimeric complex of proteins, MP1, p14 and p18, which have been linked to growth modulation in humans and mice, referred to as the 'Ragulator', links the AA-sensing complex to the lysosomal membrane lipid bilayer (57). PAT1 can also physically interact with RagC and RagD (58) and is required for recruitment of mTOR to the LEL membrane.

The surprising focus on the LELs as the location of AA-sensing has recently been explained by the observation that extracellular AAs rapidly accumulate within LELs from where they appear to mediate mTORC1 activation (59). The vacuolar H⁺-ATPase (v-ATPase) has also been highlighted as an important component of this complex (59) and it seems very likely that transport coupling with PAT1 in a multiprotein engine that we call the 'nutrisome', underlies the AA-sensing processes that activate mTORC1 on LELs (Fig 2). Interestingly, these discoveries have provided an additional explanation for the role of insulin/IGF-I/Akt signalling in mTORC1 regulation, since at least in *Drosophila*, this signalling cascade promotes the shuttling of PATs to the LELs to increase nutrisomal assembly (58).

While the work described above on AA sensing and mTORC1 control was primarily undertaken in immortalized cell lines, because all cells require mTORC1 for growth it is likely that trophoblast cells employ similar mechanisms to regulate mTORC1. The interesting conclusion from this is that while some AA transporters are important targets for mTORC1 signalling, others are key regulators of mTORC1. It will now be of particular interest to examine whether other putative intracellular AA transporters could ferry AAs into the lumen of LELs, and how these transporters are affected in IUGR.

6. Remote control of fetal metabolism by placental mTOR?

Trophoblast mTOR signalling can influence fetal growth and metabolism indirectly by regulating trophoblast AA transporter activity (34, 40). Inhibition of the TOR signalling pathway in the fat body of *Drosophila* influences whole body growth mediated by the release of a humoral factor that regulates the secretion of insulin-like peptides from the brain (60). By analogy to the role of TOR in the fat body of *Drosophila*, we hypothesized that human placental mTOR signalling regulates the release of a circulating factor that modulates metabolism in the fetal liver and endocrine pancreas (Fig 3). In a preliminary report we showed that conditioned media from cultured primary human trophoblast cells, in which mTORC1 or mTORC2 were silenced using siRNA, modulates the secretion and phosphorylation of IGFBP-I in HepG2 liver cells and the glucose-stimulated insulin secretion in a rat β -cell line (61). These results are consistent with the possibility that placental mTORC1 signalling not only controls nutrient transport across the placenta, but also regulates the release of circulating factor(s) that influence fetal metabolism and growth.

7. Conclusions and future perspectives

We have reviewed evidence implicating mTORC1 signalling as an important placental nutrient sensor. First, placental insulin/IGF-I signalling and levels of oxygen, glucose and AAs are altered in pregnancy complications such as IUGR, and all these factors are well-established upstream regulators of mTORC1. Second, mTORC1 is a positive regulator of the placental AA transporters, suggesting that trophoblast mTORC1 modulates AA transfer across the placenta. Third, placental mTORC1 is regulated in pregnancy complications associated with altered fetal growth and in animal models in which maternal nutrient availability has been altered experimentally. We propose that trophoblast mTORC1 signalling constitutes a critical link between the ability of the maternal supply line to provide oxygen and nutrients to the placenta and fetal growth, thereby influencing the long-term health of the fetus (Fig 3). The mechanisms underlying this response may include mTORC1 regulation of placental growth and function, such as AA transport. The ability of the maternal supply line to deliver nutrients and oxygen could be decreased in situations such as reduced uteroplacental blood flow and maternal under-nutrition and increased in pregnancies complicated by maternal diabetes.

Despite this emerging evidence, our understanding of placental nutrient-sensing remains limited. The data in support of mTORC1 as a placental nutrient sensor is based mostly on associations rather than cause-and-effect experiments, which are of high priority for future research. In particular, mechanistic approaches in intact animals are urgently needed. Considering the recent reports demonstrating that PAT1 constitutes a critical component of the mTORC1 AA sensing system in cell lines, it is of considerable interest to explore the role of these molecules in trophoblast AA sensing. The possibility that trophoblast mTORC1 regulates fetal metabolism by 'remote control', via the secretion of circulating factors, warrants further study. Importantly, exploring the role of other nutrient-sensitive signalling pathways in the regulation of placental growth and function may prove fruitful for future research.

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Figure 1. Signalling mechanisms of mTORC1 and other pathways involved in placental nutrient-sensing

Insulin/IGF-I phosphorylates Akt, which inhibits TSC2, thereby releasing the inhibition of Rheb by the heterodimeric TSC1/TSC2 complex. Activated Rheb then stimulates mTORC1 signalling. AMPK inhibits mTORC1 signalling by activating TSC2 and inhibiting Raptor. Rag heterodimerisation also acts immediately upstream of mTORC1 to stimulate signalling and amino acids influence mTORC1 signalling via a sensor mechanism upstream of Rag heterodimers (see Fig 2). The net effect of mTORC1 activation is phosphorylation of ribosomal S6K1/2 and the dissociation of eIF4E from 4E-BP1/2, which stimulates protein synthesis. Overall, mTORC1 activation stimulates cell proliferation and growth and promotes an anabolic metabolic phenotype. Low oxygen inhibits mTORC1 signalling. In response to amino acid deprivation there is an increase of uncharged tRNA species, which become bound by GCN2. As a result, the translation initiation factor eIF2α is phosphorylated, which leads to inhibition of global translation, but increased translation of ATF4.

ATF 4. **Abbreviations:** **mTORC1** and **mTORC2**, mammalian target of rapamycin complex 1 and 2; **TSC1/2**, tuberous sclerosis complex 1/2; **GSK-3**, Glycogen synthase kinase 3; **Rheb**, Ras homolog enriched in brain; **Rag**, Rag GTPase heterodimer; **AMPK**, AMP-activated protein kinase; **S6K1/2**, S6 kinase 1/2; **4E-BP1/2**, eukaryotic initiation factor 4E-binding protein 1/2, **GCN2**, general control non-derepressible 2 kinase; **eIF2 α** , eukaryotic initiation factor 2 α ; **REDD1**, regulated in development and DNA damage response 1; **ATF4**, activating transcription factor 4.

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Figure 2. Amino acid sensing and mTORC1 signalling from the inside – an emerging mechanism
Recent data suggests a model in which accumulation of intracellular AA in the lumen of late endosome and lysosomal compartments (LEL), mediated by currently unknown AA transporters, is 'sensed' by a protein complex on the membranes of LEL. This complex, which we call the 'nutrisome', contains PAT1, Rag, the Ragulator (a trimeric group of proteins involved in attaching the complex to the lipid bilayer), mTORC1 and the v-ATPase. Downstream signalling events result in protein synthesis and cellular growth.
Abbreviations: aminoAA, acids; AAT(s), amino acid transporter(s); mTORC1, mammalian target of rapamycin complex 1; Rag, Rag GTPase heterodimer; PAT1, Proton-Assisted Amino Acid Transporter 1; v-ATPase, vacuolar H⁺-ATPase.

Figure 3. Placental mTORC1 linking maternal nutrient availability to fetal growth

Placental mTORC1 signalling is at the nexus between maternal and fetal environments integrating nutrient availability from the mother to coordinate appropriate fetal growth. Nutritional cues from the maternal environment govern mTORC1 signalling, which influences placental size and modulate fetal growth by regulating placental nutrient transporter function. Preliminary evidence also indicates that mTORC1 signalling results in the release of placental humoral factor(s), which affect metabolism in target fetal tissues.