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Minireview

Amino Acid Transport Regulates Blastocyst Implantation[1]

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ABSTRACT

Mouse blastocyst outgrowth in vitro and probably implantation in vivo require amino acid signaling via the target of rapamycin (TOR) pathway. This signaling does not simply support protein synthesis and trophoblast differentiation. Rather, it regulates development of trophoblast protrusive activity and may act as a developmental checkpoint for implantation. Moreover, intracellular amino acids per se are insufficient to elicit TOR signaling. Instead, de novo transport of amino acids, and particularly of leucine, stimulate mTOR activity at the blastocyst stage. The activity of the broad-scope and yet leucine-selective amino acid transport system $B^{0,+}$ could produce such increases in intracellular amino acid concentrations. For example, system $B^{0,+}$ uses a Na^+ gradient to drive amino acid uptake, and the Na^+ concentration in uterine secretions increases by nearly twofold about 18 h before implantation. The resultant mTOR signaling could trigger polyamine, insulin-like growth factor II, and nitric oxide production in blastocysts and the increased cell motility sometimes associated with synthesis of these bioactive molecules.

embryo, implantation, nitric oxide, placenta, trophoblast

INTRODUCTION

At the time of implantation, the mammalian embryo has the form of a hollow sphere, the blastocyst, with a squamous cell layer at the periphery and a mass of cells inside the periphery at one end. The enclosed cells are the inner cell mass (ICM) and the squamous cells constitute the trophectoderm (TE). These two cell populations are the founder populations for embryo and placenta; the ICM gives rise to the embryo proper, and the TE forms placental tissues. In many mammalian species, including mice and humans, the TE initiates invasion by the embryo into the uterus, which anchors the embryo and eventually provides its links to the maternal circulation. Initiation of this implantation process is interesting from two additional perspectives; it involves regulated changes in cell behavior also seen in other embryonic events and it resembles, in many ways, the process by which cancer cells become metastatic.

Mammalian implantation is regulated by factors both intrinsic and extrinsic to the embryo. Intrinsic changes in gene expression regulate the lineage commitment of the TE cell population and direct their subsequent differentiation, while extrinsic signals from the uterus modulate the timing and rate of TE differentiation to coordinate the progress of implantation between embryo and uterus. The mechanisms, both intrinsic and extrinsic, that underlie the transition of epithelial TE cells to invasive trophoblast are thus the key regulators of the embryo's ability to implant. In this review, we discuss these differentiation events and focus particularly on the extrinsic regulatory functions of exogenous amino acids.

Trophoblast Protrusive Activity and Blastocyst Implantation

To penetrate the uterine epithelium, the TE cells must change their form from that of a squamous epithelium to a more motile, mesenchymal cell type. In vivo, the switch from epithelial to motile cells is manifested at E4.5 by the formation of trophoblast cell protrusions that penetrate between the uterine epithelial cells to surround and engulf those that have undergone apoptosis. A similar change in behavior can be induced in vitro by placing blastocysts on substrates of extracellular matrix (ECM), where the TE cells will attach and spread in a monolayer surrounding the ICM. When preimplantation mouse embryos are cultured in vitro after induction of ovulation using hCG, trophoblast cells acquire the ability to form outgrowths between the early blastocyst (120-h post-hCG) and late blastocyst (168-h post-hCG) stages.

This switch is characterized by changes in TE cell motility, apical membrane composition, and cell-cell adhesion [1, 2]. Changes in TE apical membrane composition and in cell-cell adhesion allow the blastocyst to attach to the apical surface of the uterine epithelium, while changes in motility permit protrusion formation for invasion. When analyzed in vitro, changes in apical membrane composition are observed as the progressive ability of fibronectin to stimulate adhesion of the TE to the ECM as blastocyst development proceeds [3–5]. At 120 h post-hCG, fibronectin has no effect on TE adhesivity, while at 168 h post-hCG, it stimulates strong ECM adhesivity. TE cell motility is concomitantly regulated, as observed by time-lapse video record-

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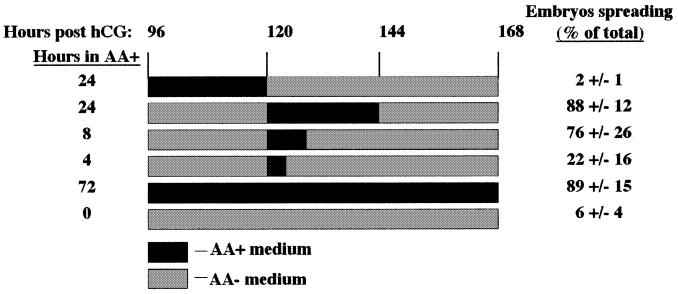

FIG. 1. Time requirement for amino acid-induced mouse trophoblast spreading. Embryos were exposed at the indicated times post-hCG to amino acid-containing medium (AA+), then rinsed and further cultured in medium lacking amino acids (AA−) after the indicated number of hours.

ings of blastocysts between 120 and 168 h post-hCG [2, 6]. At 120 h, the TE cells are nonmotile, but by 144 h, the quiescent TE cells exhibit a low rate of protrusive activity that increases in frequency to 168 h post-hCG and results in outgrowth if the blastocysts are cultured on an appropriate substrate. Changes in cell-cell adhesion become evident late, with collapse of the blastocoele cavity and retraction of TE cells to the ICM pole of the blastocyst [2, 7]. From these observations, it appears that changes in motility and apical membrane composition are coordinately regulated and required for outgrowth, while changes in cell-cell adhesion may either occur later than, or result from, changes in apical membrane composition and motility.

The changes in TE cell apical membrane composition, motility, and cell-cell adhesion are observed in vivo as well as in vitro and are crucial for the success of implantation and placentation. Changes in apical membrane composition are evident in the attachment of TE cells through their apical domains to the apices of the uterine epithelial cells. In addition, integrin receptors are relocalized from basal to apical domains as the TE cells extend protrusions [8]. These changes permit both initial attachment to the epithelium and subsequent adhesion to the underlying ECM during invasion. The onset of TE cell motility results in protrusion formation, trophoblast cell spreading on the uterine epithelial basement membrane, and engulfment of apoptotic uterine epithelial and decidual cells [9–11].

The motility changes that facilitate penetration of the blastocyst through the uterine epithelium are also critical for subsequent remodeling of the implantation chamber. The trophoblast cells that form the yolk sac placenta are highly phagocytic, and by engulfing apoptotic decidual cells, they increase the volume of the implantation chamber, allowing for growth of the embryo. Without this phagocytic activity, embryonic growth is restricted and morphogenesis is abnormal (Aeder and Sutherland, unpublished data).

Several observations have documented changes in trophoblast cell-cell adhesion during implantation in vivo. There are changes in expression of tight and adherens junction proteins, and the appearance of gaps in the layer of trophoblast cells that remain in contact with the underlying basement membrane (Reichert membrane) [10–13]. The increased permeability of the trophoblast cell layer allows diffusion of oxygen and nutrients to the embryo.

Development of Trophoblast Protrusive Activity Requires Amino Acid-Dependent Mammalian Target of Rapamycin Signaling in Early Blastocysts

Blastocyst outgrowth is profoundly affected by environmental factors and, in particular, by the availability of exogenously provided amino acids [2, 14–16]. For example, in the absence of leucine or arginine, embryos remain as expanded blastocysts and do not form outgrowths in culture [14, 15, 17]. Further studies have shown that amino acid availability specifically regulates development of protrusive activity in trophoblast cells, thus also regulating initiation of motility and the spreading behavior required for implantation [2, 16]. When embryos are cultured in medium that lacks amino acids, the TE cells undergo normal changes in adhesivity and begin to express placental lactogen I, as they would in amino acid-containing medium [2]. In contrast, they neither develop protrusive activity nor do they undergo the normal changes in cell-cell adhesion in the absence of added amino acids [2, 14, 17]. Thus, amino acid deprivation does not have a general effect on trophoblast differentiation but specifically prevents the onset of implantation behavior by limiting trophoblast motility.

Amino acids regulate intracellular signaling pathways that have been described in widely different organisms from yeast to mammals [reviewed in 18]. Amino acid signaling activates a serine-threonine kinase, mammalian target of rapamycin (mTOR), which in turn phosphorylates at least two proteins involved in regulation of translation initiation, p70S6K and PHAS-I [18, 19]. These signaling pathways regulate the balance between protein synthesis and catabolism [20] and they modulate the transduction of insulin and insulin-like growth factor signals [19].

The initiation of trophoblast cell motility also depends on amino acid signaling through mTOR [2]. Treatment with rapamycin, a specific inhibitor of mTOR, inhibits initiation of trophoblast motility and spreading behavior. Rapamycin also blocks amino acid-initiated trophoblast motility and spreading behavior, while competitive inhibition of rapamycin with FK506 restores the amino acid stimulation. Under conditions of amino acid deprivation or rapamycin treatment, p70S6K remains unphosphorylated, confirming that mTOR activation is inhibited in both cases [2]. These results demonstrate that amino acid-dependent mTOR signaling leads to development of trophoblast cell motility and initiation of implantation.

Amino Acid Transport Regulates mTOR Signaling During a 4- to 8-h Period in Early Blastocysts

Amino acids activate a program of trophoblast differentiation at a very precise time during development [2]. In embryos developing in vitro, amino acid-stimulated mTOR signaling can occur only after the early blastocyst stage (i.e., 120 h post-hCG). Prior to 120 h post-hCG, exposure to amino acids will not promote later outgrowth, while after 120 h post-hCG, the blastocyst will not progress to an implantation-competent state without contact with exogenously provided amino acids (Fig. 1) [2, 16]. Contact with amino acids is required for only a 4- to 8-h period at 120 h post-hCG, after which exogenously supplied amino acids are not needed for development of trophoblast motility (Fig. 1).

The timing and short duration of the amino acid requirement are consistent with the hypothesis that it acts as a developmental checkpoint. That is, it may be a mechanism by which the embryo and uterus coordinate trophoblast differentiation with development of a uterine epithelium that is receptive to implantation. In this regard, the time at which the TE becomes responsive to exogenously supplied amino acids is also an intrinsic property of TE cells and does not depend on previous conditions. For example, both

TABLE 1. Blastocysts developing in vitro contain low levels of leucine and isoleucine relative to blastocyst developing in vivo (mean ± SEM pmol/embryo).[a]

Amino acid	Blastocysts developing		
	In vivo		In vitro[b]
	95 h post hCG	119 h post hCG	119 h post hCG
Threonine	0.061 ± 0.018 A	0.453 ± 0.125 B	0.282 ± 0.062 B
Valine	0.175 ± 0.033 A	0.081 ± 0.040 B	0.086 ± 0.022 B
Isoleucine	0.037 ± 0.012 A	0.112 ± 0.040 A	0.006 ± 0.006 B
Leucine	0.126 ± 0.014 A	0.092 ± 0.013 A	0.055 ± 0.014 B
Histidine	0.176 ± 0.057 A	0.288 ± 0.073 A	0.292 ± 0.073 A

[a] Amino acid levels were determined as described previously [24]. Numerically adjacent values with different letters are significantly different ($P < 0.05$) for a particular amino acid (Kruskal-Wallis *H*-test, analysis of variance, or nonoverlap of 95% confidence intervals [24]).
[b] Blastocysts developed from two-cell embryos in medium not containing added amino acids [24].

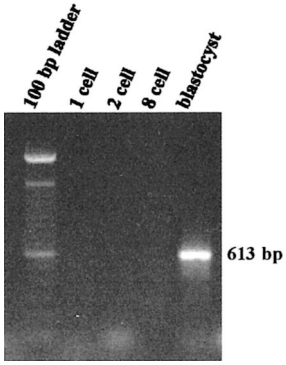

FIG. 2. Expression of mRNA encoding the ATB$^{0,+}$ protein (system B$^{0,+}$) for leucine biomembrane transport in mouse blastocysts. Polymerase chain reaction (PCR) was used to amplify a cDNA segment of the indicated size in libraries produced from oocyte, two- and eight-cell embryo, and blastocyst mRNA [105]. Procedures used for PCR are described more completely elsewhere [106].

embryos cultured in vitro from the two-cell stage and those flushed from the uterus at the morula stage respond only when exposed to amino acids at or after the early blastocyst stage [2, 16]. Hence, some property acquired at the early blastocyst stage appears to be responsible for development of the capacity to respond to amino acids. Amino acid-dependent signaling then leads to further progress toward differentiation of trophoblast cell motility.

Leucine is especially important for mTOR signaling and has been shown to activate mTOR both in vitro and in vivo [20–23]. Interestingly, blastocysts developing in vitro in the absence of added amino acids are poised to take up both leucine and isoleucine. The leucine and isoleucine levels in blastocysts developing in vitro at 119 h post-hCG are below the levels in blastocysts developing in vivo (Table 1), while the levels of other essential (Table 1) and nonessential [24] amino acids are not. Hence, transfer of blastocysts developing in vitro to medium containing essential and nonessential amino acids for 4–8 h likely leads to accumulation particularly of leucine and isoleucine. In addition, human embryos developing to the blastocyst stage in the presence of amino acids in vitro consistently accumulate leucine but not most other amino acids from the medium [25].

For blastocysts developing in vivo, an increase in amino acid transport system activity, rather than an increase in exogenous amino acid concentrations, likely raises intracellular amino acid levels to trigger mTOR signaling. Exogenous amino acids appear to be abundant in uterine secretions and their presence does not appear to be altered by development of conditions supporting implantation [e.g., 26]. Several known mechanisms could, however, stimulate leucine transport into blastocysts approaching implantation in vivo as discussed below.

Amino Acid Transport Regulation in Blastocysts In Vivo

Development of amino acid transport system expression. Cells take up and release amino acids through plasma membrane transport systems composed of proteins. Most amino acid transport systems appear to contain a single protein, although some systems are known to be heterodimers [27]. Those proteins catalyzing amino acid migration across biomembranes are termed transporters, while other subunits, if present, are called accessory proteins. Transport systems were categorized previously according to their preferences for cationic, anionic, or zwitterionic amino acids and whether they are Na^+-dependent [28]. Discovery of families and superfamilies of amino acid transporters that do not fit well into one or another of these six categories of amino acid transport systems has led to their classification instead according to phylogenetic relationships.

Mouse blastocysts likely express at least 14 amino acid transporters and two accessory proteins in seven different protein superfamilies. These transporters and accessory proteins comprise a minimum of 14 transport systems in blastocysts [29, 30]. A large proportion (probably most) of these proteins are expressed in human blastocysts as well [29]. Moreover, the activities of at least half of these systems increase greatly upon blastocyst formation sometimes from undetectable levels [31]. Most notably for the present discussion, expression of the leucine-selective system B$^{0,+}$ becomes conspicuous when preimplantation embryos form blastocysts [30]. This is likely due to changes in transcription of the gene rather than changes in translation or in the activity of preexisting protein, as the mRNA transcript can only be detected at the blastocyst stage (Fig. 2). System B$^{0,+}$ regulation in vivo could control uptake of leucine and other amino acids needed for mTOR signaling [2] and for embryo growth [32] prior to implantation. Since system B$^{0,+}$ activity appears to be controlled by regulated changes in the uterine environment, the resultant mTOR signaling and embryo growth also may be properly timed to promote implantation in a receptive uterus.

System B$^{0,+}$ regulation in vivo: Possible triggers of mTOR signaling. Experimental delay of blastocyst implantation in vivo by ovariectomy of female mice has led to discovery of at least two possible mechanisms by which amino acid transport system B$^{0,+}$ could be regulated to coordinate implantation. In progesterone-maintained ovariectomized mice, estrogen administration leads to blastocyst activation and implantation about 24 h later [33–36]. Within the first 6 h after estrogen treatment, the Na^+ concentration nearly doubles in uterine secretions [37, 38]. Such an increase in the Na^+ level would stimulate Na^+-dependent leucine uptake via system B$^{0,+}$ in blastocysts and consequently should raise the intracellular leucine concentration. If TE cells are able to maintain the resultant higher Na^+ total chemical potential gradient across their apical membranes, then their intracellular leucine levels should rise about threefold [31] because the stoichiometry of transport is likely two Na^+ with one leucine molecule [39]. In this regard, only about a 7% increase in the intracellular leucine level is needed to trigger mTOR signaling [40]. Moreover, blastocysts from progesterone-maintained ovariectomized mice remain via-

ble but make no progress toward development of trophoblast protrusive activity in low Na^+ medium otherwise designed to support trophoblast outgrowth in vitro [41]. The same blastocysts then develop normally when the Na^+ concentration in the medium is raised.

A second possible mechanism of system $B^{0,+}$ stimulation involves a transient fourfold increase in chymotrypsin-like enzyme activity in uterine secretions between 6 and 18 h after estrogen administration to progesterone-maintained ovariectomized mice [42]. Significantly, chymotrypsin stimulates system $B^{0,+}$ activity by about twofold in blastocysts from such mice [30, 43] and it hastens the onset of outgrowth in vitro [44]. In vivo, system $B^{0,+}$ activity in blastocysts increases dramatically beginning about 6 h after chymotrypsin-like enzyme activity begins to rise in uterine secretions [45]. However, this increased system $B^{0,+}$ activity appears to remain largely latent because of other factors in utero [45]. Hence, regulation of amino acid transport in blastocysts in vivo is likely more complex than that simulated, so far, in vitro.

We favor the hypothesis that an increase in the Na^+ concentration in uterine secretions shortly after estrogen administration to progesterone-maintained ovariectomized mice stimulates system $B^{0,+}$ to concentrate leucine in blastocysts. The increased intracellular leucine concentration would trigger mTOR signaling, which would lead to development of trophoblast protrusive activity about 20 h later in vivo. In our view, regulation of system $B^{0,+}$ by chymotrypsin-like enzyme activity and uterine factors 6 to 24 h after estrogen injection likely serves other functions perhaps related to coordination of the implantation process itself and protection of the conceptuses from immunologic rejection [30]. Other processes likely supported by increased amino acid uptake by blastocysts in vivo include accumulation of about 50% more protein during the 10 h preceding implantation [32] and production of arginine metabolites needed for implantation.

Why do blastocysts express so many amino acid transporters selective for arginine transport? Mouse and human blastocysts likely express at least five transporters selective for arginine transport under physiological conditions [29, 30]. Transport activities likely corresponding to three of these transporters have been detected in mouse blastocysts as systems b^+_2 (CAT1 and CAT2) [30] and system $b^{0,+}$ ($b^{0,+}$AT) [29]. Because blastocysts express mRNA sequences encoding both CAT1 and CAT2 [30], system b^+_2 may actually represent two transport activities, here provisionally designated b^+_{2a} and b^+_{2b}. We also detected sequences encoding two other arginine transporters in human and mouse blastocyst cDNA libraries [29 and unpublished data]. Transport activities corresponding to these transporters have, however, not as yet been delineated in blastocysts, possibly because they are expressed in the basal TE membrane (e.g., y + LAT2) [46] or inner cell mass (e.g., ATA3) [47]. System $B^{0,+}$ ($ATB^{0,+}$, Fig. 2) also transports arginine but with a K_m value too high to allow arginine to compete much with leucine for transport via system $B^{0,+}$ at physiological amino acid concentrations [48]. Nevertheless, system $B^{0,+}$-catalyzed leucine accumulation probably drives arginine uptake because of the exchange of intracellular leucine for arginine via system $b^{0,+}$ in blastocysts [30]. We propose that blastocysts need the conditionally essential amino acid, arginine, not only for net accumulation of protein but also for production of at least six other metabolites, each of which may be required for implantation.

First, preimplantation embryos need the arginine metabolite, creatine, and they express creatine kinase [49, 50], which may help to maintain relatively high ATP levels in metabolically active blastocysts. Blastocysts probably express a creatine transporter because a sequence corresponding to such a transporter [51] was found among ests from a mouse blastocyst cDNA library (Accession #CA551108). Blastocysts grow and develop in vitro, however, in medium without added creatine, so they likely synthesize this important free-energy buffer as well. Arginine, glycine, and methionine are consumed during creatine synthesis, and while each of these precursors could be regenerated, their transport into blastocysts also likely supports creatine synthesis.

Blastocysts also probably convert arginine to proline for prolyl residue-rich extracellular matrix protein production, as is the case in vascular smooth muscle cells [52, 53]. The presence of amino acids including arginine in culture medium fosters more normal development of the extracellular matrix in blastocysts [54]. Little or no proline likely is taken up directly by blastocysts, however, because its K_m values for transport are higher [55, 56] than most competing amino acids that are present in vivo [57] and in vitro [54]. Moreover, some culture media used for blastocyst development contain sixfold more arginine than proline [e.g., 54], and blastocysts express at least five transporters that select arginine over other amino acids as a substrate (see above). For these reasons, we favor the hypothesis that arginine rather than proline is the precursor for synthesis of prolyl residue-rich extracellular matrix proteins in blastocysts.

Finally, arginine is a substrate for the production of both nitric oxide (NO) and polyamines, through the actions of nitric oxide synthase (NOS) and arginase [58, 59]. Both NO and polyamines have been shown to be important for blastocyst outgrowth in vitro [60–65]. As discussed below, there is substantial evidence suggesting that either or both of these compounds may be downstream of mTOR in regulating the onset of TE motility.

LINKING UP AMINO ACIDS, mTOR, AND MOTILITY

The link between amino acid signaling and development of trophoblast motility is not yet clear. It may result from a change in translation of a specific mRNA or group of mRNAs, as mTOR activity strongly affects protein translation both through p70S6K activation and through PHAS-I inactivation. Phosphorylation by mTOR activates p70S6K and results in an increase in translation of a group of mRNAs known as 5′TOP mRNAs [reviewed in 66]. These messages have a characteristic polypyrimidine tract at their 5′ ends and typically encode proteins that are important components of the translational machinery. In contrast, PHAS-I is inactivated by mTOR phosphorylation. When dephosphorylated, it forms a complex with eIF4E, which is the cap-binding protein in the eIF4F initiation complex. Phosphorylation of PHAS-I leads to dissociation of the complex and an increase in free eIF4E, which can then participate in forming active initiation complexes [reviewed in 67, 68].

The role of eIF4E in regulating cell behavior is of particular interest in the context of TE differentiation. This protein participates in Ras transformation of cells and overexpression of eIF4E in cultured cells leads to malignant transformation [69–71]. In addition, overexpression of eIF4E in *Xenopus* animal caps promotes mesoderm induction, indicating that it can play a role in cell differentiation during development [72]. The availability of eIF4E has

been found to be less important for general protein synthesis than for enhanced translation of specific messages [73, 74]. In particular, translation of mRNAs that have complex 5′-untranslated regions, which often code for proteins associated with growth control and differentiation, is preferentially increased several fold over the average [69, 70, 75, 76]. Based on these observations, we hypothesize that amino acid-stimulated mTOR activity in TE cells promotes translation of mRNA(s) encoding a protein (or proteins) important in regulating the onset of motility. The cellular events triggered by amino acid signaling are likely to be downstream either of p70S6K phosphorylation, PHAS-I phosphorylation, or both.

From this perspective, the mRNAs encoding insulin-like growth factor II (IGFII) and ornithine decarboxylase (ODC) are particularly interesting candidates. Translation of each is regulated by mTOR [73, 77]. IGFII is expressed specifically in TE and trophoblast cells at the time of implantation [78] and has been shown to induce nitric oxide production in myoblasts [79]. Ornithine decarboxylase is the rate-limiting enzyme in the synthesis of polyamines from ornithine (a product of arginase action on arginine), and polyamines are required for embryo outgrowth [65].

Nitric oxide and trophoblast motility. A role for NO in regulating trophoblast motility is supported by many observations. To begin with, TE motility and outgrowth are inhibited in medium lacking the NO precursor arginine (Arg) [14, 15, 17]. Arg is used to generate NO by the enzyme nitric oxide synthase (NOS) [58, 59]. NO can act in both a paracrine and an autocrine manner, and signals through activation of soluble guanylyl cyclase to produce cGMP. NO produced by the TE could be important in vivo to initiate TE motility as well as to promote maternal capillary dilation during implantation. Very interestingly, NO has been shown to promote cell motility in several different cell types through modification of cell-substrate adhesion. In endothelial cells and smooth muscle cells, NO inhibits focal adhesion and stress fiber formation by activating protein tyrosine phosphatase 1b (PTP-1b or SHP2) in a cyclic GMP-dependent fashion [80–85].

Several studies have shown that inhibitors of NOS arrest blastocyst development and trophoblast outgrowth in the mouse [64, 86, 87]. Consistent with these observations, in vivo administration of NO inhibitors into the uteri of rats significantly decreases the number of embryos that implant [62, 88]. In addition, hepatocyte growth factor-induced motility of human trophoblast cells is activated by NO signaling through PI3K and mTOR [89]. Blastocysts in delay of implantation express eNOS and iNOS within 1 h of the estrogen injection that triggers their reactivation and implantation [63]. The iNOS expression is highest in periimplantation blastocysts relative to earlier or later stages of development [90].

Polyamines and trophoblast motility. Changes in motility may also be the result of an increase in ODC in TE cells, as increased eIF4E activity specifically enhances the translation of this mRNA [73]. ODC is the rate-limiting enzyme in polyamine synthesis from arginine [58]. Polyamines associate with DNA and nuclear proteins to produce normal chromatin and are thus required for new cells in the polar TE and for polyploid nuclei in the mural TE during giant cell formation. In addition to their well-known roles in chromatin organization, polyamine signaling has also been shown to affect intracellular signaling pathways such as those involving tyrosine and MAP kinases and the protooncogenes, c-myc, c-jun, and c-fos [91]. Many studies have found a link between ODC activity, cellular transformation, and cell motility. Overexpression of ODC leads to higher motility and invasiveness, while depletion of polyamines results in arrested migration, changes in cytoskeletal organization, and a decrease in integrin signaling through FAK [92–97]. In the intestinal epithelial cell line, IEC-6, increased polyamine synthesis upon wounding leads in turn to greater K_v channel gene expression, membrane hyperpolarization, increased cytosolic [Ca^{2+}], greater GTP-RhoA and Rac 1 activity, Rho-kinase activation, myosin phosphorylation, myosin/F-actin stress fiber formation, and cell migration [98, 99]. The effect of polyamines on cell motility depends ultimately on Rac 1 activation, providing the outline of one potential pathway from amino acids to motility [99].

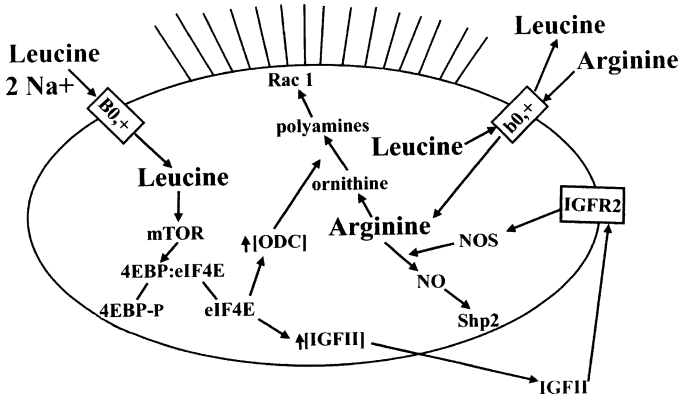

FIG. 3. A model for the potential actions of leucine and arginine in initiating motility in the trophectoderm. Leucine uptake by $B^{0,+}$ leads to concentration within the cell, where it can activate mammalian target of rapamycin (mTOR). In addition, it can act as a counter transporter for uptake of arginine. Phosphorylation of 4EBP by mTOR leads to increased levels of free eIF4E, which not only elevates protein translation generally, but specifically increases that of ornithine decarboxylase (ODC) and insulin-like growth factor II (IGFII). Increased internal arginine provides the substrate for arginase (to form ornithine) and nitric oxide synthase (NOS) (to form nitric oxide, NO). IGFII can act through its receptor to increase NOS activity, while increased levels of ODC lead to increased synthesis of polyamines. Polyamines can activate cell motility through Rac 1, while NO can affect cell motility through the phosphatase Shp-1 and its subsequent effects on adhesion complexes.

SUMMARY AND FUTURE DIRECTIONS

Amino acids are critical not only as nutrients for the mammalian embryo but also as regulators of its ability to implant and continue development. The data suggest that this is a very sophisticated system that specifically regulates motility without affecting many other aspects of TE differentiation and effectively controls the ability of the embryo to initiate invasion. Amino acid signaling in the embryo is regulated through the uterine environment, most likely by $B^{0,+}$ amino acid transporter activity. This mechanism provides one way for the uterus and the embryo to more precisely coordinate their progression to implantation; as the uterus becomes receptive, it in turn signals the embryo to progress to an invasive phenotype.

The exact mechanism by which the uterus regulates amino acid transport in the embryo is not known, but is quite likely part of the normal process of induction of uterine receptivity. In the case of experimental delay of implantation, administration of estrogen both initiates uterine activation and receptivity and the changes in amino acid uptake and motility in the blastocyst [34, 101]. Two potential regulatory factors are an increase in sodium concentration, which would stimulate the uptake of leucine by the $B^{0,+}$ transporter [37, 38], or alternatively, an increase in chymotrypsin-like enzyme activity, which would activate the $B^{0,+}$ transporter for uptake [30, 42–44]. We still need to learn how estrogen initiates changes in the concentration of sodium and chymotrypsin-like enzyme activity in uterine secretions. The latter activity may act on blastocysts to stimulate system $B^{0,+}$ activity via a novel serine protease-activated receptor [30].

Downstream of amino acid transport, the pathway leading from amino acids to increased TE motility is emerging, and some of the components have now been identified. Leucine and arginine are clearly the key amino acids, and the dynamics of their transport in the blastocyst suggest that leucine may function to drive arginine transport. Arginine has many functions in cells, but its roles in NO and polyamine production are clearly of great interest in relation to regulation of motility. The activation of mTOR is required, and several downstream targets, such as IGFII and ODC, are candidates for effectors in regulating motility. The link between mTOR activation and the requirement for arginine needs, however, to be determined. It is possible that mTOR and arginine have parallel functions in regulating trophoblast motility or that both pathways act in concert (Fig. 3). Leucine may increase IGFII signaling and ODC activity through activation of mTOR as well as promoting uptake of arginine. IGFII may then both promote NO production from arginine to stimulate motility and induce growth and differentiation. The increase in ODC may promote an increase in polyamine production from arginine, which in turn both activates Rac 1 and other aspects of motility, as well as supporting the necessary chromatin requirements of proliferation and endoreduplication.

Interestingly, there are two mouse mutants that exhibit defects in implantation consistent with an effect on amino acid and/or mTOR signaling. The best characterized of these is the knockout of leukemia inhibitory factor (LIF), which results in a defect in uterine receptivity. Female mice that lack LIF are sterile because the uterus does not become receptive and the embryos do not implant [102]. Recent studies have shown that LIF expression in the uterus is induced by estrogen and that LIF itself can substitute for estrogen to induce decidualization and implantation [103]. These observations suggest that changes in sodium and/or chymotrypsin-like activity in the uterine fluid may be triggered either directly or indirectly by the action of LIF, and thus LIF-deficient mice may be an ideal model system in which to study this question.

The other mutant of interest is the knockout of the T-box transcription factor Tbr-2, or eomesodermin, which results in a defect in blastocyst activation. Embryos lacking Tbr-2 develop normally to the blastocyst stage but then remain as fully expanded blastocysts, unable either to form outgrowths in vitro or to implant into the uterus [104]. These observations suggest that some component(s) of the amino acid to mTOR signaling system may be a transcriptional target of Tbr-2. Alternatively, it is possible that Tbr-2 controls transcription of the genes encoding proteins needed to initiate motility. This interpretation is supported by the fact that when Tbr-2-null embryos are rescued by aggregation with tetraploid wild-type embryos, the wild-type-derived trophoblast allows them to implant, but they fail at gastrulation due to lack of migration of the prospective mesoderm. Thus, lack of Tbr-2 has the same effect on both trophectoderm and mesoderm cell behavior. Further definition of the targets of Tbr-2 and of the targets of mTOR activation should shed light on this question.

While much remains to be learned, we have begun to draw a molecular and physiological link between the intertwined processes of uterine receptivity and embryo activation. Further investigation should provide important insights into the regulation of implantation.

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