

Nutrient transport across the placenta

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Abstract

The placenta forms a selective barrier that functions to transport nutrients that are of critical use to the fetus. Nutrient transport across the placenta is regulated by many different active transporters found on the surface of both maternal and fetal facing membranes of the placenta. The presence of these transporters in the placenta has been implicated in the facilitation of nutrient diffusion and proper fetal growth. In this review, recent developments concerning nutrient transporters that regulate glucose, amino acid, fatty acid, and nucleoside transplacental movement are discussed. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Rat placenta; Human placenta; Nutrient transporters; Amino acids; Glucose; Fatty acids; Nucleoside

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Abbreviations:

RT-PCR, reverse transcription polymerase chain reaction; GLUT, glucose transporter; MCAT-1, murine cationic transporter-1; CAT-4, cationic amino acid transporter-4; 4F2HC, antigen heavy chain; SATT1, system A amino acid transporter 1; hATB^o, human system B^o amino acid transporter; EAAC1, excitatory anionic amino acid carrier 1; EAAT4, excitatory anionic amino acid transporter 4; GLAST1, glutamate/aspartate anionic amino acid transporter 1; GLT1, glutamate anionic amino acid transporter; hFABP, heart fatty acid binding protein; FAT, fatty acid translocase; FATP, fatty acid transport protein; FABPpm, plasma membrane fatty acid binding protein; RT-PCR, reverse transcription polymerase chain reaction; rENT, rat equilibrative nucleoside transporter; hENT, human equilibrative nucleoside transporter

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1. Introduction

Proper fetal development is dependent on the ability of the fetus to obtain nutrients. The fetus derives its nutrients from both maternal diet and circulation. Critical to meeting these requirements is the ability of nutrients to permeate the placenta. Since nutrient transfer occurs from the maternal circulation, the placenta's ability to facilitate this transfer is of critical importance to the development of a healthy fetus.

While passive diffusion does account for some nutrient transfer, the fetal requirement for these nutrients is so great that passive diffusion alone is not adequate. Therefore, specific nutrient carriers, or transport proteins are located in the placenta that act to facilitate transfer and meet the increased nutrient demands of the fetus during gestation. It is apparent that the size of the fetus increases during gestation, and therefore, the need for these nutrients also

greatly increases [1]. Based on the increased nutrient demands with increasing gestational age, it would be expected that placental expression of these nutrient transporters would also increase to meet these needs.

Several classes of nutrient carriers have been identified in the mammalian placenta and comprise the basis for this review. We will discuss aspects of nutrient transport including: glucose, amino acid, fatty acid, and nucleoside transporters in the placenta. Our review will focus on molecular aspects of nutrient transporters and their expression patterns in human and rodent placentas. Several excellent reviews on nutrient transport across the placenta are also available [2–5]. The significance of a review of nutrient transporters lies in the possibility of the future feasibility of utilizing the placental nutrient transporters for controlling drug delivery to the placenta. It is clear that these transporters contribute to nutrient and xenobiotic transport from the mother to the fetus. Consequently, the use of prodrug

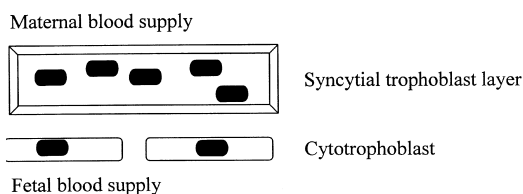
strategies or rational drug design to enhance or reduce the affinity of pharmaceuticals to these specific transporters will open new avenues for potentially selective fetal therapeutic interventions.

2. Placental organization

As mentioned previously, central to the transport of nutrients from the mother to the fetus is the presence of nutrient transfer proteins in the placenta. Since we have focused our discussions on placental nutrient carriers found in human and rodent (primarily the rat) models, a brief description of placental structure in these two systems is presented. The organization of human and rodent placentas has been more comprehensively reviewed [6,7] and is also discussed by others in this volume.

The placenta is comprised of highly specialized trophoblast cells, which arise from the embryo and differentiate to perform specialized functions. These functions include invasion of the uterine wall, nutrient and waste transport, metabolism, evasion of the maternal immune system, and cytokine and hormone production. Table 1 lists the various trophoblast cell types found in human and rodent placental model systems, and some generalized functions attributed to each of these trophoblast cells types. Both the human and rodent placentas are hemochorial, where trophoblast cells are directly bathed by maternal blood. In this review we will focus on the syncytial trophoblast cells, based on their prominent role in the regulation of fetal to maternal and maternal to fetal transport. Syncytial trophoblast cells are multinucleated and arise by fusion of precursor cytotrophoblast cells. The apical surface of the syncytial

Human placenta



Rat placenta

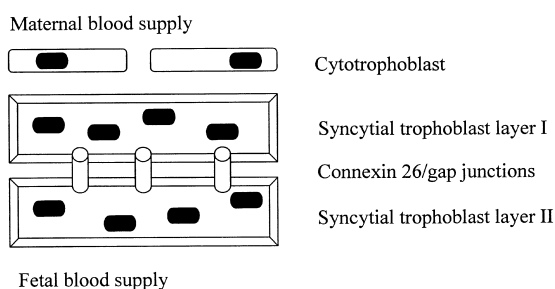


Fig. 1. A schematic representation of the nutrient transport barrier in human and rat chorioallantoic placentas.

trophoblast layer is referred to as the maternal facing membrane and makes direct contact with the maternal environment. The basal aspect of the syncytial trophoblast layer is situated in apposition to the fetal environment and is referred to as the fetal facing membrane.

2.1. Human placenta

Cytotrophoblast and syncytial trophoblast cells of the human placenta are found in villous and extravillous locations. Human syncytial trophoblast cells not only function as a transport barrier (see Fig. 1), but are also responsible for hormone production.

Table 1
Human and rat trophoblast cell types of the chorioallantoic placenta

Species	Cell type	Placental location	Function
Rodent	Trophoblast giant cells	Junctional zone	Endocrine, invasive
		Labyrinth zone	
	Spongiotrophoblasts	Junctional zone	Endocrine
	Glycogen cells	Junctional zone	Energy storage
	Syncytial trophoblast cells	Labyrinth zone	Transport barrier
Human	Cytotrophoblast cells	Villous and extravillous	Progenitor cells, invasive
	Syncytial trophoblast cells	Villous and extravillous	Transport barrier, endocrine

Primary cytotrophoblast and syncytial trophoblast cell culture systems can be utilized for studying human placental transfer, as reviewed recently [8,9]. In addition, several *in vitro* choriocarcinoma cell culture models of the human placenta also exist, including BeWo, JEG, and JAr cell lines. BeWo cells have been shown to be useful for the study of trans-trophoblast transport [10]. Each of the choriocarcinoma cell lines possesses 'cytotrophoblast-like' characteristics with somewhat variable abilities to differentiate into syncytial trophoblast cells.

2.2. Rat placenta

In the rat, the placenta is comprised of two structures, the choriovitelline and chorioallantoic placenta [6,11]. The choriovitelline placenta is important in the early stages of gestation. At mid-gestation, the chorioallantoic placenta is established and becomes more influential in helping to regulate the development of the fetus as gestation advances [11]. Eventually, the morphology of the chorioallantoic placenta changes and becomes divided into the functionally distinct junctional and labyrinth zones [11]. The junctional zone is positioned at the maternal interface, whereas, the labyrinth zone is at the fetal interface. These two zones differ in function, where the junctional zone is involved in endocrine and invasive functions, the labyrinth zone forms the main transport barrier and controls maternal to fetal transfer of nutrients [11]. Both the labyrinth and junctional zones are comprised of trophoblast cells, as outlined in Table 1. Syncytial trophoblast cells, of the labyrinth zone are the main type of trophoblast cell that regulates the transport of nutrients and wastes across the placenta [11]. The labyrinth zone is comprised of two syncytial trophoblast cell layers (I and II) and a cytotrophoblast cell layer forming a hemotrichorial placenta (see Fig. 1).

Several *in vitro* models of the rat placenta exist, including HRP-1 and Rcho-1 trophoblast cell lines [12–14]. HRP-1 trophoblast cells closely mimic the phenotype of labyrinth cell progenitors, whereas the Rcho-1 trophoblast cell model represents precursors found in the junctional zone that are capable of differentiation into trophoblast giant cells. In addition, HRP-1 trophoblast cells grown as a confluent

monolayer on a collagen-coated filter support have been established as a model to investigate nutrient and drug transport across the placenta [15]. Rcho-1 trophoblast cells have been more widely utilized for studying endocrine and invasive functions of trophoblast giant cells [13,16,17].

3. Placental glucose transporters

The metabolism of carbohydrates and, in particular, glucose fulfills a large portion of the fetal energy requirements during gestational development [1]. These glucose demands are substantial. For example, it has been shown that to satisfy human fetal glucose consumption, the fetus requires approximately 4–8 mg/kg/min of glucose [1]. Simple passive diffusion of glucose across the placenta is not adequate to meet these needs. Therefore, specific carrier mechanisms are required to facilitate placental transport of glucose

Placental glucose transporters have long been implicated in the transport of glucose to the fetus [1,18–22]. In one of the earliest studies of placental hexose transport, Eaton et al. [23] demonstrated, using the perfused guinea pig model, that trophoblasts can actively transport hexoses. Later studies further implicated the presence of active transporters with the ability to facilitate the transplacental transport of glucose and glucose analogs [19–22,24].

Six glucose transporters (GLUT1, GLUT2, GLUT3, GLUT4, GLUT5, and GLUT7) have been described in mammalian tissues [18,25–29]. These facilitative-diffusion glucose transporters are expressed separately in a tissue specific manner [25,26]. The involvement of glucose transporters in the maintenance of glucose homeostasis and their regulation have been reviewed previously [18]. Transplacental glucose transport has been demonstrated to be a stereospecific, carrier mediated, saturable process [30–34]. These transporters facilitate hexose (particularly glucose) transport and some are regulated by insulin [18,31,35].

Two GLUT isoforms have been identified in both human and rodent placentas, GLUT1 and GLUT3 [18,35–41]. The remainder of this section will focus on the placental patterns of expression and function

of GLUT1 and GLUT3. More comprehensive reviews of transplacental glucose transport have been presented elsewhere [42,43].

3.1. GLUT1

GLUT1 was first demonstrated to be active in human erythrocyte ghosts, and was first cloned from a human hepatoma cell cDNA library [28,42]. Glucose transport by GLUT1 is concentration dependent and bidirectional across the plasma membrane [42,44]. GLUT1 is a 55 kDa protein that is widely expressed in tissues and is independent of insulin control [18,42]. The regulation of GLUT1 expression has been reviewed elsewhere [18]. In the placenta, it has been proposed that GLUT1 is primarily responsible for placental glucose uptake from the maternal blood supply [43].

3.1.1. Human

GLUT1 mRNA is abundantly expressed by the human placenta in both cytotrophoblast and syncytial trophoblast cells [39,42,45–47]. Immunohistochemical analyses have demonstrated that abundant amounts of GLUT1 are present in syncytial trophoblast cells [42,48]. These studies clearly showed GLUT1 expression on both the apical and basal plasma membranes of the syncytial trophoblast cells that comprise the placental villous [48]. Further investigations determined that apical expression of GLUT1 was 20-fold higher than its basolateral expression [49]. This finding strongly supports the fact that glucose transport is highly directional from mother to fetus [42,49]. In addition, since GLUT1 is a bidirectional transporter, such an expression pattern suggests a protective mechanism preventing glucose transport from the fetus to the mother during maternal hypoglycemia.

Under *in vitro* conditions, GLUT1 expression has been observed in primary cytotrophoblast cultures and in choriocarcinoma cells [39]. Recently, it has been found that sustained hyperglycemia downregulates GLUT1 expression in trophoblast cells [50]. GLUT1 downregulation was proposed as a protective mechanism to control normal fetal development in the presence of high glucose [50].

3.1.2. Rodent

GLUT1 is highly expressed in both the junctional and labyrinth zones of the rat chorioallantoic placenta [26,30,38,43,51,52]. Junctional zone GLUT1 expression is highest at midgestation and actually decreases in concentration by the end of pregnancy [43,51]. GLUT1 in the junctional zone likely is associated with metabolic requirements for rapid placental growth at midgestation rather than transplacental glucose transport. Within the labyrinth zone, GLUT1 was abundantly expressed on both maternal and fetal facing membranes of syncytial trophoblast layers I and II [26,38]. Extracellular glucose concentrations positively regulate placental GLUT 1 expression in a time and concentration dependent manner [30]. In fact, increased placental GLUT1 expression in diabetic pregnancies may be responsible, at least in part, for increased delivery of glucose to the fetus and the resulting fetal macrosomia.

Some insight into the regulation of placental glucose transporters has been obtained by examining GLUT1 expression and glucose uptake in mixed mouse placental cell cultures [53]. Activation of the cyclic AMP/protein kinase A pathway was associated with a decrease in GLUT1 expression and glucose uptake [53]. Unfortunately, the mixed nature of the placental cell culture precludes an assessment of the specific cellular target and its involvement in transplacental glucose transport.

3.2. GLUT3

GLUT3 is a 42-kDa protein that has been found to be abundantly expressed in several different human tissues [42] and in the rodent brain [26]. GLUT3 has a very low K_m for glucose and has been shown to be responsible for neuronal uptake of glucose [25]. Specific roles for GLUT3 in placental glucose transport have not been firmly established.

3.2.1. Human

The intraplacental pattern of GLUT3 expression differs from the expression pattern found for GLUT1 [39]. GLUT3 mRNA is abundant; however, reports on the detection of GLUT3 protein have been equivocal, ranging from localization to the basal membrane of syncytial trophoblasts [39] to an

inability to detect GLUT3 protein associated with trophoblast cells of the placenta [43,49].

In the Jar choriocarcinoma cell model, GLUT3 mRNA and protein expression are differentiation dependent [39]. Proliferative JAr cells express high levels of GLUT3. In contrast, following differentiation, induced by cyclic AMP treatment, GLUT3 mRNA and protein are not detectable [39]. These findings suggest that GLUT3 may be important in meeting metabolic glucose needs during trophoblast cell proliferation [39].

The relative absence of GLUT3 protein expression in the syncytial trophoblast layer of the human placenta and the apparent decrease in GLUT3 expression accompanying *in vitro* trophoblast cell differentiation imply that GLUT3 is not a major facilitator of glucose transport to the developing fetus.

3.2.2. Rodent

GLUT3 is expressed in the labyrinth zone of the rat chorioallantoic placenta [35,40,43,51,52]. Within the labyrinth zone, GLUT3 is predominantly localized to the apical, or maternal facing, membrane of the syncytial trophoblast layer [26]. Expression of GLUT3 increases in amount as gestation progresses [43,51].

Functionally, GLUT3 has been proposed to participate in the protection of the fetus from maternal hypoglycemia and maternal hyperglycemia [26]. Recall, GLUT1, with a relatively higher K_m , is widely expressed on both the maternal and fetal facing membranes of the syncytial trophoblast layers of the rat placenta. In addition, both GLUT1 and GLUT3 can facilitate bi-directional transport of glucose across the placenta [42,26]. Given these facts, it appears that glucose can readily accumulate in the placenta and be rapidly cleared into the fetus under normoglycemic conditions. However, glucose transport is concentration driven and during maternal hypoglycemia glucose will move from the fetus to the mother, provided that the fetus is normoglycemic. Since the K_m of GLUT3 is low relative to GLUT1, and GLUT3 is only present on the maternal facing membrane of the labyrinth zone, glucose exit from the placenta will proceed at a slower rate from the fetus to the mother. Thus creating a situation where the concentration of

glucose in the placenta, in particular the labyrinth zone, will exceed the maternal and fetal glucose concentrations. Since GLUT1 has a higher K_m than GLUT3, transport will again be driven towards the fetus [26]. Therefore, the predominantly maternal facing membrane expression of GLUT3 serves a protective role by its decreased ability to bi-directionally facilitate glucose transport into the mother, leading to placental glucose accumulation [26]. During maternal hyperglycemia, the predominantly maternal facing membrane overexpression of GLUT3 and its low K_m for glucose, acts to prevent glucose from entering the syncytial trophoblast layers. The result of this action limits glucose transport into the syncytial trophoblast cell layer and protects the fetus from excessive glucose [26].

In streptozocin-induced diabetic rats, placental GLUT3 mRNA and protein levels are elevated 4–5-fold [35]. In contrast, the level of GLUT1 expression remains relatively unchanged when compared to control rats. Such evidence has been used to suggest a pivotal role for GLUT3 in facilitating glucose transport across the rat placenta, especially as a protective mechanism in maintaining normoglycemic conditions in the fetus [35].

3.3. Overview

In summary, the involvement of placental glucose transporters in regulating fetal glucose homeostasis is somewhat complex. It appears that GLUT1 expression is fairly uniform throughout development and GLUT1 is the main glucose transporter in the syncytial trophoblast layers. However, the asymmetric distribution of GLUT3, especially in the rodent placenta, implicates GLUT3 as an important contributor to the maintenance of fetal glucose homeostasis during periods of maternal hypoglycemia or hyperglycemia.

At this juncture, we have not yet addressed the specialized transport requirements from syncytial trophoblast layer I to syncytial trophoblast layer II in the rat or mouse placenta. Connexin 26 has been implicated as an important component in the facilitation of glucose transfer across syncytial trophoblast cells by mediating transfer from syncytial trophoblast layer I to layer II in the rat. Connexin 26 is a component of gap junctions and connects syncytial

trophoblast layer I with syncytial trophoblast layer II, allowing them to function as one layer (see Fig. 1). Therefore, diffusion of glucose through the connecting gap junctions is as critical to glucose transport across the placenta as is the glucose transporters [36,38]. The role of connexin 26 in mediating glucose transfer has been further demonstrated in the mouse placenta, where an embryonic lethal connexin 26-deficient mouse showed decreased placental glucose uptake [36]. Lethality in these mutant mice represented a placental defect and was presumably attributable to the failure of nutrient transport between the two syncytial trophoblast layers. This role of connexin 26 would not be expected in the monochorial syncytial trophoblast layer of the human placenta (see Fig. 1).

4. Placental amino acid transporters

Amino acids serve as the building blocks of proteins in the fetus. In addition, amino acids serve as a source of oxidative energy and as a source of carbon and nitrogen through metabolic interconversion [1]. Placental uptake of amino acids is an

energy dependent process that can be disrupted by treatment with glycolysis and aerobic metabolism inhibitors [1,54–56]. Amino acid transport across the placenta has been shown to be carrier mediated, and to progress down a concentration gradient from mother to fetus [57,58]. Placental amino acid transport has been extensively reviewed elsewhere [59–61].

The active transport of amino acids across cellular barriers is regulated by several different transporter systems [54,55,57,60–85]. Amino acid transporter systems are identified by their charge specificities and also by their dependency on Na^+ [54,55,57,86,87]. These transporters can and sometimes do have overlapping specificities and function [59,61]. Several amino acid transporter systems have been functionally identified in trophoblast cells [60,62–72,85]. A summary of the amino acid transporter systems operative in the placenta is presented in Table 2. This overview is based on several other excellent reviews [61,67,83,88].

In this section we will focus our review on the systems that have been functionally characterized in human and rodent placentas. Only a few of these transporter activities have been ascribed to specific

Table 2
Amino acid transport systems kinetically and molecularly identified in human and/or rat placentas

Charge	System	Amino acid specificity	Sodium	Transporter protein	Intraplacental location
Cationic	y^+	Basic: Lys, Arg, His	Independent	CAT-4 (Human) MCAT1 (Rat)	Human: not known Rat: labyrinth zone
	y^+L	Basic: Lys, Arg, His	Dependent	4F2HC (Human) 4F2HC (Rat)	Human: not known Rat: labyrinth zone
Neutral	A	Small aliphatic: Ala, Gly, Ser, Pro, Thr aminoisobutyric acid (AIB)	Dependent	–	–
	ASC	Small Aliphatic: Ala, Ser, Cys, Val, Gln, Thr, Ile	Dependent	SATT1 (Human)	Human: not known
	L	Branched and Aromatic: Leu, Ile, Phe, Met, Trp, Tyr, Val, Thr	Independent	–	–
Neutral/cationic	$B^{o,+}$	Broad specificity	Dependent	hATB ^o	Human: villous trophoblast
	$b^{o,+}$	Basic: Lys, Arg	Independent	–	–
	N	Gln, Asn, His	Dependent	–	–
Anionic	X_{AG}^-	Acidic	Dependent	EAAC1 (Rat) GLAST1 (Rat) GLT1 (Rat) EAAT4 (Rat)	Rat: junctional and labyrinth zones Rat: junctional and labyrinth zones Rat: junctional and labyrinth zones Rat: not determined

proteins. Thus, our current state of knowledge in this area is fragmentary. Transport systems will be presented according to charge specificities.

4.1. Cationic amino acid transporters

Cationic amino acid transporters have an affinity for transporting amino acids that have basic side chains (e.g. arginine, lysine). Many of these cationic amino acid transporters have been functionally identified in the placenta [61,65,67,70,72,75,76,89,90]. Two cationic amino acid transporters, system y^+ and y^+L , have been widely accepted as being present in both the maternal and fetal facing membranes of the placenta [75,89,90]. System y^+ and system y^+L , are specific for cationic amino acids only. Other amino acid transport systems, including systems N, $B^{0,+}$, and $b^{0,+}$, possess overlapping specificities for both cationic and neutral amino acids, and will be discussed in the neutral amino acid section of this review [61].

Systems y^+ and y^+L have different amino acid specificities, transport capacities, sodium dependencies, and temperature sensitivities. System y^+ is Na^+ -independent, has a relatively low affinity, but a high capacity and specificity for cationic amino acids [61]. System y^+L activity is distinguished from system y^+ activity by the fact that system y^+ is inhibited by *N*-ethylmaleimide. System y^+L is highly temperature sensitive and system y^+L has a much higher affinity for lysine and neutral amino acids [61]. System y^+ activity has been ascribed to proteins referred to as MCAT1 [76] and CAT-4 [91], whereas system y^+L activity has been ascribed to a protein called 4F2 antigen heavy chain (4F2HC) [65,72].

4.1.1. Human

MRNAs for CAT-4 and 4F2HC have been detected in the human placenta [72,91]. Their cellular localization within the placenta and the roles of their encoded proteins in cationic amino acid transport remains to be elucidated.

4.1.2. Rodent

Cationic amino acid transporters have been found in the rat placenta [76]. Initial functional characterizations showed three kinetically distinct cationic

amino acid transporters to be present in the rat placenta. Two different Na^+ -independent cationic amino acid transporter systems are present in both maternal and fetal facing plasma membranes of the rat chorioallantoic placenta [76]. The two Na^+ -independent cationic amino acid transporter systems were distinguished by their differing sensitivities to competition from leucine [76]. The leucine sensitive cationic transporter activity was shown to increase in both maternal and fetal facing membranes with increasing gestational age [76]. However, the leucine insensitive cationic transporter (system y^+) activity increases in the apical, maternal facing membrane but remains fairly constant in the basal membrane, as a function of gestation [76]. MCAT1, which is a system y^+ cationic amino acid transporter, increases in concentration in the placenta from days 14–20 of gestation in the rat [76].

System y^+L , having Na^+ -independent cationic amino acid transport activity, was demonstrated in the fetal facing, basal membrane of the rat placenta [65]. Expression of 4F2HC, a cationic amino acid transporter with y^+L activity, increases with advancing days of gestation and was localized in the syncytial trophoblast cells of the labyrinth zone [65].

4.2. Neutral amino acid transporters

In the placenta it is generally accepted that three main neutral amino acid carrier systems are present: types A, ASC, and L [92]. System A, a Na^+ -dependent transporter, has been shown to be the most reactive for amino acids containing short, polar, or linear side chains; alanine, proline, glycine, α -aminoisobutyric acid, serine, threonine, and glutamine [54,59,71]. System ASC, a Na^+ -dependent transporter, has somewhat restricted specificity to serine, cysteine, threonine, and alanine and is largely pH insensitive as compared to system A [59]. A protein possessing some system ASC activity is referred to as SATT1 [66]. SATT1 has a very high affinity for L-serine and L-alanine. System L, a Na^+ -independent transporter, has a high affinity for transporting large aromatic or apolar branched side chains; valine, alanine, serine, phenylalanine, glutamine, and isoleucine [59].

As indicated above, some amino acid transporters have a broader specificity, with capabilities of trans-

porting neutral and cationic amino acids, including systems N, B^{o,+}, and b^{o,+} [61]. System N is a Na⁺-dependent transporter with a high specificity for transporting glutamine and histidine [61,83,88]. System B^{o,+} is identified as a Na⁺-dependent transporter with a broad specificity for most neutral and basic amino acids [61,83,88]. The protein corresponding to system B^{o,+} activity is referred to as hATB^o [85]. System b^{o,+} is identified as a Na⁺-independent transporter with a broad specificity for most neutral and basic amino acids [61,83,88].

4.2.1. Human

SATT1, a transporter with system ASC activity, is expressed in the human placenta [66]. ASC system activity decreases when cytotrophoblast cells undergo syncytialization [78]. However, all of the placental ASC activity is probably not entirely attributable to SATT1 [78].

A Na⁺-dependent, broad specificity, neutral amino acid transporter (hATB^o) with system B^{o,+} activity has been cloned from a human choriocarcinoma (JAR) cell cDNA library [85]. Evidence for system B^{o,+} activity in the maternal facing membrane has been demonstrated [62,76], and may be related to the expression of hATB^o.

4.2.2. Rodent

Several of the neutral amino acid transport systems have been kinetically identified in rodent placentas [61,85]. However, evidence for the existence of specific neutral amino acid transporter proteins or their corresponding mRNAs in rodent placentas have not been reported.

4.3. Anionic amino acid transporters

There are currently two transport systems, X_{AG}⁻ and X_c⁻, that have been demonstrated to have anionic amino acid specificities [61,83,85]. System X_{AG}⁻ activity is characterized as being electrogenic, Na⁺-dependent transport with a high specificity for acidic amino acids [61,83,85]. Several proteins have been identified with System X_{AG}⁻ activity including EAAC1, GLAST1, GLT1, and EAAT4 [61,81,83]. System X_c⁻ activity is characterized as being electroneutral, Na⁺-independent transport with a high

specificity for acidic amino acids and cysteine [61,83,85]. System X_c⁻ activity has not been identified in either human or rodent placentas.

4.3.1. Human

Information on the existence of specific anionic amino acid transporters in the human placenta is not available.

4.3.2. Rodent

System X_{AG}⁻ activity [79–81] transporters that confer an anionic amino acid preference, have been identified in the rat placenta. The expression patterns of several system X_{AG}⁻ transporters (EAAC1, GLAST1, GLT1, and EAAT4) have been studied in gestational days 14 and 20 rat chorioallantoic placentas by Northern, Western, and immunocytochemical analyses [81]. At gestational day 14, EAAC1 is expressed in all major trophoblast cell types throughout the rat chorioallantoic. The levels of EAAC1 increase from gestation day 14 to 20, with the greatest increase in expression occurring in the syncytial trophoblast layers [81]. GLAST1 had a similar pattern of expression, with two exceptions: (i) GLAST1 was not detected in the trophoblast giant cells at gestational day 14, and (ii) GLAST1 expression increased more significantly from gestational day 14 to 20 than EAAC1 [81]. The pattern of GLT1 mRNA expression was similar to EAAC1 [81]. However, the greatest increase in GLT1 expression on gestational day 20 was found in the spongiotrophoblast cells of the junctional zone, and relatively no change in GLT1 expression levels was found in the syncytial trophoblast cells [81]. EAAT4 was only weakly expressed in the rat gestational day 20 placenta [81]. Taken together, these results provide a framework for understanding specific anionic amino acid transport proteins potentially involved in regulating the transfer of anionic amino acids to the fetus. GLAST1 and EAAC1 would appear to be excellent candidates for regulating anionic amino acid delivery to the fetus.

4.4. Overview

In summary, it is apparent that several classes of amino acid transporters exist in human and rodent placentas. The molecular identification of some of

these transporters has been demonstrated. It is also apparent that there is a clear deficit in our understanding of the localization of the amino acid transporters within the placenta. Until this is rectified, the exact extent to which each of these transporters function to regulate transplacental movement of amino acids will not be fully appreciated.

5. Placental fatty acid transport

Fatty acids, especially essential fatty acids, are nutrients that significantly contribute to the normal development of the fetus [93–97]. Unsaturated fatty acids of the n-3 and n-6 classification are considered to be essential. This is attributable to the fact that mammals are unable to introduce a double bond in the aliphatic side chains at the n-3 and n-6 positions, counting from the carboxylic acid moiety [97]. Therefore, the fetus must rely on maternal circulation and transfer across the placenta as its source of these essential fatty acids [96]. Fatty acid transfer is highly directional from mother to fetus [93–97].

Essential fatty acids are involved in the synthesis of phospholipids and biological membranes, myelin biosynthesis, gangliosides, glycolipids, and sphingolipids [97]. Essential fatty acids of both the n-3 and n-6 series (e.g. linoleic and linolenic acid) are also involved in the synthesis of several compounds involved in cell–cell signaling including: prostaglandins, prostacyclins, leukotrienes, thromboxanes, eicosanoids, and lipoxins [97]. The ability of fatty acids to cross the placenta is crucial for proper fetal brain development [93,94,97], fetal growth [95], and cardiovascular and lung development [96,98–101]. The role of placental fatty acid transfer in the proper development of the fetus has been reviewed elsewhere [1,93–98,100–109].

We will restrict our presentation to the proteins that mediate the transfer of fatty acids across the placenta. Fatty acid transport has been shown to be regulated by several key proteins (Table 3); including plasma membrane fatty acid binding protein (FABPpm), fatty acid translocase (FAT), fatty acid transporter protein (FATP), and the cytoplasmic fatty acid binding protein family (FABP) [110]. FAT,

Table 3
Fatty acid transport systems located in the placenta and their cellular localization

Transporter	MW (kDa)	Function	Intraplacental location	Placental cell culture models
Plasma membrane fatty acid binding protein (FABPpm)	40	Membrane bound fatty acid transporter	Human: maternal facing membranes Rat: junctional and labyrinth zones	BeWo, HRP-1, Rcho-1
Fatty acid translocase (FAT)	88	Membrane bound fatty acid transporter	Human: maternal and fetal facing membranes Rat: labyrinth zone	HRP-1, Rcho-1
Fatty acid transport protein (FATP)	63	Membrane bound fatty acid transporter	Human: maternal and fetal facing membranes Rat: not determined	HRP-1, Rcho-1
Cytoplasmic fatty acid binding protein family (FABP)	12–16	Intracellular fatty acid trafficking protein, role in metabolism and transport of fatty acids		
(a) Heart isoform	15		Human: villous trophoblast Rat: labyrinth zone	BeWo, HRP-1, Rcho-1
(b) Liver isoform	15		Human: villous trophoblast Rat: not determined	BeWo

FATP, and FABPpm are localized to the plasma membrane and have been identified as transporters of fatty acids [111]. FABP is a family of cytoplasmic proteins responsible for the intracellular trafficking of fatty acids.

5.1. Plasma membrane fatty acid binding protein (FABPpm)

FABPpm is a 40-kDa membrane protein that has been identified in various tissues and has been demonstrated to regulate the uptake of long chain fatty acids [112–117]. FABPpm has also been shown to possess mitochondrial aspartate aminotransferase activities [118,119].

5.1.1. Human

A unique placental isoform of FABPpm (pFABPpm) has been described in the human placenta [111,120]. The pFABPpm isoform differs from FABPpm in its enzymatic specificities, its amino acid composition, and its isoelectric point [111,120]. pFABPpm preferentially binds essential long chain fatty acids, arachidonic and decosahexaenoic acid, which suggests its possible role in mediating their transfer across the placenta [120,121]. The binding specificities of pFABPpm are not in complete accordance with the specificities of fatty acid permeation across the placenta [121], indicating the potential involvement of other fatty acid transport systems.

5.1.2. Rodent

In the rat placenta, FABPpm has been detected by Western and Northern analysis [122]. FABPpm has a broad expression pattern in both the junctional and labyrinth zones of the rat placenta, with a modest increase in expression with increasing gestation [122]. FABPpm is also present in trophoblast cell culture models of the rat placenta. Both the HRP-1 labyrinthine and Rcho-1 trophoblast cell lines express FABPpm [122]. More specifically, FABPpm levels increase following Rcho-1 trophoblast cell differentiation toward the trophoblast giant cell phenotype [122].

5.2. Fatty acid translocase (FAT) and fatty acid transport protein (FATP)

FAT, an 88-kDa membrane protein, was first identified and characterized based on its ability to facilitate uptake of fatty acids in rat adipocytes [123,124]. FAT was also shown to be homologous to human CD36 glycoprotein [123]. FAT has also been linked to an increase in the uptake of oxidized fatty acids in macrophages [125–128]. FATP is a 63-kDa protein that was originally identified by expression cloning in adipocytes [129] and has been shown to be involved in facilitating lipid transport [129].

5.2.1. Human

Both FAT and FATP are expressed in trophoblast cells of the human placenta, however, their location and gestational expression patterns have yet to be determined [111].

5.2.2. Rodent

FAT and FATP mRNAs have been identified in the rat placenta [122]. FAT is predominantly localized in the labyrinth zone of the rat chorioallantoic placenta by *in situ* hybridization [122]. FAT is expressed in HRP-1 labyrinthine trophoblast cells and Rcho-1 trophoblast cells. FATP is also expressed in HRP-1 labyrinthine trophoblast cells and in Rcho-1 trophoblast cells, where it exhibits a differentiation dependent increase in expression [122].

5.3. Cytoplasmic fatty acid binding proteins (FABP)

Of the proteins involved in fatty acid transport, the FABPs are the most abundant in all cell types, comprising as much as 4% of total cytosolic protein [130]. There are several different FABPs (MW range from \approx 12 000–16 000) which are identified by the tissue in which they were originally discovered [131]. It has been proposed that each FABP serves unique cell/tissue-specific functions. FABPs are responsible for the intracellular distribution of fatty acids [132,133] and are believed to play an integral role in metabolism, transport, and membrane incorporation of fatty acids [132].

5.3.1. Human

Both heart and liver FABP isoforms are expressed in human trophoblast cells [111]. The cellular location(s) of expression within the placenta remains to be determined.

5.3.2. Rodent

The heart FABP (hFABP) isoform has been identified in the rat placenta by Northern Western, in situ hybridization, and immunocytochemical analyses [122,134,135]. Expression is restricted primarily to the labyrinthine trophoblast of the rat placenta, and increases in abundance as gestation progresses [122]. HFABP is also expressed in HRP-1 labyrinthine and Rcho-1 trophoblast cell culture models [122]. In contrast to FABPpm expression in the Rcho-1 trophoblast cell line, which increases accompanying differentiation, hFABP decreases accompanying giant cell differentiation [122].

5.4. Overview

It is clear from this discussion that there are at least five proteins that potentially participate in placental fatty acid transfer. The conservation of their expression in both human and rat placentas suggests that they may be critical for proper fetal development. Based on our localization data for the rat model [122], it would appear that FAT and hFABP may play a greater role in the delivery of fatty acids to the fetus. Elucidation of the specific involvement of each of the fatty acid transfer proteins in placental fatty acid transport is of critical importance.

6. Placental nucleoside transporters

Nucleoside uptake and transport by cells is critical to their ability to make nucleotides and maintain their ability to grow and function. In the case of fetal growth, the demand for nucleosides is very great. Placental nucleoside transporters help meet the fetal requirements for the nucleosides. In mammals, two classes of nucleoside transporters have been identified, equilibrative and concentrative. Equilibrative nucleoside transporters exhibit broad substrate specificity for both purine and pyrimidine bases, and

have been identified in the placenta [136–140]. There are two classes of equilibrative nucleoside transporters, which are distinguished from one another by their sensitivity to nitrobenzylthioinosine (NBMPR). As the name would indicate, the equilibrative NBMPR-insensitive transporters are unaffected by micromolar NBMPR (IC_{50} values $> 1 \mu\text{M}$), whereas the equilibrative NBMPR sensitive transporters are readily inhibited by NBMPR (IC_{50} values $< 5 \text{ nM}$) [136–140]. To date, no reports have linked the concentrative nucleoside transporters to either the rat or human placentas, and therefore, they will not be discussed in this section. Readers are referred to the excellent review of concentrative nucleoside transporters written by Wang et al. [141], for more information about these transporters. In this section, we will focus on the equilibrative nucleoside transporters.

6.1. Human

Equilibrative nucleoside transport activity has been shown to be present on both the maternal and fetal facing membranes of the human placenta [142]. Both classes of equilibrative nucleoside transporters have been characterized and identified in the human placenta [136,137] and in the BeWo trophoblast cell culture model [138]. Initially, the presence of nucleoside transporters in maternal and fetal facing membranes of human placental syncytial trophoblasts was suggested by assessment of NBMPR binding [142,143]. In latter studies in the human placenta, two isoforms of human equilibrative nucleoside transporters (hENT) were cloned and characterized, hENT1 and hENT2 [136,137]. The hENT1 isoform has been found to be an NBMPR sensitive type of equilibrative nucleoside transporter, with an apparent molecular weight of 55 kDa [137]. Immunoblots of human syncytial trophoblast maternal facing membranes showed that the hENT1 isoform is abundantly expressed in human trophoblast [137]. Immunoreactive hENT1 was not found in the basolateral membranes, despite an equal number of NBMPR binding nucleoside transporters. This suggested that hENT1 is present only in the maternal facing membranes of the syncytial trophoblasts, and that a second nucleoside transporter is present in the

trophoblast basolateral membranes [137,138,142]. The identity of the second NBMPR sensitive equilibrative nucleoside transporter is yet to be reported.

A human NBMPR-insensitive nucleoside transporter, hENT2, has been isolated from the human placenta [136]. HENT2 possesses a molecular weight of 50 kDa and has 46% amino acid identity with hENT1 [136]. In addition, hENT2 was found to mediate transport of pyrimidine and purine nucleosides, in a saturable, NBMPR-insensitive manner [136]. The location of hENT2 has not been determined.

Some insights regarding the regulation of nucleoside transport have been generated from studies with ethanol. Adenosine transport across the human placenta is significantly lowered (25–50%), in a dose-dependent manner, in the presence of ethanol [144]. Since adenosine transport across the human placenta is NBMPR-sensitive [145], a potential role for hENT1 in adenosine transport may be inferred. It is interesting to speculate that the developmental abnormalities associated with fetal alcohol syndrome may be associated with decreased expression or perturbed function of nucleoside transporters in the placenta [144].

6.2. Rodent

Homologues for ENT1 and ENT2 have been identified in the rat [139], however, their expression pattern and function in the rodent placenta has not been reported.

6.3. Overview

It is apparent that there are at least three equilibrative nucleoside transporters present in the human placenta, two NBMPR-sensitive and one NBMPR-insensitive. The presence of rat homologues for two of these human ENT proteins has been confirmed in several tissues, however, their expression in the rat placenta has not been demonstrated. Describing specific roles of these nucleoside transporters in placental nucleoside transport remains to be determined.

7. Conclusions

Nutrient transfer is a fundamental property that defines a placenta. Classically, nutrient transport across the placenta was studied by kinetic and functional observations of solute transfer. Michaelis–Menten kinetics and competitive inhibitors were employed to decipher whether transport phenomena was active or passive in nature. In recent years, the development of *in vitro* and *in vivo* models has accelerated research on nutrient transport across cellular barriers. In particular, cell culture models are now routinely utilized to investigate placental transport [10,15,39,70,72,120,145]. Some nutrient transporters have been isolated and their cDNAs cloned. The availability of immunological and molecular reagents for monitoring expression of these transporters has provided more precise information about specific cell types involved in the transport process and enabled the postulation of mechanisms controlling placental nutrient transfer. Additional proteins responsible for placental nutrient transporter activities require isolation. This may involve utilizing probes generated for nutrient transporters already identified in other tissues. Alternatively, it is important to appreciate that there may be transport mechanisms or transporter isoforms that are unique to the placenta.

It is also necessary to move from the current situation dominated by phenomenological investigations to include mechanistic experimentation. We now know some of the transporters expressed by trophoblast cells but we do not have a clear understanding as to how individual transporters contribute to the control of placental nutrient transfer. The stage is set for implementation of molecular manipulation strategies. Specific genes involved in the transport process need to be manipulated in cell culture systems and in mouse models. *In vitro* paradigms will permit investigations of placental nutrient transporter structure–function relationships, while *in vivo* analyses will provide insight regarding the relative importance of a nutrient transporter to embryonic/fetal development. The latter approach involving transgenesis and gene targeting should be quite promising given the conservation of nutrient transporters between humans and rodents. We also need to gain insight into possible roles for placental nutrient

transport processes in the etiology of various disease states impacting on fetal development. Improved understanding of these relationships should lead to therapeutic strategies for treating conditions leading to intrauterine fetal growth retardation.

Finally, the utilization of these placental nutrient transporters for pharmaceutical drug delivery to the placenta may also hold great potential. It is clear that these transporters function to facilitate transport from the mother to the fetus. Therefore, the ability of medicinal chemists to use prodrug strategies or rational drug design to enhance the affinity of pharmaceuticals to these specific transporters will open new avenues for potentially selective fetal therapeutic interventions.

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