Glucose transporters in preimplantation development

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The inability of the embryo to utilize glucose as a fuel before compaction has been an area of much speculation. It is suggested that limitations in glucose transporter processes are the prime reasons for this. The recent identification of GLUT3 as the transporter responsible for the uptake of maternal glucose after compaction may provide the missing link in this puzzle. Furthermore, the coincidence of its expression with the onset of embryonic glucose utilization suggests that GLUT3 may be involved in the determination of metabolic priorities of the embryo. A model for the uptake of glucose by the blastocyst based on the function of two facilitative glucose transporters, GLUT3 and GLUT1, is proposed which can accommodate growth factor regulation of embryonic processes and is consistent with both the well established biochemical characteristics of GLUT proteins and the physiology of the embryo.

Embryos of many species do not rely on glucose as a major energy source until compaction and blastocyst formation. Cleaving embryos derive their energy from the more oxidized sources, pyruvate and lactate, although they possess all the requirements for glucose oxidation, including glucose transport systems. The reasons for the pyruvate dependency of early embryos and the nature of the metabolic switch to glucose at compaction–blastocyst formation are unclear. While a block in glycolysis before compaction has been suggested (Barbehenn et al., 1974), recent evidence implicates glucose transport as a factor contributing to the inability of early embryos to derive energy from glucose (Pantaleon et al., 1997a). This article focuses on glucose transport in the preimplantation embryo and the molecules involved in the passage of this important fuel across embryonic plasma membranes.

Active glucose uptake

There are two classes of glucose carrier: the concentrative Na+-glucose cotransporters (SGLT), which couple glucose uptake with influx of Na+ down a concentration gradient generated by Na+-K+ ATPases, and the energy independent facilitative glucose transporters (GLUT).

Evidence for the presence of SGLT in the mouse blastocyst is conflicting. Immunological studies detected a SGLT on apical trophodermal membranes (Wiley et al., 1991), while pharmacological studies placed a SGLT on basolateral membranes (Chi et al., 1993). However, the antiserum used in the immunological study bound to two embryonic antigens (one consistent with SGLT, the other unidentified, but of mobility similar to GLUT). The kinetic study relied on a glucose analogue, 2-deoxy glucose, to measure glucose accumulation. However, upon entry into cells, this analogue may be phosphorylated, preventing subsequent efflux. Given that others have failed to detect active glucose transport (Gardner, D. K. and Leese, 1988; Gardner, H. G. and Kaye, 1995) and that the glucose concentration of blastocoel fluid approximates half that of the external environment (Brison et al., 1993), a physiological role for a SGLT in the blastocyst is elusive.

Facilitative glucose transporters

In a family of seven isoforms, only five have been characterized as functional transporters (GLUT1–5). These exhibit a high degree of sequence homology but vary in their kinetic characteristics, sensitivity to hormonal and environmental stimuli, and tissue and subcellular distribution. The key to understanding the functional role of these transporter molecules lies in their kinetic characteristics. The exhibition of typical Michaelis Menten saturation kinetics defines the rate of glucose uptake by a given cell as a function of external glucose concentration and transport efficiency by the transporter Km value or affinity for glucose. The saturaibility of uptake reflects the presence of a limited number of carrier molecules at the plasma membrane. In a passive system such as this, which is not under any form of allosteric regulation, the partitioning of regulatable isoforms (GLUT1 and GLUT4) between the membrane and intracellular sites allows cells to increase glucose uptake by translocation of carriers from intracellular stores to the plasma membrane to increase Vmax without a change in Km value. Collectively, these properties allow specific isoforms to perform varied, distinct functions in maintaining whole body glucose homeostasis (Table 1).

GLUT expression in early embryos

Facilitated glucose uptake had been reported in several early studies using radiolabelled glucose or by assessing uptake via loss of glucose from the medium (Wales and Brinster, 1968; Gardner and Leese, 1988). However, when molecular characterization of GLUT provided sequences and isoform specific antisera, it became possible to examine expression of individual isoforms during preimplantation development. Immunoelectron microscopy of rabbit blastocysts revealed
GLUT1, predominantly on basolateral trophectoderm membranes (Robinson et al., 1990). In mice, mRNA encoding GLUT1 was found in embryos of all preimplantation stages. While mRNA encoding GLUT2 was detected from the eight-cell stage, GLUT3 and GLUT4 were not found (Hogan et al., 1991). In contrast to the case in rabbits, immunoelectron microscopy showed GLUT1 was randomly distributed along apical, basolateral and intercellular membranes of the trophectoderm, as well as membranes of the inner cell mass (ICM). Intriguingly, in this study, GLUT2 was detected only in blastocysts, where it appeared on basolateral trophectoderm membranes facing the blastocoel, intracellular vesicles and all membranes of the ICM (Aghayan et al., 1992). The ubiquitous distribution of GLUT1 in the mouse blastocyst (Aghayan et al., 1992) is inconsistent with its reported polarized distribution in rabbit trophectoderm (Robinson et al., 1990) and other polarized epithelia (Pascoe et al., 1996). GLUT1 is generally accepted as a basolateral transporter in polarized cells with a $K_m$ value higher than that reported for glucose uptake in mouse blastocysts (Gardner and Kaye, 1995). Moreover, the 300-fold increase in uptake rate during compaction is not matched by the 20-fold parallel increase in GLUT1 expression (Morita et al., 1992). These inconsistencies indicate that GLUT1 is not responsible for transport of maternal glucose across the apical membranes of the trophectoderm. The alternative isoform expressed, GLUT2, has an even higher $K_m$ value than GLUT1 and is also accepted as a basolateral transporter and, thus, is unlikely to provide that function either.

**Blastocyst glucose transport: a model**

The absorptive nature of the trophectodermal epithelium requires apical expression of a transporter with a suitably low $K_m$ value, linked to basolateral expression of a transporter capable of efflux, to provide glucose from the low uterine concentrations to inner cells. While an earlier model drawing an analogy with intestinal epithelium cast GLUT1 and GLUT2, respectively, in these roles (Aghayan et al., 1992), the subcellular locations reported above do not support such a disposition. Furthermore, in the gut, apical absorption of glucose is via an SLGT, not GLUT1.

New insight into this problem comes from high resolution confocal microscopy of whole mount blastocysts stained with mouse isoform specific antisera. This study identified GLUT3 in apical membranes, supported by expression of GLUT1 on basolateral trophectoderm and uniformly on the ICM (Pantaleon et al., 1997a). The polarized expression of these two GLUT isoforms can provide the necessary delivery of uterine glucose to cells of the blastocyst (Fig. 1). The very low $K_m$ value and high capacity of GLUT3 are ideally suited to the low glucose concentrations in uterine fluid.

The uniform GLUT1 distribution in the ICM suggests that it also transports glucose from the blastocoel cavity and interstitium for utilization by these cells. The ability of GLUT1 to provide both influx and efflux modes of transport reflects its asymmetric kinetics. While the $K_m$ value for glucose influx is 1–2 mmol l$^{-1}$, that for efflux is 20–30 mmol l$^{-1}$, providing an efficient efflux mechanism (Thorens, 1996).

Thus, consistent with its kinetic characteristics, basolaterally targeted GLUT1 in its low affinity–high $K_m$ value mode would efflux apically GLUT3-derived glucose to the blastocoel. This reservoir could then provide glucose for the glycolytic activity of the ICM by influx through GLUT1 in its higher affinity–low $K_m$ value mode.

**GLUT function**

**GLUT3**

The inhibition of blastocyst formation after GLUT3 ablation suggests an integral role for this transporter in early development (Pantaleon et al., 1997a). The coincidence of GLUT3 expression with the switch to glucose utilization at compaction.

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**Table 1. Mammalian glucose transporters: major sites of expression and physiological functions**

<table>
<thead>
<tr>
<th>Name</th>
<th>Tissue distribution</th>
<th>Proposed function</th>
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<tbody>
<tr>
<td>SGLT1</td>
<td>Kidney, intestine</td>
<td>Na$^+$-dependent active transport; concentration apical epithelial membranes</td>
</tr>
<tr>
<td>GLUT1</td>
<td>Ubiquitous; abundant in human erythrocytes, placenta and fetal tissues, brain, endothelia, and many immortalized cell lines</td>
<td>Basal and growth factor-stimulated transport; blood–tissue barrier transport</td>
</tr>
<tr>
<td>GLUT2</td>
<td>Liver, kidney, pancreatic $\beta$-cells, small intestine</td>
<td>Low-affinity transporter Transepithelial transport (basolateral membrane) in kidney and gut, hepatic glucose output, part of the glucose sensor in islets and liver</td>
</tr>
<tr>
<td>GLUT3</td>
<td>Neurones, placenta</td>
<td>High-affinity transporter</td>
</tr>
<tr>
<td>GLUT4</td>
<td>Skeletal and cardiac muscle, brown and white adipose tissue</td>
<td>Insulin-stimulated glucose transport</td>
</tr>
<tr>
<td>GLUT5</td>
<td>Small intestine, spermatozoa, smaller amounts in adipose, muscle, brain and kidney</td>
<td>High-affinity fructose transporter</td>
</tr>
<tr>
<td>GLUT7</td>
<td>Liver</td>
<td>Mediates glucose release from endoplasmic reticulum coupled to glucose-6-phosphatase</td>
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suggests a functional relationship. GLUT3 expression may be important in determining the metabolic priorities of the embryo.

While early studies demonstrated a block to glucose utilization at the phosphofructokinase stage (Barbehenn et al., 1974), they did not exclude a block in glucose transport. The correlation between hexokinase and glucose uptake activities (Brinster, 1968) suggests that the block to glucose utilization before blastocyst formation may arise from insufficient capacity of GLUT1 and hexokinase to produce enough glucose-6-phosphate to activate phosphofructokinase allosterically. Expression of high capacity-low $K_m$ value GLUT3 and its accumulation in apical membranes of outer cells at compaction would explain the vast increase in glucose uptake and release of phosphofructokinase from inhibition by low glucose-6-phosphate.

The predominant expression of GLUT3 in cells with a high glucose dependency (trophectoderm and neurones) tends to support this speculation. Similar to the events occurring in early development, GLUT3 ontogeny in rat brain and retina is associated closely with the morphological and functional development of these tissues (Bondy et al., 1992), supporting the suggestion that GLUT3 expression is a required phenotype of glucose dependent cells. As compaction approaches, increasing expression of GLUT3 and its localization to apical membranes of polar outer cells may reflect the metabolic dependence of the developing blastocyst on glucose.

**Role of GLUT3 in blastocyst formation**

The inability of the embryo to form a blastocyst after GLUT3 ablation (Pantaleon et al., 1997a) may stem from this putative metabolic coupling. However, blastocysts can develop from two-cell embryos in the absence of glucose _in vitro_, suggesting that flexible metabolic adaptation is in place (Martin and Leese,
Blastoscyt formation may occur in the complete absence of glucose because this absence activates pyruvate uptake and metabolism to provide the energy for blastocyst formation (Martin and Leese, 1995). Glucose and GLUT1 were both present in the GLUT3 ablation experiments (Pantaleon et al., 1997a), so access of the cleaving embryos to glucose via GLUT1 may have prevented increased pyruvate uptake and energy supply in morulae so preventing blastocyst formation. This suggests that GLUT1 plays a role in signalling the surrounding glucose concentration, before compaction, so that the decision to switch to glucose for energy can be made at the same time as GLUT3 expression is activated.

**GLUT1 and regulation of glucose transport**

The proliferative and metabolic effects induced by insulin, insulin-like growth factor (IGF) and other growth factors suggest that the receptors for these factors co-ordinate nutrient transport with the modified metabolic requirements of stimulated proliferation. The confirmed absence of the key insulin target, GLUT4, explained the results of earlier studies that failed to show an increase in glucose transport in response to insulin and led to the widespread assumption that embryonic glucose transport is not regulated by insulin. However, this view ignored the rapidly growing body of evidence demonstrating GLUT1 responsiveness to a number of growth and environmental stimuli in *vivo*. Stimulation of GLUT1 occurs by a combination of transporter recruitment (initial response) and transporter synthesis (chronic response) and is much smaller than the GLUT4 response to insulin, due to differences in sub-cellular partitioning of the two isoforms. In the absence of GLUT4, expressed mainly in muscle and fat, GLUT1 responsiveness to growth factors and cellular stress is, therefore, extremely important as a mechanism for providing increased substrate to cope with increased cellular demand arising from growth, proliferation and stress (for review see Baldwin, 1993).

Glucose uptake by the blastocyst is increased by IGF-I and insulin, both acting through the type 1 IGF receptor (Pantaleon and Kaye, 1996). Growth hormone also stimulates glucose transport in the blastocyst under conditions designed to identify the cumulative effects of recruitment and synthesis of transporter *de novo* (Pantaleon et al., 1997b). The model can accommodate these responses. The differential expression of GLUT3 and GLUT1 creates an affinity-concentration gradient that allows the developing embryo to increase its glucose uptake rate in response to growth and proliferative stimuli within the ICM. The only known growth factor responsive transporter present is GLUT1. Thus, in the presence of growth factors, increased GLUT1 efflux at the basolateral trophoectoderm membrane would drive increased apical glucose entry via GLUT3. This is permitted by the low $K_m$ value of GLUT3 for glucose. Thus, an increase in maximal rate of uptake by the ICM, as a result of growth factor-induced GLUT1 recruitment, coupled with the GLUT1–GLUT3 affinity gradient, would result in increased trans-trophoectodermal influx in response to growth factor stimulation.

Inclusion of GLUT1 stimulation in the array of events activated by the type 1 IGF receptor may be a consequence of the increased energy and biosynthetic requirements of actively dividing cells (for example, ICM where proliferative effects of IGF are manifested). As such, it represents an example of co-ordination of proliferation and metabolism.

**GLUT2**

There are conflicting reports of GLUT2 expression. While Hogan et al. (1991) found evidence of the mRNA encoding GLUT2 using the highly sensitive polymerase chain reaction, and Aghayan et al. (1992) were able to detect GLUT2 immunologically, Morita et al. (1992) failed to demonstrate GLUT2 expression in 500 blastocysts using an immunoblotting method sensitive enough to detect it in 25 ng of liver membrane protein (equivalent to one blastocyst). The model proposed is consistent with embryonic physiology and transporter biology without the need to involve GLUT2.

A trophectodermal location for GLUT2 would be inconsistent with its role in other epithelia, where it is normally expressed opposite SGLT2 and mediates the flux of glucose from areas of high glucose concentration, unlike regions of lower glucose concentrations, where the higher affinity SGLT1 works opposite GLUT1 to this end. Moreover, epithelial cells expressing GLUT1 are glycolytic (like ICM cells) in contrast to the cells expressing GLUT2 (Thorens, 1996).

Expression of GLUT2 in the ICM is also problematic. The ~20 mmol l$^{-1}$ $K_m$ value for glucose is the key to understanding the functional niche occupied by GLUT2. As a high $K_m$ transporter, it could not function efficiently in the low glucose environment prevailing in the blastocoel. Furthermore, its general association with gluconeogenic rather than glycolytic cells in the kidney and liver is also incompatible with a role in the glycolytic ICM.

The concentration of glucose in uterine fluid is about 1 mmol l$^{-1}$ (Wales and Edirisinghe, 1989) and that in the blastocoel cavity is approximately half the value in the external environment (Brison et al., 1993). GLUT2 would be the least efficient isoform to supply glucose to morulae and blastocysts. The absence of glucokinase, exclusively coupled to GLUT2 expression, from early mouse embryos (Houghton et al., 1996) further supports this conclusion. In view of the conflicting evidence about GLUT2 in preimplantation embryos, it is difficult to propose a significant function for GLUT2 in the blastocyst. Perhaps it is expressed at concentrations so low as to be functionally meaningless.

**Conclusion**

The discovery of expression of GLUT3 on the apical trophoectoderm membranes, where it enables the blastocyst to obtain large quantities of glucose efficiently and rapidly from the low concentration in the uterine milieu, has filled a gap in our understanding of glucose uptake by the blastocyst. Together with basolateral GLUT1, these transporters deliver glucose to the cells of the ICM without any need for other facilitative or active transporters. Moreover, expression of GLUT1 provides a coordinating link with the proliferative and morphological effects demonstrated by growth factors such as IGF-I and growth hormone. In the cleaving embryo, which does not utilize glucose for fuel, GLUT1 may signal maternal glucose concentrations, enabling the switch from pyruvate to glucose that coincides with the beginning of blastocyst formation. The
GLUT3 expression required for blastocyst formation in the presence of glucose may be linked to the initiation of events involved in the adaptive response of embryos to their substrate environment.

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