Glucose transporter gene expression in early mouse embryos

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Summary

The glucose transporter (GLUT) isoforms responsible for glucose uptake in early mouse embryos have been identified. GLUT 1, the isoform present in nearly every tissue examined including adult brain and erythrocytes, is expressed throughout preimplantation development. GLUT 2, which is normally present in adult liver, kidney, intestine and pancreatic ß cells is expressed from the 8-cell stage onward. GLUT 4, an insulin-recruitable isoform, which is expressed in adult fat and muscle, is not expressed at any stage of preimplantation development or in early postimplantation stage embryos. Genetic mapping studies of glucose transporters in the mouse show that Glut-1 is located on chromosome 4, Glut-2 on chromosome 3, Glut-3 on chromosome 6, and Glut-4 on chromosome 11.

Key words: mouse embryos, glucose transporters, polymerase chain reaction

Introduction

Glucose can be transported across membranes by two different mechanisms: via a sodium-coupled active carrier system that is expressed in epithelial cells (Esposito, 1984) or by a sodium-independent facilitative glucose transporter system (Wheeler and Hinkle, 1985). Five facilitative glucose transport proteins (GLUT 1 to GLUT 5) have been described to date and their cDNAs have been cloned (reviewed in Bell et al. 1990; Thorens et al. 1990; Kasanicki and Pilch, 1990). In adult tissues, the GLUT 1 isoform that is expressed in many tissues and transformed cells and the GLUT 3 isoform that is expressed primarily in human brain and kidney appear to be responsible for constitutive glucose uptake. The GLUT 2 isoform mediates glucose transport in hepatocytes and insulin-producing ß cells. The GLUT 4 isoform is the major transporter responsible for the insulin-stimulated uptake that occurs in muscle and adipose tissue although GLUT 1 expression is also modulated by insulin in muscle and fat cells, but the effect is less significant because GLUT 4 is the primary and most abundant transporter expressed in these cells (Walker et al. 1989; de Herreros and Birnbaum, 1989; Kasanicki and Pilch, 1990). This stimulation by insulin of glucose influx is caused by the translocation of intracellular transporter molecules to the plasma membrane (Cushman and Wardzala, 1980; Suzuki and Kono, 1980; James et al. 1988). GLUT 5, the most recently described isoform, is involved in glucose uptake in the small intestine (Bell et al. 1990). GLUT 2, which has a high Km for glucose (15–20 mM) in the concentration range that stimulates insulin release, is involved in the glucose sensing mechanism of ß cells as well as in glucose uptake (Orci et al. 1989; Thorens et al. 1990; Unger, 1991).

The consensus GLUT molecule is a protein of about 500 amino acids with twelve membrane-spanning domains. There is considerable sequence conservation in these domains between the five members of the GLUT family characterized so far. It is probable that the transmembrane domains are necessary to form the channel through which glucose is carried. The specific mechanism of glucose transport and the molecules involved during early development are of considerable interest since the intracellular level of glucose has a direct bearing on metabolic activity (Leese, 1990). From in vitro studies, glucose does not seem to be essential for embryonic growth until the time of compaction and may even be inhibitory for early
cleavage-stage embryos (Seshagiri and Bavister, 1989; Chatot et al. 1989). However, from the late morula stage onwards, glucose becomes increasingly important to development and is the preferred energy substrate (Biggers and Borland, 1976; Wordinger and Brinster, 1976).

Previous studies have shown that preimplantation embryos in culture respond to exogenous insulin with increased rates of DNA, RNA and protein synthesis (Harvey and Kaye, 1988; Heyner et al. 1989a; Rappolee et al. 1990) and that insulin receptor gene expression can be detected from the 8-cell stage onwards (Rosenblum et al. 1986; Mattson et al. 1988; Rappolee et al. 1990). One aim of this study was to determine which class of glucose transporter molecule was responsible for uptake of glucose by the preimplantation embryo and at which stage the corresponding genes were expressed. Using the reverse transcription–polymerase chain reaction technique (RT–PCR) as well as immunohistochemistry, we have shown that the GLUT 1 isoform is expressed throughout preimplantation development, that GLUT 2 expression is switched on at the 8-cell stage, and that the insulin-recruitable GLUT 4 isoform is not expressed at any stage of preimplantation development.

In the mouse, at least two genetic loci for diabetes mellitus and obesity have been mapped, db and ob (Coleman, 1978). Both of these mutations give rise to obese, insulin-resistant animals and are associated with embryonic malformation and lowered fertility. It was of considerable interest to map the glucose transporter genes (Glut-1–Glut-4) in the mouse genome to determine linkage to the above markers of diabetes. The results of these analyses are presented below.

Materials and methods

Embryo recovery

Random-bred CD1 mice (Charles River Breeding Laboratories) were used for the RT–PCR and immunofluorescence studies. Procedures for superovulation and recovery of oocytes and preimplantation embryos were as described by Giebelhaus et al. (1983) and Rosenblum et al. (1986). Procedures for dissection of 7.5 to 9.5 day preimplantation stage embryos and culture of F9 embryonal carcinoma cells were as described by Telford et al. (1990).

RNA extraction

RNA was extracted from pools of 100–300 preimplantation embryos in the presence of 10 µg E. coli ribosomal RNA (Boehringer-Mannheim) as described by Hahnel et al. (1990). Embryos in 100 µl of buffer (0.2 M NaCl, 1 mM EDTA, 25 mM Tris–HCl pH 7.4) were vortexed with an equal volume of phenol and chloroform and phases were separated by centrifugation. The aqueous phase was re-extracted with chloroform, recovered after centrifugation and precipitated in ethanol. RNA pellets were re-dissolved in 5 µl of sterile distilled water and reverse transcribed. RNA from adult tissues was extracted by lysis in guanidinium isothiocyanate followed by ultracentrifugation through CsTFA (Berger and Kimmel, 1987). RNA was purified from postimplantation stage embryos and cultured F9 cells exactly as described by Telford et al. (1990).

Reverse transcription and polymerase chain reaction

RNA from embryos was reverse transcribed into cDNA as described by Telford et al. (1990). In brief, purified RNA was incubated at 43°C for 60 min with a mixture of 20 units of AMV reverse transcriptase (Molecular Genetics Resources), 0.2 µg oligo-dT (Pharmacia), 3 mM MgCl2, 50 mM Tris–HCl pH 8.3, 60 mM KCl, 1 µg nuclease-free bovine serum albumin (BSA), 1 mM each of dATP, dCTP, dGTP and dTTP (Pharmacia), and 5 units of RNAguard (Pharmacia) in a volume of 10 µl. The reverse transcription reaction was then repeated by addition of 12.5 units of fresh AMV reverse transcriptase after a 95°C, 3 min denaturation step and flash cooling to 4°C. The reaction mix was diluted with sterile distilled water to 30 µl and 3 µl aliquots were used for each PCR reaction. PCR (Saiki et al. 1988) was performed as described previously (Rappolee et al. 1986; Telford et al. 1990). One tenth of the reverse transcription reaction (3 µl) was amplified with 1 unit Taq polymerase (Perkin Elmer-Cetus) in a final volume of 50 µl containing 10 mM Tris–HCl pH 8.3, 2.5 mM MgCl2, 50 mM KCl, 5 µg nuclease-free BSA, 1.5 µM of each sequence-specific primer, and 0.6 mM of each dNTP. The mixture was overlaid with mineral oil to prevent evaporation and then amplified by PCR for 40 cycles in a DNA thermal cycler (Perkin Elmer-Cetus), where each cycle included denaturation at 94°C for 1 min, reannealing of primers to target sequences at 55°C for 2 min, and primer extension at 72°C for 2 min. The PCR products (20 µl) were run on 2% agarose gels containing 0.5 µg ml−1 ethidium bromide and photographed.

Primer pairs

The Glut-1 primer pair was constructed from the sequence of the rat Glut-1 cDNA (Birnbaum et al. 1986). The 5′ primer is identical to nucleotides 1263 to 1286 (24-mer) except for nucleotide 1280 which was changed from a T to an A residue in order to create a PstI site for cloning purposes. The 3′ primer is the reverse complement of nucleotides 1600 through 1623 (24-mer). The Glut-2 primers were derived from the corresponding mouse cDNA sequence (Asano et al. 1989). The 5′ Glut-2 primer is identical to nucleotides 1211 through 1231 (21-mer); its 3′ partner is the reverse complement of nucleotides 1606 through 1628 (23-mer). The Glut-3 primer pair was derived from the human Glut-3 cDNA sequence (Kayano et al. 1988). The 5′ primer is identical to nucleotides 1290 through 1319 (30-mer); the 3′ primer is the reverse complement of nucleotides 1647 through 1670 (24-mer). The Glut-4 primer pair was derived from the sequence of the rat cDNA (Charron et al. 1989). The 5′ primer is the same as nucleotides 1104 to 1127 (24-mer) of the cDNA and its 3′ partner is the reverse complement of nucleotides 1324 through 1347 (24-mer).

A primer pair for mouse β-actin cDNA was included as an internal control. Cytoplasmic β-actin transcripts are expected to be present in all cell types, and the particular primer pair design was such that it spanned the first intron of the rodent β-actin gene (Nudel et al. 1983), which is 87 bp in length. This primer pair can, therefore, be used to detect both the presence of β-actin mRNA (cDNA) via a predicted 243 bp PCR fragment and contaminating genomic DNA, if present, as a 330 bp fragment. The 5′ primer (21-mer) is identical to nucleotides 182 to 202 in the cDNA sequence (Tokunaga et al. 1986). The 3′ primer is the reverse complement of nucleotides 403 through 424 (22-mer). The target sequence bracketed by the β-actin primer pair is approximately 2000 bp from the 3′.
end of the cDNA; therefore, detection of a β-actin PCR fragment indicates that reverse transcription produced β-actin cDNAs at least 2 kb in length.

All primers were obtained from the Regional DNA Synthesis Laboratory, University of Calgary.

Antibodies
All antibodies were anti-peptide antibodies produced in rabbits. Antibodies to the GLUT 1 transporter were directed against the COOH-terminal 16 amino acids (Kahn et al. 1991); antibodies to the murine GLUT 2 were also raised against COOH-terminal amino acids (a.a. 512–523) using the procedure outlined by Thorens et al. (1988). Two antibodies to GLUT 4 were raised against the COOH-terminal 16 amino acids (Baldini et al. 1991) and the NH2-terminal 13 amino acids, respectively (Kahn et al. 1991). Control sera were either preimmune sera (GLUT 1, 4) or reconstituted rabbit IgG (GLUT 2). In addition, experiments were designed so that several developmental stages were examined under identical conditions, thus providing an internal control. The specificity of the antisera to GLUT 1 and GLUT 4 was checked by immunoblotting from target tissues, and from Xenopus oocytes after microinjection with an mRNA containing the appropriate transcript (Baldini et al. 1991). In the case of GLUT 2, preabsorption of the antibody with the immunizing peptide completely blocked the reactivity of the antisemur to GLUT 2 as detected by western blotting of total liver lysate.

Immunofluorescent labelling
All manipulations were carried out in microdrops with the exception of a few early experiments in which the embryos were immobilized on a lawn of fibroblasts. Oocytes and developmental stages were fixed in 3% neutral buffered formaldehyde for 30 min. They were then permeabilized by incubating for 60 min in 20% donkey serum in phosphate-buffered saline containing 2% bovine serum albumin (PBS–BSA). Oocytes and embryos were then washed three times with PBS–BSA, and incubated in the primary antibody at dilutions ranging from 1:200 to 1:1000 for 30 min. The developmental stages were then washed extensively with PBS–BSA and incubated with the secondary antibody, rhodamine-conjugated swine anti-rabbit IgG (Accurate Chemical and Scientific Corp.) at a concentration of 1:80 for 20 min. Following a final wash, the developmental stages were mounted in drops of 30% glycerol in PBS under a supported coverslip, and evaluated with a Zeiss photomicroscope equipped for epifluorescence. Developmental stages were photographed using Kodak Tri X film using a standard exposure time for all fluorescent records and were also recorded under bright-field phase contrast.

Cloning and sequencing
The GLUT 1 PCR fragment was isolated from an agarose gel using GeneClean (Bio 101), cut with PstI, which cleaved within the 5’ primer, and Sau3A, which cleaved 31 bp from the 3’ end, then cloned into PstI–BamHI-cleaved pBluescript KS+(Stratagene). The gel-purified GLUT 2 PCR fragment was blunt ended with Klenow fragment in the presence of dNTPs, cut with PstI which cleaved 77 bp from the 3’, and cloned into PstI–HindIII-cleaved pBluescript KS+. Sequencing was done by the dideoxy method as described by Sanger et al. (1977) using the M13 universal primer for one strand and the M13 reverse sequencing primer for the opposite strand.

Interspecific backcross mapping
Interspecific backcross progeny were generated by mating (C57BL/6J×M. spretus) F1 females and C57BL/6J males as previously described (Copeland and Jenkins, 1991). A total of 205 N2 progeny were obtained; a subset of these N2 mice were used to map the Glut loci (see text for details). DNA isolation, restriction enzyme digestion, agarose gel electrophoresis, Southern blot transfer and hybridization were performed essentially as described (Jenkins et al. 1982). All blots were prepared with Zetabind nylon membrane (AMF-Cuno); probes were labelled with ([α-32P]dCTP using a nick translation labelling kit (Boehringer Mannheim). The probes and RFLPs for the Glut loci are as follows. The Glut-1 probe (Birnbaum et al. 1986) detected a 2.4 kb M. spretus-specific HindIII fragment that was followed in these studies. The Glut-2 locus was mapped by following the inheritance of a 9.0 kb PstI fragment present in M. spretus DNA when probed with a rat cDNA clone (Thorens et al. 1988). The Glut-3 probe (Kayano et al. 1988) detected 7.8 and 2.7 kb PstI M. spretus-specific fragments that cosegregated in this analysis. TagI digestion of M. spretus DNA and hybridization with the Glut-4 probe (Charron et al. 1989) revealed a 2.6 kb fragment that segregated in backcross mice.

A description of probes and RFLPs for the loci linked to the Glut loci have been reported (Ceci et al. 1989; Mucenski et al. 1988; Siracusa et al. 1991; Cox et al. 1991; de Lapeyriere et al. 1990; Goodwin et al. 1991; and Buchberg et al. 1989) with the exception of the Ret proto-oncogene. The probe for the Ret locus (Takahashi and Cooper, 1987) detected 3.3 and 2.0 kb TagI M. spretus-specific fragments that cosegregated and placed the locus on mouse chromosome 6. Recombination distances were calculated as described (Green, 1981) using the computer program Spretus Madness. Gene order was determined by minimizing the number of recombination events required to explain the allele distribution patterns.

Results
RNA was isolated from batches of oocytes and from embryos at the 2-cell, 8-cell, morula and blastocyst stages of development. The purified RNA was primed with oligo-dT and reverse transcribed. One-tenth of the product was used for amplification by PCR for each primer pair specific for actin cDNA or for cDNAs for the various glucose transporters. The assays were repeated at least three times with different embryo batches. Each cDNA preparation was tested for genomic DNA contamination by PCR amplification using primers that flank a target sequence of the actin gene that contains an 87 bp intron. If genomic DNA was present in the cDNA, a 330 bp target sequence would be amplified as well as the 243 bp fragment representing the cDNA (Telford et al. 1990). No 330 bp PCR product for actin was detected in any of the RT–PCR reactions with embryo RNA samples (see Fig. 1A).

The primers designed to amplify target sequences of the glucose transporter cDNAs were chosen from the 3’ region of the coding sequences. The amino acid sequences of GLUT 1 and GLUT 4 share 65% identity and the amino acid sequences of GLUT 1 and 2 and GLUT 2 and 4 share 55% and 54% identity, respectively (Gould and Bell, 1990). Thus, the nucleic
Fig. 1. Detection of transcripts in RNA from preimplantation embryos by RT–PCR. RNA from 200 oocytes (Oc), 225 two-cell (2), 200 eight-cell (8), 238 morula (M), and 184 blastocyst (B) stage embryos was reverse transcribed with oligo-dT, and one-tenth of the reverse transcription product was amplified by 40 cycles of PCR with oligonucleotides specific for β-actin or GLUT cDNAs as described in Materials and Methods. Part (20 µl) of the PCR product was resolved on 2% agarose gels along with 2 µg of 1 kb ladder (Bethesda Research Laboratories; lanes marked L). (A) RT–PCR using β-actin primers. (B) RT–PCR with GLUT 1 primers. The arrow marks the position of the expected 361 bp PCR product for GLUT 1. (C) RT–PCR using GLUT 2 primers. The arrow marks the position of the expected 418 bp PCR product for GLUT 2. (D) RT–PCR with GLUT 4 primers. The lane marked LM contains the 244 bp PCR product of reverse transcribed leg muscle RNA using GLUT 4 primers. Abbreviations are as follows: L=1 kb ladder; Li=liver; LM=leg muscle; BR=brain.

Acid sequences are sufficiently dissimilar to allow the design of primers that do not cross-react during amplification. The primers were tested on liver, muscle and brain cDNA to ensure that the appropriate-sized target sequence was amplified. The primers for this study spanned the regions from the 3' end of the ninth transmembrane domain to 60 bp from the end of the coding sequence in the case of GLUT 1; from the 3' end of the ninth transmembrane domain to the end of the coding sequence for GLUT 2; and from the 3' end of the ninth to the 5' end of the twelfth transmembrane region for GLUT 4.

Examples of results of PCR amplification of cDNA from preimplantation mouse embryos are shown in Fig. 1. Each PCR assay contained reverse transcribed RNA from roughly equivalent numbers of embryos because total embryo mass does not change greatly during the preimplantation period (Schultz, 1986). Glut-1 mRNA was detected at all stages of development from egg through to blastocyst as is shown by a band of the expected 361 bp target size (see arrow, Fig. 1B). To verify that this band was a PCR product of Glut-1 cDNA, it was excised, cloned and sequenced (Fig. 2A). A comparison of the cloned GLUT 1 sequence from mouse embryos and the rat Glut-1 sequence (Birnbaum et al. 1986) is shown (Fig. 2A). Over the 321 bp of sequence presented, which includes the Psrl and the internal Sau3A restriction sites used for cloning, there is 96% nucleotide sequence identity and 99% amino acid sequence identity between the mouse and the rat sequences.

Glut-2 mRNA was detectable after the 8-cell stage as shown by the expected 418 bp band (see arrow, Fig. 1C). The identity of the band was confirmed by cloning and sequencing (Fig. 2B). The cloned 342 bp component of the mouse Glut-2 PCR fragment shares 100% nucleotide sequence identity with the published mouse sequence (Asano et al. 1989) and 94% nucleotide sequence identity and 96% amino acid sequence identity with the corresponding rat cDNA sequence (Thorens et al. 1988). Other amplified products were
Fig. 2. Sequences of the cloned Glut-1 and Glut-2 PCR products. (A) The PCR target sequence of the mouse Glut-1 cDNA is shown in the upper row in bold face. The corresponding rat cDNA is shown in the upper row in bold face. The amino acids corresponding to the nucleotide sequence are identified above and below each codon with differences in the mouse sequence relative to the rat sequence underlined.

observed in some of the PCR assays but the identification of these bands was not attempted.

In preliminary RT-PCR experiments, no expression of GLUT 3 was detected in preimplantation embryos but antibodies are not yet available to verify this result. The nucleotide sequence of Glut-5 has not been published so the design of primers for RT-PCR assays is not yet possible.

No expression of GLUT 4 was detected at any stage of preimplantation development. However, a strong band of the expected target size (244 bp) was generated when cDNA made from leg muscle RNA was used in the PCR assay (Fig. 1D). The identity of the Glut-4 PCR band generated from muscle cDNA was verified by cleavage with AluI which cut the fragment at a site predicted from the cDNA sequence (data not shown).

The pattern of GLUT expression in postimplantation embryos at 7.5 to 9.5 days post coitum and in F9 embryonal carcinoma cells was also analysed. As was the case during preimplantation development, GLUT 1 and GLUT 2 RT-PCR products were detected but no expression of GLUT 4 was observed (see Fig. 3A for 8.5 day embryo RNA and Fig. 3B for F9 cell RNA). At 9.5 days of development, the mouse embryo has about 20 somites (muscle primordia) but these cells apparently do not express GLUT 4 characteristic of differentiated muscle cells. A possible explanation may be that GLUT 1 is sufficient for glucose uptake at this stage of development since Glut-1 mRNA has been detected in most tissues (Bell et al. 1990). Alternatively, Glut-3 was originally cloned from fetal muscle (Kayano et al. 1988) and could be mediating the transport of glucose.

Indirect immunofluorescence studies carried out with antibodies directed against mouse GLUT 1 transporter peptides showed that developmental stages from the oocyte through to the blastocyst reacted positively (Fig. 4). Similar studies with antibodies directed against the GLUT 2 transporter peptides showed positive reactions in mouse embryos at the morula through blastocyst stages (Fig. 5). These results show that the protein products of Glut-1 and Glut-2 are present as well as their mRNAs. No immunofluorescence was detected when antibodies to GLUT 4 were reacted with mouse embryos of similar developmental stages (Fig. 5).

The mouse chromosomal locations of the four genes encoding glucose transporter proteins (designated Glut-1, Glut-2, Glut-3 and Glut-4) were determined by interspecific backcross analysis using progeny derived from matings of [(C57BL/6J x Mus spretus)F1 x C57BL/6J] mice (see Materials and methods). This interspecific backcross mapping panel has been typed for over 675 loci that are well distributed among all the autosomes as well as the X chromosome (Copeland and Jenkins, 1991). C57BL/6J and M. spretus DNAs were digested with several restriction endonucleases and analyzed by Southern blot hybridization for informative restriction fragment length polymorphisms (RFLPs) using the GLUT probes. The strain distribution pattern (SDP) of each RFLP in the interspecific backcross mice
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Fig. 3. Detection of Glut transcripts in RNA from postimplantation embryos and from F9 embryonal carcinoma cells by RT-PCR. (A) 1 μg of RNA from embryos at 8.5 days post coitum was reverse transcribed using oligo-dT, and one-tenth of the reverse transcription product was amplified by 40 cycles of PCR with oligonucleotides specific for Glut-1 (1), Glut-2 (2), and Glut-4 (4). The 1 kb ladder (L) is used as molecular weight marker. (B) 1 μg of RNA from F9 embryonal carcinoma cells was reverse transcribed as in the panel above and one-tenth of the RT product was used for PCR with primers for Glut-1 (1), Glut-2 (2) and Glut-4 (4). was then determined and used to position the Glut loci on the interspecific map (Fig. 6).

Each locus segregated independently. The Glut-1 locus was assigned to mouse chromosome 4, 1.7 cM proximal of Lmyc. This placement of Glut-1 is consistent with the recent results of Bahary et al. (1990). The Glut-2 locus was placed on mouse chromosome 3. This gene did not recombine with Evi-1 in 150 mice typed in common for both probes. This suggests that Glut-2 and Evi-1 reside within 2.0 cM (upper 95% confidence level). Glut-3 was placed on mouse chromosome 6, 2.0 cM distal to Ret and 0.7 cM proximal of Ly.

Fig. 4. Immunofluorescent localization of GLUT 1 transporter protein. The left-hand panel shows a developmental series of preimplantation mouse embryos under bright-field phase-contrast optics and the right-hand panel shows the image under epifluorescence. Stages from oocyte (1a,b), two-cell (2a,b), eight-cell (3a,b), and blastocyst (4a,b) showed positive reactivity, while a blastocyst treated under the same conditions with preimmune serum was negative (5a,b). The bar represents 40 μm.
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Finally, Glut-4 was placed in the middle region of mouse chromosome 11, tightly linked to Trp53-1. The fact that no recombination between the two genes in 135 mice typed in common was observed suggests that they reside within 2.2cM of each other (upper 95% confidence level).

Discussion

The facilitative glucose transporters are encoded by a small multi-gene family and various members have distinct tissue distributions (Bell et al. 1990; Thorens et al. 1990). This study focuses on the three most extensively characterized isoforms: GLUT 1, GLUT 2, and GLUT 4. Human Glut-3 cDNA has been isolated from a fetal skeletal muscle library and is expressed in various adult tissues, primarily brain and kidney (Kayano et al. 1988). The murine Glut-3 cDNA has now been cloned and the sequence (as yet unpublished) is significantly divergent from the human Glut-3 cDNA (G. Bell, personal communication). This may explain, in part, our inability to detect this mRNA using oligonucleotides based on the human cDNA sequence. When the murine Glut-3 sequence becomes available, we will be able to address this question accurately.

Preimplantation mouse embryos require pyruvate and lactate as energy sources until the 8-cell stage and then switch to a glucose-based metabolism (Gardner and Leese, 1986). Glucose is a precursor of a number of macromolecular cell constituents: complex sugars for mucopolysaccharides, ribose moieties for nucleic acid synthesis and glycerol phosphate for phospholipids. Using the selective inhibitors phlorizin and phloretin, it was determined that glucose uptake in preimplantation embryos is mediated by sodium-independent facilitative glucose transporters (Gardner and Leese, 1988) so it is important to examine the functional role of GLUT proteins in early development. GLUT 1 is expressed throughout preimplantation development and appears to be responsible for the low levels of glucose uptake observed from the 1-cell stage onwards by Gardner and Leese (1988). Since the zygotic genome in the mouse is not activated until the 2-cell stage, the Glut-1 PCR band from oocyte RNA is presumably due to the presence of maternal mRNA. At the 8-cell stage of development, GLUT 2 begins to be expressed and stays switched on through the morula and blastocyst stages. This may be responsible, along with an increase in GLUT 1, for the rise in glucose uptake that begins at the 8-cell stage (Gardner and Leese, 1988). Since insulin receptor gene expression can be detected in embryos from the 8-cell stage onwards (Heyner et al. 1989a; Schultz et al. 1990), it seemed possible that the insulin-recruitable GLUT 4 isoform might also be involved in glucose transport after the 8-cell stage. However, no expression of GLUT 4 was detected with either RT-PCR methods or

Fig. 5. Immunofluorescent localization of GLUT 2 transporter protein. The left-hand panel shows a developmental series of preimplantation mouse embryos under bright-field phase-contrast optics and the right-hand panel shows the image under epifluorescence. Embryos at the two-cell (1a,b) and eight-cell (2a,b) stages were negative, while morula and blastocyst stages showed positive reactions (3a,b, 4a,b). All stages were negative when incubated with antisera directed against GLUT 4; 5a,b shows a representative result of incubating blastocyst stage embryos. The bar represents 40 μm.
antibodies specific to GLUT 4 at any stage up to the blastocyst.

A number of studies on the anabolic and proliferative effects of insulin on the early mouse embryo have been reported (Harvey and Kaye, 1988, 1990; Heyner et al, 1989b; Gardner and Kaye, 1991) but the effects of insulin on glucose uptake by preimplantation mammalian embryos is not entirely clear. Since insulin causes an increase in glucose uptake in adult cells such as adipocytes (Charron et al, 1989) and since expression of the insulin receptor gene has been detected in preimplantation embryos (Schultz et al, 1990), an association between insulin and glucose uptake during early development was suspected. Insulin at concentrations ranging from 0.0017 to 170 nM has been reported to stimulate labelled glucose uptake in vitro in preimplantation mouse embryos (Gardner, 1990). In addition, blastocysts collected from streptozotocin-induced diabetic mice (Beebe and Kaye, 1991) and rats (Brison and Leese, 1990) show impaired development. The uptake of glucose in rabbit blastocysts is also mediated by sodium-independent glucose transporters but in this species, glucose transport appears to be unaffected by the addition of insulin at concentrations of 100 nM (Robinson et al, 1990). Our data show that the glucose transporter isoforms GLUT 1 and GLUT 2 are the likely mediators of glucose uptake in the early mouse embryo and are in agreement with observations obtained using western blot analysis and high resolution immunocytochemistry (Rao et al, 1990). Further studies are needed to establish the conditions under which transport of glucose in preimplantation embryos by GLUT 1 and GLUT 2 might be influenced by insulin and to determine whether the mitogenic and other physiological effects of insulin are distinct from its role in sugar metabolism.

In this report, we also present the genetic mapping of Glut-1-Glut-4 in the mouse genome. Glut-1 is located on chromosome 4, Glut-2 on chromosome 3, Glut-3 on chromosome 6 and Glut-4 on chromosome 11. Alignment of the interspecific map with the composite map compiled by M. T. Davisson, T. H. Roderick, A. L. Hillyard, and D. P. Doolittle and provided by GBASE (a computerized data base maintained at The Jackson Laboratory, Bar Harbor, ME) has failed to identify any known mouse mutation that maps in the vicinity of a Glut locus that has a phenotype consistent with a defect in one of these genes. Finally, many regions of homology have been identified between mouse and human chromosomes and it is often possible to predict where a gene will map in one species based on its position in the other. From the mouse mapping studies, we would suggest that Glut-1 is located on human 1p, Glut-2 on human 3q, Glut-3 on human 12p, and Glut-4 on human 17p. These expectations have been confirmed from human mapping studies and the precise location of the GLUT loci in human chromosomes is summarized in Fig. 6. These studies also demonstrate
that the GLUT loci are dispersed in the human genome as well.

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