KUOPION YLIOPISTON JULKAISUJA C. LUONNONTIETEET JA YMPÄRISTÖTIETEET 140
KUOPIO UNIVERSITY PUBLICATIONS C. NATURAL AND ENVIRONMENTAL SCIENCES 140

HELI TEERIJOKI

Fish Glucose Transporters
Molecular Cloning and Functional Characterization

Doctoral dissertation

To be presented by permission of the Faculty of Natural and Environmental Sciences of the University of Kuopio for public examination in Auditorium L23, Snellmania building, University of Kuopio, on Saturday 18th May 2002, at 12 noon

Institute of Applied Biotechnology
University of Kuopio

KUOPION YLIOPISTO

KUOPIO 2002

Distributor: Kuopio University Library
P.O.Box 1627
FIN-70211 KUOPIO
FINLAND
Tel. +358 17 163 430
Fax +358 17 163 410

Series editor: Professor Lauri Kärenlampi, Ph.D.
Department of Ecology and Environmental Science

Professor Jari Kaipio, Ph.D.
Department of Applied Physics

Author´s address: Institute of Applied Biotechnology
University of Kuopio
P.O.Box 1627
FIN-70211 KUOPIO
FINLAND
Tel. +358 17 163 061
Fax +358 17 163 752
E-mail: Heli.Teerijoki@uku.fi

Supervisors: Professor Leena Alhonen, Ph.D.
A.I. Virtanen Institute

Senior Assistant Hannu Mölsä, Lic.Phil.
Institute of Applied Biotechnology

Researcher Aleksei Krasnov, Ph.D.
Institute of Applied Biotechnology

Reviewers: Professor Emerita Erika M. Plisetskaya
School of Fishery and Aquatic Sciences
University of Washington
Seattle, USA

Professor Mikko Nikinmaa
Department of Biology
Laboratory of Animal Physiology
University of Turku

Opponent: Professor Gwyn W. Gould
The Henry Wellcome Laboratory of Cell Biology
Division of Biochemistry and Molecular Biology
University of Glasgow
Glasgow, UK

ISBN 951-781-238-8
ISSN 1235-0486

Kuopio University Printing Office
Kuopio 2002
Finland

Teerijoki, Heli. Fish glucose transporters: molecular cloning and functional characterization. Kuopio University Publications C. Natural and Environmental Sciences 140. 2002. 75 p.
ISBN 951-781-238-8
ISSN 1235-0486

ABSTRACT

The facilitative glucose transporters (GLUTs) play an important role in sugar metabolism and belong to one of the most ubiquitous and diverged multi-gene family. Glucose transporters have been extensively studied in bacteria, protozoa and higher vertebrates but their existence in teleost fish has been unclear. Carnivorous fish have limited ability to utilize carbohydrates when compared to mammals. Feeding of salmonid fish like rainbow trout (*Oncorhynchus mykiss*) with a high level of digestible carbohydrates results in persistent hyperglycaemia due to the slow clearance of the glucose load from the blood. This can be accounted for by lack of or by low expression of facilitative glucose transporters. When this study was initiated, results of sugar uptake analyses hinted at the existence of facilitative glucose transporters in fish but no genes had been cloned. The aim of this study was to clone and identify fish glucose transporters and to study their structure, phylogeny, expression and functional properties.

Three GLUTs of two types (OnmyGLUT1, CyiGLUT1 and OnmyGLUT2) were cloned from rainbow trout and carp (*Cyprinus carpio*) EPC cell line and dot blot analyses suggested that new isoforms are likely to be found in fish. Phylogenetic studies showed that the multi-gene family of glucose transporters became diversified at an early stage of vertebrate evolution. OnmyGLUT1 is the predominant embryonic isoform, being especially abundant in neural tissues of embryos. An abundant expression of OnmyGLUT1 was found in heart and low-level expression in skeletal muscle, liver, spleen, blood and brain of adult rainbow trout. OnmyGLUT2 was expressed in the liver, kidney and intestine. The high level of CyiGLUT1 expression in the EPC cell line suggests that as is the case in mammals, glucose metabolism of fish cells may be enhanced by malignant transformation. The functional properties of fish GLUT1 were studied in detail. Recombinant OnmyGLUT1 was expressed in *Xenopus* oocytes and glucose transport mediated by the transporter present in EPC cells (CyiGLUT1) was analysed. The results showed that these proteins are fully functional and similar to mammalian GLUT1 in their kinetics and substrate selectivity. The ability of OnmyGLUT2 to transport glucose was confirmed by gene transfer into rainbow trout embryos and its activity was demonstrated in isolated hepatocytes. These results suggested that the poor utilization of carbohydrates in carnivorous fish is not due to the lack or abnormal functional properties of glucose transporters, the role of these proteins in glucose metabolism remains to be elucidated.

Universal Decimal Classification: 597.5, 577.113, 577.124, 591.133
CAB Thesaurus: fish feeding; rainbow trout; carp; carbohydrate metabolism; dietary carbohydrate; glucose; glucose tolerance; nutrient transport; gene expression; DNA cloning; phylogeny; characterization

ACKNOWLEDGEMENTS

This study was carried out in the Institute of Applied Biotechnology in 1999-2001. This work has been generously supported by the Technology Development Center, Finland (TEKES) and grants from the University of Kuopio Foundation, Olvi Foundation and Kuopio Naturalists's Society are also gratefully acknowledged.

I wish to thank my supervisor, Professor Leena Alhonen, Ph.D., to taking me and my fish under her guidance and for her support throughout the work. My sincere thanks are offered to my supervisor and our Research Director Hannu Mölsä, Lic.Phil., for giving me the opportunity to work with genes and for his warm and constant encouragement during these years and also for his marvellous ability to initiate new projects. I express my deepest gratitude to my closest supervisor Dr. Aleksei Krasnov, Ph.D., for his enormous patience and willingness to explain things to me a million times. Thank you for all your guidance and optimisms even during those days when I was so pessimistic :)

I also wish to thank the reviewers of this thesis Professor Erika M. Plisetskaya and Professor Mikko Nikinmaa for their constructive comments.

I have enjoyed collaboration and friendship with Dr. Tiina Pitkänen-Arsiola, who has shown me that it is possible to be a woman with both a career and a "big" family. You have given me the greatest support and a shoulder to lean on during these years and you will always be in my heart even though there may be an ocean between us. My thanks belong also to former and current members of our research group Heikki Koskinen, M.Sc. and Heikki Ryynänen, M.Sc. for providing pleasant working conditions in the lab.

The whole staff of the Institute of Applied Biotechnology, not forgetting the men in the Aquaculture Research Unit, are also gratefully acknowledged for the warm atmosphere, especially Helena Könönen, Marketta Lämsä, Elina Reinikainen and other working pals around the coffee table for their crazy humour and passion for cakes…….. ;)

I wish to thank my co-authors: Dr. Sanjeev Krishna and his group in London for fruitful collaboration and keeping me as a member of their research team during my stay in London and Dr. Yuri Gorodilov for his enormous knowledge of fish embryos.

I am also grateful to all my friends in Kuopio, Kemi, and Helsinki. My special thanks belong to my friends Hansu and Riitta for all those happy and marvellous study years in Kuopio and the times thereafter, and also to my cousin Minna who has always been there for me for as long as I can remember.

My humble thanks go to my mother, Ritva and my sister, Pirkko and her "boys" for always supporting and believing in me, and to my little brother, Jani who has shown me that it is possible to enjoy the simple things in life. I wish to thank also my other relatives who have supported me during these years. My biggest and warmest thanks belong to my life companion Mika, for his endless understanding and support for my "boring" work and our precious, "always too short time" together.

Kuopio, April 2002

ABBREVIATIONS

ATB-BMPA	2-N-4- (1-azi-2, 2, 2-tri-fluoroethyl) benzoyl-1, 3-bis (D-mannos-4-yloxy)-2-propylamine
bt	brown trout
cAMP	cyclic adenosine monophosphate
cDNA	complementary DNA
CHO	Chinese hamster ovary cells
CMV	cytomegalo virus
COS	African green monkey kidney cells
Cyi	Cyprinus carpio
CRE	cyclic AMP-responsive element
EPC	epithelioma papulosum cyprini
GLUT	glucose transporter
h	human
HK	hexokinase
K_i	inhibition constant
K_m	Michaelis constant
KCN	potassium cyanide
mRNA	messenger RNA
Onmy	Oncorhynchus mykiss
SRE	serum response element
TRE	12-O-tetradecanoylphorbol-13-acetate responsive element
PCR	polymerase chain reaction
RT-PCR	reverse transcription PCR
TM	transmembrane
UTR	untranslated region
V_{max}	maximum velocity
1-DOG	1-deoxy-D-glucose
2-DOG	2-deoxy-D-glucose
6-DOG	6-deoxy-D-glucose
3-OMG	3-O-methyl-D-glucose

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following papers, referred to in the text by their Roman numerals:

I Teerijoki, H., Krasnov, A., Pitkänen, T.I., Mölsä, H. (2000). Cloning and characterization of glucose transporter in teleost fish rainbow trout (*Oncorhychus mykiss*). Biochim Biophys Acta 1494: 290-294.

II Teerijoki, H., Krasnov, A., Pitkänen, T.I., Mölsä, H. (2001). Monosaccharide uptake in common carp (*Cyprinus carpio*) EPC cells is mediated by facilitative glucose carrier. Comp Biochem Physiol B 128: 483-491.

III Krasnov, A., Teerijoki, H., Mölsä, H. (2001). Rainbow trout (*Oncorhychus mykiss*) hepatic transporter. Biochim Biophys Acta 1520: 174-178.

IV Teerijoki, H., Krasnov, A., Gorodilov, Y., Krishna, S., Mölsä, H. (2001). Rainbow trout glucose transporter (OnmyGLUT1): functional assessment in *Xenopus* oocytes and expression in fish embryos. J Exp Biol 204: 2667-2673.

1 **INTRODUCTION** **13**

2 **REVIEW OF THE LITERATURE** **15**

2.1 Superfamily of sugar transporters 15
2.2 Facilitative glucose transporters 16
2.2.1 Structure 16
2.2.2 Tissue distribution and regulation of expression 24
2.2.3 Functional properties 30

2.3 Glucose metabolism in fish 35
2.3.1 Carbohydrate nutrition in teleost fish 35
2.3.2 Digestion and absorption of carbohydrates 35
2.3.3 Intermediate glucose metabolism in fish 36
2.3.4 Existence of facilitative glucose transporters 39

3 **AIMS OF THE PRESENT STUDY** **42**

4 **MATERIALS AND METHODS** **43**

4.1 Fish and cell cultures 43
4.2 PCR-cloning 43
4.3 Sequence analysis 43
4.4 DNA dot blot and PCR analysis (I) 43
4.5 Expression studies 44
4.6 Transport assays in embryos and cells 44
4.7 Functional assessment of recombinant GLUTs 45
4.8 Statistics 45

5 **RESULTS** **46**

5.1 Cloning of fish glucose transporters (I-III) 46
5.2 Structure and phylogeny of fish glucose transporters (I-III) 47
5.3 Expression of fish glucose transporters (I, III, IV) 49
5.4 Transport activity and functional properties of fish GLUTs (I-IV) 49

6 **DISCUSSION** **52**

6.1 Identification and structure of fish glucose transporters 52
6.2 Tissue expression of fish glucose transporters 54
6.3 Activity and functional properties of fish glucose transporters 56

7 **SUMMARY AND CONCLUSIONS** **60**

8 **REFERENCES** **62**

APPENDIX: ORIGINAL PUBLICATIONS

1 INTRODUCTION

Glucose is required as a nutrient by nearly all cells for energy production and synthesis of sugar-containing macromolecules. In nature, the ubiquitous use of glucose as a common currency of metabolism is linked to the abundance of glucose units in the form of cellulose and starch. The facilitative glucose transporters (GLUTs) play a key role in sugar metabolism and belong to one of the most ubiquitous and diverged multi-gene families. Glucose transporters have been extensively studied in bacteria, protozoa and higher vertebrates, and mammalian glucose transporters have been one of the most important models to study the structure to function relationships of membrane proteins. In the past few years, an exponential increase in the amount of information regarding the molecular structures and function of glucose transporter proteins involved in the transport of glucose across the plasma membrane has provided new insight into the physiological basis of glucose homeostasis (Mueckler, 1994a).

At an early stage of the present work, the existence of facilitative glucose transporters in teleost fish was still unsolved. Predator fish such as salmonids, are known for their limited ability to utilize dietary carbohydrates. In general, not more than 20% of digestible carbohydrates are recommended for farmed coldwater fish (Wilson, 1994). Feeding the salmonid fish, such as rainbow trout with a high level of digestible carbohydrate results in persistent hyperglycaemia due to the very slow clearance of the glucose load from the blood (reviewed in Legate et al., 2001). Numerous attempts to find facilitative glucose transport in fish cells and tissues have provided contradictory results and no GLUT-like genes have been cloned from fish.

Identification and characterization of fish facilitative glucose transporters are important if we are to achieve a better understanding of the evolution of this multi-gene family and the structure to function relationship of the transporters. Characterization of fish glucose transporters may also provide an explanation at a molecular level for the limited ability of fish to use carbohydrates. This problem is of major importance, both for fish physiology and aquaculture. Carbohydrates are the least expensive form of dietary energy as compared to proteins and lipids. Due to the poor nutritional value of carbohydrates, fish protein and fat represent the major source of energy in salmonid feeds. The predicted demand for fish oil is expected to exceed its availability in the immediate future. Better carbohydrate utilization would decrease feed costs and reduce environmental pollution, which are a result of the high nitrogen/phosphorous meals (reviewed in Wilson, 1994; Moon, 2001).

This thesis presents an overview of mammalian facilitative glucose transporters and a summary of glucose metabolism in fish with special reference to hexose transport, which has been studied only in a few fish species. The cloning of facilitative glucose transporters from rainbow trout (*Oncorhychus mykiss*) and carp (*Cyprinus carpio*) cell line and studies into their structure, phylogeny, expression and functional properties are described.

2 REVIEW OF THE LITERATURE

2.1 Superfamily of sugar transporters

The capability to transport glucose across the plasma membrane is a common feature in virtually all cells, from bacteria to highly specialized mammalian cells. In mammalian cells, there are two distinct families of glucose transporter proteins; first the sodium-dependent or Na^+/glucose transporters, that mediate the secondarily active uptake of glucose against its concentration gradient, and secondly the facilitative glucose transporters, which mediate the saturable, bidirectional passive transport of glucose down its concentration gradient. These structurally and functionally different protein families together ensure efficient uptake of glucose to cells (Hediger et al., 1987; 1995).

At least three different Na^+-dependent glucose transporter isoforms (SGLT1-3) have been cloned from mammalian kidney, small intestine, lung and liver (Hediger et al., 1995). The uptake of glucose does not occur in the absence of a Na^+ -gradient since this system utilizes energy from an extracellular to intracellular Na^+ -gradient. The Na^+/glucose -transporter is not able to mediate the bidirectional transport of glucose and its saturable transport activity is inhibited by phloridzin (Ikeda et al., 1989; reviewed in Burant et al., 1991).

The facilitative glucose transporters belong to a transporter superfamily, which contains over 300 individual proteins. These proteins are expressed in organisms ranging from archaebacteria and lower eukaryotes to plants and vertebrates. The proton-dependent, sugar/H^+ symporters from higher plants, green algae, protozoans, yeasts, cyaonobacteria and eubacteria belong also to this superfamily, which contains both passive and secondary active transporters, transporters other than sugar transporters such as the tetracycline and citrate transporters of bacteria (reviewed in Baldwin, 1993; Marger and Saier, 1993; Sato and Mueckler, 1999). Many of these transporter proteins have 12-transmembrane (TM) segments as predicted by hydropathic analysis. The topology of the proteins consists of six transmembrane α-helical segments, followed by a dispensable central cytoplasmic loop and by six additional transmembrane α-helical segments. This structural similarity points to a common evolutionary origin, thus these proteins are assumed to be derived from a common ancestor (reviewed in Marger and Saier, 1993). The similarity of the amino acid sequence between the members of the superfamily is weak, but many of these proteins have conserved motifs in their structure. Maiden et al. (1987) discovered the presence of a pentametric motif (RXGRR, where X is any amino acid) in many members of the transporter superfamily. For example, this sequence can be found in the Lac permease of *Escherichia coli*, which does not have other similarities with members of the superfamily except for its 12-transmembrane

structure (Calamia and Manoil, 1990; Jessen-Marshall et al., 1995). The presence of this motif at equivalent positions in the two halves of these proteins in the cytoplasmic loops connecting TM segments 2-3 and 8-9 suggests that these proteins have a common ancestor comprised of six TM segments, whose gene underwent a duplication event (reviewed in Gould and Holman, 1993).

2.2 Facilitative glucose transporters

The facilitative glucose transporters (GLUTs) are probably the most extensively studied of all the facilitated diffusion transport systems. The GLUT isoforms were named by numbers according to their chronological order of cDNA isolation and publication. The first facilitative glucose transporter protein, hGLUT1, was isolated in 1977 from human erythrocytes (Kasahara and Hinkle, 1977) and eight years later the amino acid sequence was elucidated (Mueckler et al., 1985). Since every cell type has its own demand for glucose, it was anticipated that different cell types might have other transporters. This hypothesis received support in the early 1980s, when researchers discovered five different glucose transporters from different cells. Since that time, many new isoforms have been identified also in non-mammalian species, such as birds, fruit flies (*Drosophila melanogaster*) and parasitic protozoans (Tetaud et al., 1997; Escher and Rasmuson-Lestander, 1999; Thomas-Delloye et al., 1999).

These facilitative glucose transporters have similar structures but distinct kinetics and different substrate specificities. The structural and functional properties of mammalian glucose transporters are discussed below.

2.2.1 Structure

Each of the glucose transporters contains about 500 amino acids and the predicted pattern of folding for each transporter has 12 membrane spanning segments. The 12-transmembrane segment topological model for GLUT1, originally postulated on the basis of hydrophobicity analysis (Mueckler et al., 1985), is strongly supported by enzymatic (Cairns et al., 1987) and immunologic studies (Davies et al., 1987, 1990), and by glycosylation-scanning mutagenesis experiments (Hresko et al., 1994). Figure 1 presents the basic structure of the facilitative glucose transporter. The amino- and carboxy-termini are located in the cytosol, which also contains a large hydrophilic loop between transmembrane regions TM6 and TM7 dividing this structure into two halves, the N-terminal domain and the C-terminal domain. Both halves are important in the formation of a stable protein (Cope et al., 1994). There is also a large extracellular loop between the first and second transmembrane segments. The predicted length of loops and TM domains is dependent on which programs are used. In general, the loops in

the cytoplasmic surface are very short and the length of these loops is conserved in the whole family. The length of loops at the extracellular surface is highly varied but they are usually longer than the loops at the cytoplasmic surface (reviewed in Gould and Holman, 1993). There is little sequence similarity in the extracellular domains but conservation is considerable in the intracellular loops connecting the membrane spanning helices. These loops have been proposed to simply maintain the structural integrity of the protein, but sequence conservation may also reflect a functionally important role for these regions (reviewed in Gould and Bell, 1990; Burant et al., 1991; Lienhard et al., 1992; Baldwin, 1993; Gould and Holman, 1993; Barrett et al., 1999). Since the highest similarity of GLUTs is found in the TM domains, it is reasonable to postulate that they contain recognition sites for glucose (Kayano et al., 1990; Burant et al., 1991). Of the TM segments, the sequences of TM4, TM5 and TM6 are most highly conserved (38%, 48% and 38% of identical amino acids between GLUT1-5). No functional role has been identified for the extracellular loop between TM1 and 2 or the intracellular loop between TM6 and TM7 (Wu et al., 1998). Sato and Mueckler (1999) found that the positive charges in pentametric motifs (RXGRR) located within loops 2 and 8 of facilitative glucose transporter type 1 (GLUT1) are important in determining the proper orientation of the two cytoplasmic loops as well as each of their flanking TM segments. This provides one possible explanation for the presence of this highly conserved pentametric motif in proteins of the transporter superfamily although they differ radically from each other in terms of substrate specificity and mode of transport.

Due to the high level of sequence similarity, there is likely to be a common mechanism for facilitative transport. Little is know about the regions of the protein responsible for substrate binding or the conformational changes during transport. It has been assumed that protein creates an oscillating pore through the membrane and the chemically reactive groups of the amino acids will be arrayed periodically along the surface. The transporter is believed to hold glucose by weak hydrogen bonds and TM segments 3, 5, 7, 8, 11 contain many amino acids having hydroxyl (OH) and carbamino (CONH2) groups that could participate in hydrogen bonding with the hydroxyl groups on glucose. It is believed, in general, that the transport of glucose across the plasma membrane requires substrate binding at the outward-facing site, conformational changes to reorientate the substrate to inward-facing site, followed by the dissociation of the sugar from the binding site (Baldwin, 1993; Colville et al., 1993; Walmsley et al., 1998).

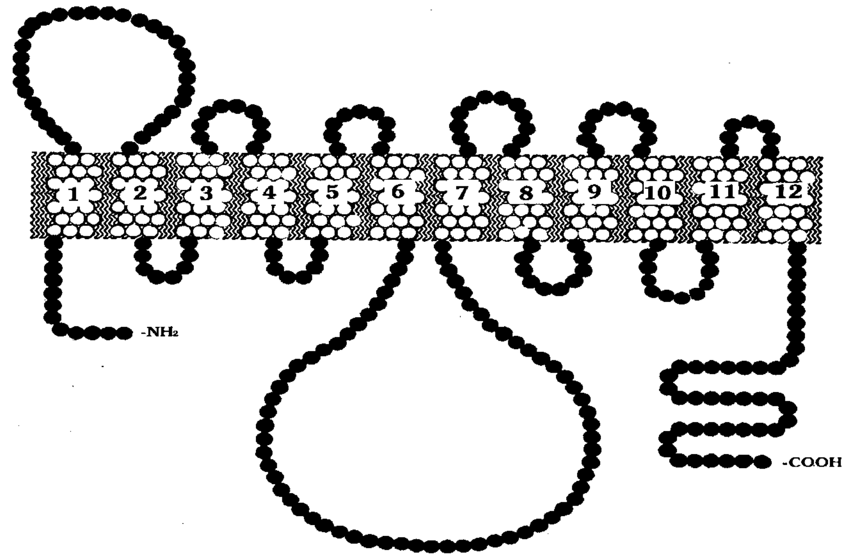

Figure 1. Proposed model for organization of GLUT1 in the plasma membrane. Amino acids are presented as circles. Transmembrane domains are labelled 1 through 12. Modified from Kaestner et al. (1989).

The sugar transporter signatures have been identified by sequence comparison as well as by site-directed mutagenesis (reviewed in McGowan et al., 1995; Barrett et al., 1999). There are several sequence motifs, which have been found to be important for transport activity by using mutagenesis and expression of these mutated constructs in different cell lines or *Xenopus* oocytes. The introduction of point mutations or large domain changes are efficient ways to determine the regions of transporters associated with substrate binding and other functions. The expression of chimeric or mutated transporters permits the determination of the structural basis for their kinetic properties and sugar specificities. The problem of the mutagenesis approach is the possibility that amino acid substitutions may disrupt the correct folding of the peptide chain, leading to a nonspecific disruption of the secondary structure of the protein and altered functional properties (Schürmann et al., 1997; Buchs et al., 1998). GLUT1 is the most intensively studied isoform and many regions have been found to be important for all GLUTs. Table 1 summarises some of these motifs. For example, GLUT1, GLUT3 and GLUT4 contain the QLS motif in TM7, which is involved in high affinity recognition of D-glucose, due to its interaction with the C-1 hydroxyl of the in-coming D-glucose molecule at the exofacial site. In GLUT2 (glucose/fructose transporter) this motif is replaced by HVA (Arbuckle et al., 1996; Seatter et al., 1998). Tryptophan is the rarest and most highly conserved amino acid residue, and plays an important role in the structure and catalytic activity of the transporters. Of the six tryptophan residues in GLUT1, three (Trp_{65}, Trp_{186} and Trp_{412}) are conserved in

GLUT1-5 and a fourth (Trp_{388}) is conserved in four of these five isoforms (reviewed in Mueckler, 1994a).

Table 1. Glucose transporters structure to function relationships

Motif or amino acid	Localization and/or function	References
Central hydrophilic/ C-terminal regions	targeting of the protein to the plasma membrane/ important for function	Baldwin et al., 1980 Katagiri et al., 1992 Due et al.,1995 Oka et al., 1990
TM7	substrate selectivity	Arbuckle et al., 1996
TM 10-11	part of the endofacial binding site	Inukai et al., 1994
455-480 Lys_{451}	transport activity/ conformational sensitive domain	Oka et al., 1990 Lin et al.,1992 Mueckler, 1985
$Q_{282}QXSGXN_{288}XXXYY$ in TM7	part of the exofacial binding site	reviewed in McGowan et al., 1995
PES(/T)PR in loop 6 GW/PGPIPW in TM 10	transport activity	reviewed in Doege et al., 2000b
RXGRR(K) in loop 2 and 8	conformational stability	Gould and Holman, 1993
VPETKG in C-terminal tail	transport activity	Oka et al., 1990
STS in loop 7	conformational alteration	Doege et al., 1998
QLS in TM7	substrate selectivity exofacial binding site	Seatter et al., 1998
Conserved Arg/Glu	cytoplasmic surface/conformation	Schürmann et al., 1997
Pro residues in TM 6 and 10	side chains of the amino acids important for transport activity	Wellner et al., 1995
Phe_{467} and Arg_{468} in C-tail	conformation and function/ not individually essential	Muraoka et al. 1995
Gln_{161} in TM5	conformational changes/ part of the exofacial binding site	Mueckler et al., 1994c
Gln_{282} in TM7 Pro_{385} in TM10	part of the exofacial binding site/ binding of ATB-BMPA	Hashiramoto et al., 1992 Tamori et al., 1994
Val_{165} in TM5	transport activity/within the sugar translocation pathway	Mueckler and Makepeace, 1997, 1999
Tyr_{143} in TM4 Tyr_{293} in TM7	transport activity	Wandel et al., 1994 Mori et al., 1994
Trp_{388} in TM10	binding of cytochalasin B/ transport activity	Garcia et al., 1992 Kasahara and Kasahara, 1998
Trp_{412} in TM11	transport activity	Inukai et al., 1994 Schürmann et al., 1993
Phe_{379} in TM10	transport activity	Kasahara and Kasahara, 1998
Asn_{415} in TM11	transport activity/inward facing binding site	Ishihara et al., 1991

GLUT1

The first facilitative glucose transporter GLUT1 was cloned from human erythrocytes and a hepatoblastoma cell line (Mueckler et al., 1985), and subsequently rat (Birnbaum et al., 1986), rabbit (Asano et al., 1988), mouse (Kaestner et al., 1989; Reed et al., 1990) and pig (Weiler-Guttler et al., 1989). This isoform is also called as an erythrocyte, brain or HepG2-type glucose transporter. All of these proteins have highly conserved 492 amino acid structures and there is 98% identity between human and rat GLUT1 sequences, and 97% identity between human and mouse, rabbit or pig GLUT1. This high degree of sequence conservation suggests that all regions of this protein are functionally important, either for transport of glucose or for the regulation of transporter activity (reviewed in Gould and Bell, 1990).

GLUT2

Many studies provided hints that glucose transport in hepatocytes was different from that mediated by GLUT1. The facilitative glucose transporter GLUT2 was cloned from human liver and kidney (Fukumoto et al., 1988) and rat liver (Thorens et al., 1988) cDNA libraries by using human GLUT1 as the probe. Human and rat GLUT2 contain 524 and 522 amino acids, respectively, having 55% amino acid identity with sequence of the GLUT1, being 82% identical to each other. The overall structure of the transporter is similar to GLUT1. The difference in size between GLUT1 and GLUT2 is due to the difference in size of the extracellular loop connecting TM1 and TM2, which are 33 and 65 residues in GLUT1 and GLUT2, respectively. This loop in GLUT1 and GLUT2 contains a potential site of aspargine-linked glycosylation. The most divergent regions of GLUT2 are the large extracellular loop 1 and the intracellular C-terminal domain (reviewed in Gould and Bell, 1990; Burant et al., 1991). Buchs et al. (1995) proposed that TM7-12 and C-terminus of the protein are responsible for the distinct glucose affinity of GLUT2. Using chimeric studies, Arbuckle et al. (1996) and Wu et al. (1998) showed that the TM segments 8-12 have an overall role of substrate selection and the TM segments 7-8 are crucial for the ability of GLUT2 to transport D-fructose. Arbuckle et al. (1996) noted that the QLS motif in TM7 was absent in GLUT2 and GLUT5, which are also capable of transporting D-fructose. Both of these research groups presented evidence that the N-terminal region of GLUT2 imposed strict structural requirements on the C-terminus of the glucose transporter protein and that interactions of the C-terminus were required for protein stability. The Val_{197}-to-Ile mutation of GLUT2 (equivalent residue 165 in GLUT1) has been shown to impair glucose transport activity and this mutation was first detected from a patient with type 2 diabetes (Mueckler et al., 1994b).

GLUT3

The facilitative glucose transporter GLUT3 was first cloned from a human fetal skeletal muscle cDNA library (Kayano et al., 1988). Human GLUT3 is 496 amino acids in length and has 64 and 52% sequence identity with human GLUT1 and GLUT2, respectively. The sequence of GLUT3 is not as highly conserved as that of GLUT1 and the greatest difference occurs in the extracellular loop 1 and intracellular C-domains (reviewed in Burant et al., 1991). Mutagenesis studies showed that GLUT3, which had a mutated Trp_{410} was unable to mediate glucose transport in *Xenopus* oocytes despite normal expression levels (Burant and Bell, 1992). The orientation of GLUT3 in the membrane is predicted to be similar to that of GLUT1. Recently, Dwyer (2001) published a model for the 3-D structure of GLUT3. In the proposed structure, the 12 TM helices form a right-hand barrel with a central hydrophilic pore. A network of polar and aromatic amino acids lines the pore region and may facilitate the movement of glucose along the channel. A putative binding site for inhibitory ligands like cytochalasin B, was identified on an intracellular aspect of the protein.

GLUT4

GLUT4 protein was first identified from rat adipocytes (James et al., 1988) followed by isolation of cDNA clones from rat and mouse (James et al., 1989; Birnbaum, 1989; Charron et al., 1989; Kaestner et al., 1989). The human GLUT4 was cloned from small intestine and muscle cDNA libraries (Fukumoto et al., 1989). This protein has a 509 amino acids having 65%, 54% and 58% sequence identity with GLUT1, 2 and 3, respectively, and 95% identity with rat GLUT4 (reviewed in Burant et al., 1991).

GLUT4 differs from other isoforms in its extended N-terminus, which is 7-14 residues longer than the corresponding region of the GLUT1-5 (reviewed in Burant et al., 1991). Piper et al. (1992; 1993) reported that the N-terminal cytoplasmic tail of GLUT4 is necessary for its intracellular sequestration and Garippa et al. (1994) proposed that one major function of the N-terminus is to promote effective internalisation of the protein from cell surface rather than retention of the transporter within the intracellular structure where the transporter is located in the basal state. Marshall et al. (1993) suggested that both the N-terminus and the C-terminal cytoplasmic tail contribute to the intracellular sequestration of GLUT4 and Araki et al. (1996) proposed that neither domain alone is entirely sufficient for proper trafficking behaviour. In the C-terminus of the GLUT4 protein, a Leu_{489}-Leu_{490} sequence has been implicated in targeting this protein to an intracellular location instead of the plasma membrane (Verhey and Birnbaum, 1994; Corvera et al., 1994). Garippa et al. (1996) reported the importance of Ser_{488} (before Leu_{489}-Leu_{490} motif) in internalisation and Marsh et al. (1998) noticed that this motif is not involved in regulated movement of GLUT4 to the plasma membrane. Glu_{491} and Glu_{493}

also play an important role in the sub-endosomal trafficking of GLUT4 (Cope et al., 2000) and Shewan et al. (2000) found an additional intracellular targeting signal TELEYLGP $_{498\text{-}505}$ from the C-terminus. Martinez-Arca et al. (2000) reported that removal of Tyr_{502} and Pro_{505}-Asp-Glu-Asn-Asp_{509} prevented the storage of GLUT4 near the Golgi complex. There are highly conserved tyrosine residues in mammalian GLUTs and at least two (Tyr_{143} and Tyr_{293}) in TM4 and 7 are essential for GLUT4 transport activity (Wandel et al., 1994). Schürmann et al. (1997) proposed an important role of the conserved arginine and glutamate residues in GLUT4 (Arg $_{92,\ 153,\ 333,\ 334,\ 400}$ and Glu $_{146,\ 329,\ 393}$). These are highly conserved throughout the family of facilitative sugar transporters and therefore do not determine the substrate specificity of the transporter. Instead these residues are important in the interaction of acidic and basic residues on the cytoplasmic surface, providing the structural basis for the correct arrangement of the TM segments.

GLUT5 - GLUT10

The facilitative glucose transporter GLUT5 was first identified from a human jejunal cDNA library (Kayano et al., 1990) and later from rat jejunum and rabbit cDNA libraries (Rand et al., 1993; Miyamoto et al., 1994). The predicted amino acid sequence of human GLUT5 (501 amino acids) has 42, 40, 39, and 42% identity with human GLUT1, GLUT2, GLUT3 and GLUT4, respectively. GLUT5 exhibits the weakest inter-isoform homology of any of the members of the GLUT family. Hydropathic and secondary structure analyses suggest that the GLUT5 has 12 TM segments and an extracellular loop of 33 amino acids between TM1 and TM2. This extracellular loop contains a potential N-linked glycosylation site Asn_{51}. Two protein domains from the N-terminus to the first intracellular loop and TM segments 5-11 are required for fructose transport and at least part of the C-terminal is also required for transport function (Buchs et al., 1998).

GLUT6 has been isolated from a human fetal skeletal muscle cDNA library. This glucose transporter has a pseudogene-like sequence, which is part of a mRNA that appears to be expressed in all human tissues. Conceptual translation of the GLUT6 cDNA indicated that the 1495-bp glucose transporter-like segment (which has 79.6, 60, 46, 52 and 39% identity to GLUT3, GLUT1, GLUT2, GLUT4 and GLUT5, respectively) lacked a significant open reading frame due to the presence of multiple stop codons and frame shifts. This indicated that it could not encode a functional glucose transporter protein. The similarity with the GLUT3 sequence suggested that the glucose transporter-like region might have been generated by insertion of cDNA copy of GLUT3 mRNA into the non-coding region of an unknown gene (Kayano et al., 1990).

GLUT7 was cloned from a rat liver cDNA library and it shares 68% sequence identity at the amino acid level with rat GLUT2. It encodes a protein of 528 amino acids, which is six amino acids (KKMKND) longer than rat GLUT2. This extension at the C-terminal end contains a consensus motif (KK-K--) for retention of GLUT7 proteins in the endoplasmic reticulum. The hydrophobicity plot suggests that the structure of GLUT7 with 12 TM domains is very similar to that of GLUT2 (Waddell et al., 1992).

The GLUT8 cDNA from mouse and human tissues contains open reading frames encoding a protein of 477 amino acids. The deduced amino acid sequence of human GLUT8 is 29.4% identical with that of GLUT1 (Doege et al., 2000b). Ibberson et al. (2000) cloned also GLUT8 -like transporters, named as GLUTX1, from rat, human and mouse. Their rat GLUTX1 isoform is similar with the GLUT8 cloned by Doege et al. (2000b). A third group (Carayannopoulos et al., 2000) has cloned a GLUT8 like cDNA from murine embryonic teratocarcinoma cell line F9; it encodes a 478 amino acid protein. The protein sequence shows 22-25% identity with mouse GLUT1-3 and like other GLUT8 isoforms, it has a shorter cytoplasmic C-tail as compared to other isoforms. Similarly to other GLUTs, these three transporters contain 12 TM spanning domains with a large cytoplasmic loop between TM6 and TM7. They have a shorter loop 1 than GLUT1 and this loop does not contain a N-glycosylation site, instead this site is located in loop 9. These proteins also contain many important conserved areas critical for glucose transport; such as two repeated sequence motifs GRR(K) in loops 2 and 8, the Gln_{282} in TM7 and tryptophan residues corresponding to Trp_{388} and Trp_{412} in GLUT1. The conserved STS motif in loop 7 is replaced by AET in human GLUT8 and by ANT in rat GLUTX1. The transport activity of GLUT8 in COS-7 cells was similar to that of GLUT4, which indicated that this motif was not so important for the transport activity (Doege et al., 2000b). All of these three GLUT8 isoforms have a highly conserved PES(/T)PR motif in loop 6 and VPETKG(R) motif in the C-terminal tail and they have 82% identity in their amino acid sequence.

GLUT9 was cloned from human leucocytes by Doege et al. (2000a). The transporter has similarities with GLUT8/GLUTX1 and they share 43.6% identity. The protein also has 12 TM domains and its sequence contains many of the conserved areas needed for proper function, such as PES(/T)PR/VPETKG(R) motif in loop 6 and C-terminal tail, the GRR motifs in loops 2 and 8 and tyrosine residues in TM4 and 7 corresponding to Tyr_{146} and $Tyr_{292/293}$. Tryptophan residues corresponding to Trp_{388} and Trp_{412} in GLUT1 are also found. Like GLUT8, this protein has a short loop 1 without any glycosylation site, instead this site is located in loop 9.

McVie-Wylie et al. (2001) have cloned a new member of the facilitative glucose transporter family, GLUT10 from a human cDNA library. The full length cDNA predicts a 541 amino acid protein. The amino acid sequence has the lowest similarity with GLUT3 (29.7%) and highest with GLUT8 (33.6%) protein. Hydropathy analysis indicates that GLUT10 possesses the 12 TM structure with a hydrophilic intracellular loop between TM6 and TM7, and a large extracellular loop containing a potential glycosylation site between TM 9 and TM10. GLUT8 and GLUT10 are considered to be part of a novel subgroup of mammalian facilitative transporters (Ibberson et al., 2000; McVie-Wylie et al., 2001).

2.2.2 Tissue distribution and regulation of expression

Most cell types express two or more different isoforms of facilitative glucose transporters. One example of the cooperative action of two glucose transporters is in the insulin-sensitive tissues, such as fat and muscle. In these tissues, GLUT1 is present constitutively in the plasma membrane and it provides the low level of glucose required for basal cellular activity, whereas GLUT4 is present in a special intracellular membrane compartment in the basal state and is recruited to the plasma membrane in response to insulin or other stimuli that signal the need for higher levels of glucose transport (reviewed in Mueckler, 1994a). Another example of this cooperative action is in human muscle where basal uptake of glucose is provided by GLUT1, GLUT3 and GLUT4 (Stuart et al., 2000). Table 2 summarises the major expression sites of the various glucose transporters.

In addition to tissue specific gene expression of glucose transporters, there are multiple mechanisms by which hormones and other agents, such as growth factors and metabolites can control the net flux of glucose across the plasma membrane. The mechanisms can be divided in acute regulation (the translocation of pre-existing transporters to the plasma membrane, modulation of transporter intrinsic activity and alterations in transporter trafficking) or chronic regulation (regulation of the transporter synthesis by transcription, mRNA stability and protein levels). The response of a specific glucose transporter isoform to hormonal or metabolic changes depends upon the tissue in which it is expressed (for review see Baldwin, 1993; McGowan et al., 1995; Qi and Pekala, 1999). Studies of the mechanisms regulating the rate of glucose transport into cells have important implications in our understanding of diseases. GLUTs are involved in a number of pathological conditions such as diabetes and cancer. Altered expression levels of transporters can also be related to other diseases, for example GLUT1 protein levels are reduced at the blood-brain barrier of Alzheimer´s disease patients (reviewed in Qi and Pekala, 1999). Additionally, expression of glucose transporters is developmentally regulated, highlighting the importance of an adequate supply of glucose for metabolism and energy production during embryogenesis and the functional importance of

different transporter isoforms during development (Werner et al., 1989; Aghayan et al., 1992; Santalucia et al., 1992; Rand et al., 1993; Postic et al., 1994; Smoak and Branch, 2000).

Table 2. Major expression sites of the facilitative glucose transporters

Isoform	Tissue	Function
GLUT1	Fetal tissues, placenta, adults brain, blood-brain barrier, erythrocytes, kidney, colon, transformed cells	Basal uptake of glucose in cells, transport across blood-brain barrier Constitutive glucose transporter
GLUT2	Liver, pancreatic β-cell, kidney, small intestine	Transport of glucose in hepatocytes, β-cell glucose sensor, release of absorbed glucose in small intestine and kidney
GLUT3	Brain/nerve cells in rodents. Brain, nerve, low levels in placenta, kidney, liver, muscle, heart in humans.	Basal high affinity uptake of glucose in cells, especially in brain
GLUT4	Brown/white adipocytes, heart, skeletal muscle	Insulin-stimulated glucose uptake
GLUT5	Small intestine, low levels in brain, muscle, adipocytes, kidney, heart, erythrocytes, testis	Absorption of glucose from the lumen of the small intestine. Fructose transport, low affinity for glucose
GLUT7	Endoplasmic reticulum of hepatocytes	Mirosomal glucose tranport in liver
GLUT8	Testis, low levels in brain, spleen, heart, liver, small intestine, muscle, adipocytes	Glucose/fructose transport in testis and brain.
GLUT9	Brain, spleen and leucocytes	
GLUT10	Liver, pancreas, low levels in heart, brain, placenta, lung, skeletal muscle, kidney	

Data is collected from Mueckler et al., 1985, Burant et al., 1991, Baldwin, 1993; Gould and Holman, 1993, Concha et al., 1997, Doege et al., 2000b, Ibberson et al., 2000, McVie-Wylie et al., 2001.

GLUT1

The erythrocyte-type transporter, GLUT1 appears to be the most ubiquitously distributed isoform, which is expressed in most tissues. The highest levels of GLUT1 are found in fetal tissues including placenta, erythrocytes, brain, endothelial cells, kidney and colon (reviewed in Gould and Bell, 1990; Burant et al., 1991; Gould and Holman, 1993). In human erythrocytes, GLUT1 represents about 6% of the total membrane protein and it is expressed together with GLUT2 and GLUT5 (Allard and Lienhard, 1985; Concha et al., 1997). GLUT1 is the major isoform present in most cell lines and it is well established that transformation of cells results in a pronounced elevation of GLUT1 mRNA, protein and activity levels (Mueckler et al., 1985; Gould and Holman, 1993; Onetti et al., 1997).

GLUT1 promoter studies have provided novel insights into the regulation of the GLUT1 gene expression. Mouse GLUT1 gene was isolated and characterized by Murakami et al. (1992) and there were at least two enhancer elements containing SREs (serum response element), TREs (12-O-tetradecanoylphorbol-13-acetate responsive element) and CREs (cyclic AMP-responsive element) motifs and GC boxes. Regulation of mRNA stability plays a major role in control of GLUT1 expression and a variety of unrelated hormones, pharmaceuticals and physiological conditions such as hypoxia have been demonstrated to alter the stability of the GLUT1 transcripts in various cell lines and tissues (reviewed in Ebert et al., 1995; McGowan et al., 1995; Jung, 1998). GLUT1 expression in general, is induced by growth stimuli responding to the increased energy and biosynthetic requirements of dividing cells. The expression of GLUT1 in different cell cultures is altered by glucose, fibroblast growth factor, tumor necrosis factor α, oncogenes, hypoxia, cAMP, thyroid hormone, serum, insulin, insulin-like growth factor-I, growth hormone, mitogens, starvation and many other factors. All these factors, except glucose, increase GLUT1 expression (for reviews see Gould and Holman, 1993; Mueckler, 1994a; McGowan et al., 1995). The cellular distribution of GLUT1 is regulated by hormones. Insulin induces the translocation of GLUT1 to the surface of the cell from an intracellular location in 3T3-L1 adipocytes, 3T3-L1 fibroblasts and in skeletal-muscle-derived L6 cells (Calderhead et al., 1990; Walker et al., 1990; Koivisto et al., 1991; Yang et al., 1992). The level of GLUT1 in the intact animal is altered by blood glucose concentration, hypothyroidism, hypoxia and denervation of muscle (Mueckler, 1994a; Jones et al., 1998; Zhang et al., 1999). During development, embryos have the capability to control the uptake of glucose by regulating the expression of GLUT1 (Leppens-Luisier et al., 2001) and expression of GLUT1 has been shown to change dramatically during embryonic development. In rat, GLUT1 is the predominant glucose transporter expressed in muscle during fetal and early postnatal life but in later postnatal life, repression of GLUT1 and an increase of GLUT4 expression is observed (reviewed in Viñals et al., 1997).

GLUT2

The liver-type transporter, GLUT2 is expressed in the liver, small intestine, kidney and cells of the endocrine pancreas. GLUT2 is also expressed at a low level in human erythrocytes together with GLUT1 and GLUT5 (Concha et al., 1997). The size of the expressed protein differs in these tissues, probably due to differences in glycosylation (Fukumoto et al., 1988; Thorens et al., 1988, 1990). The tissue distribution suggests that GLUT2 mediates the uptake and release of glucose by hepatocytes and participates in the transepithelial transport of absorbed and reabsorbed glucose by the small intestine and kidney, respectively. On the other hand, Guillam et al. (1998) found that hepatic glucose release does not require the presence of GLUT2 or any plasma membrane facilitative diffusion mechanism though there seems to be

some unknown pathway that may be based on a membrane traffic mechanism. In intestine and kidney, the high-capacity low-affinity transport system is probably required to handle the large transepithelial substrate flux that may occur after a high carbohydrate meal (Mueckler, 1994a). The low affinity probably prevents transport from becoming a rate limiting step in hepatocytes and it enables glucokinase to act as the "glucose sensor" by ensuring that intracellular glucose concentrations rapidly change in response to any change in extracellular sugar concentrations. The presence of GLUT2 in pancreatic cells suggests that it may take part in the regulation of glucose-stimulated insulin secretion (reviewed in Burant et al., 1991).

The regulation of GLUT2 expression in cells is unclear but there is evidence that in liver, portal hyperinsulinemia decreases GLUT2 gene expression and that hyperglycaemia induces expression of this gene. Chronic insulin-induced hypoglycaemia results in a profound reduction in GLUT2 mRNA levels and transport activity in pancreatic β-cells (reviewed in Baldwin, 1993; Mueckler, 1994a), whereas over-expression of the c-Myc transcription factor increases liver glucose utilization and leads to the increased GLUT2 expression in mice (Valera et al., 1995). Expression of GLUT2 is developmentally regulated and can be detected from the blastocyst stages onwards during mouse embryogenesis and also from early liver tissues, where expression increases throughout development (Hogan et al., 1991; Aghayan et al., 1992; Postic et al., 1994).

GLUT3

The brain-type transporter, GLUT3 is present in various tissues of adult human with the highest levels being found in brain, kidney and placenta. Its ubiquitous distribution in human tissues suggests that it may be responsible for noninsulin-stimulated glucose transport together with GLUT1. It has been suggested that GLUT3 may be involved together with GLUT1 in brain glucose transport in a way that GLUT1 is responsible for transport of glucose across the blood-brain barrier and GLUT3 transports glucose into neuronal cells. GLUT3 is also present in human cardiac myocytes but its role there is unknown (Grover-McKay et al., 1999). The expression pattern of GLUT3 in other species like mouse differs from humans, being highest in brain and very low or even undetectable in other tissues (Nagamatsu et al., 1992).

GLUT4

The muscle/fat-type transporter, GLUT4 mediates insulin-stimulated glucose uptake in fat, skeletal muscle and cardiac muscle. The expression of GLUT4 is highest in adipose and muscle tissues but low levels can be also detected in kidney (Charron et al., 1989). Glucose

transport in muscle and fat has received considerable attention because of the importance of this process in the maintenance of whole-body glucose homeostasis.

A great deal of information is available about the hormonal, nutritional and metabolic regulation of GLUT4 expression (for review see McGowan et al., 1995). Studies on GLUT4 gene regulation and regulatory elements in promoter of gene are underway and new studies with GLUT4 knockout models are likely to promote understanding of regulation of this gene expression under different conditions (reviewed in Charron et al., 1999). Insulin is one of the best studied regulatory agents being the primary acute regulator of the GLUT4 transport system needed for activation of translocation of a latent pool of glucose transporters from an intracellular site to the plasma membrane. In the absence of insulin, GLUT4 is localized to tubulovesicular elements that are clustered either in the region of *trans*-Golgi, endosomes or in the cytoplasm. For example in muscle cells and adipocytes, over 90% of GLUT4 are located within the cell during the basal state (Cushman and Wardzala, 1980; Haney et al., 1995; Cope et al., 2000; Shewan et al., 2000).

Since, glucose transport is most commonly rate-limiting for glucose disposal, and because insulin-induced GLUT4 translocation is the route by which insulin stimulates glucose transport, studies of the mechanism of this process will be the key to understanding insulin resistance and diseases like type 2 diabetes in humans. When the insulin signal arrives at the intracellular locus, it activates the intracellular vesicular trafficking machinery. This involves an interaction of many proteins, specialized vesicular compartments and sub-compartments, and regulatory events (reviewed in McGowan et al., 1995; Olefsky, 1999; Czech and Corvera, 1999). Like insulin, growth hormone has been shown to enhance glucose uptake by stimulating the rapid translocation of the GLUT4 transporter to the plasma membrane but on the other hand in rat adipocytes, growth hormone down-regulates the amount of GLUT4 protein in plasma membrane (Kilgour et al., 1995). Thyroid hormone may also have a positive regulatory effect on GLUT4 mRNA and protein levels (reviewed in McGowan et al., 1995).

Chronic exercise training increases skeletal muscle GLUT4 protein levels in the rat (Rodnick et al., 1990). There is evidence that both insulin and muscle contractions increase glucose transport activity in skeletal muscle by increasing V_{max} with no significant alterations occurring in K_m (Hansen et al., 1995). Derave et al. (1999) noted that glucose transport and the cell surface GLUT4 content is also dependent on the muscle glycogen content. Glucose deprivation in cultured 3T3L1 adipocytes decreases mRNA levels but at the same time glucose transport activity is increased due to an increased amount of GLUT4 protein at the plasma membrane without there being any changes in total cellular GLUT4 protein levels

(Tordjman et al., 1990). Glucose deprivation also increases the plasma membrane content of GLUT4 in skeletal-muscle-derived L6 cells (Koivisto et al., 1991). Glycaemia regulates glucose transport in rat skeletal muscle independently of insulin, via changes in plasma membrane GLUT4, and diabetes and fasting can also affect GLUT4 expression in insulin sensitive tissues by decreasing the amount of GLUT4 mRNA and protein by heterogeneous manner depending of type of tissue (Camps et al., 1992; Mathoo et al., 1999). Hansen et al. (1998) found that impaired GLUT4 translocation to the cell surface decreased glucose transport when rats were fed with a high fat diet. During embryogenesis in the mouse, GLUT4 is not expressed at the preimplantation or early postimplantation stages (Hogan et al., 1991; Aghayan et al., 1992).

GLUT5 - GLUT10

GLUT5 is expressed mainly in the jejunal region of the small intestine and low levels are also detected in human kidney, four different skeletal muscle groups, heart, brain, testis, erythrocytes and adipose tissue (Kayano et al., 1990; Shepherd et al., 1992; Concha et al., 1997). In muscle and fat cells, GLUT5 is constitutively present in the plasma membrane and it is not regulated by insulin (Shepherd et al., 1992). GLUT5 expression and localization in human intestine seems to be under developmental regulation (Davidson et al., 1992). The expression of fructose-specific transporter GLUT5 in the plasma membrane of mature spermatozoa is consistent with the known ability of sperm cells to utilize fructose (Burant et al., 1992). In rat intestine, expression has been shown to be regulated by diurnal factors (Rand et al., 1993; Corpe et al., 1998).

GLUT7 is found in internal membrane of endoplasmic reticulum and nuclear membrane in hepatocytes but not in the plasma membrane. GLUT7 is a part of glucose-6-phosphatase system transporting released glucose from the lumen of the endoplasmic reticulum (Waddell et al., 1991; 1992).

GLUT8 transcripts have been detected in human testis and at a low level from most of other analysed tissues such as spleen, prostate, small intestine, heart, brain, and skeletal muscle (Doege et al., 2000b). GLUTX1 expression was detected in adult rat testis and also in the cerebellum, brain stem, hippocampus, adrenal gland, liver, spleen, brown/white adipose tissues, lung, kidney, tongue and stomach (Ibberson et al., 2000). Surprisingly, rat GLUTX1 was not found on the cell surface and it was proposed that this location was due to presence of a Leu_{12}-Leu_{13} internalisation motif at the amino-terminal end of the protein. Researchers suggested that GLUT8/GLUTX1 might be involved in the provision of glucose/fructose required for DNA synthesis in male germ cells during spermatogenesis. Doege et al. (2000b)

proposed that GLUT8 expression in testis was controlled by gonadotropins since estrogen, which is known to suppress gonadotropin secretion, fully suppressed the expression in testis. They analysed rats of different ages and found GLUT8 expression in adult and pubertal but not in prepubertal rats, concluding that GLUT8 expression was under hormonal regulation by gonadotropins and/or was related to spermatogenesis. Rat GLUTX1 expression was relatively high in the brain stem and hypothalamus, where glucose-sensing neurons were located. Expression of mouse GLUT8, cloned by Carayannopoulos et al. (2000), was similar to that reported by others. However, they found high level expression in mouse blastocysts and concluded that GLUT8 might be an embryonic transporter offering an additional mechanism for glucose transport into mammalian blastocyst during critical turning point in development. They also observed increased glucose transport and translocation of GLUT8 to the plasma membrane during insulin treatment in the mouse blastocyst.

The expression of GLUT9 has been detected in human brain, spleen and peripheral leucocytes. The highest levels of GLUT10 expression have been found in human liver and pancreas and lower levels were also found from heart, brain, placenta, lung, skeletal muscle and kidney. No functional studies of GLUT10 transporter have been published so far and the physiological role of both these proteins remains unknown (Doege et al., 2000a; McVie-Wylie et al., 2001).

2.2.3 Functional properties

Mammalian glucose transporters have been one of the most important models to study structure to function relationships of membrane proteins. Characterization of their properties and physiological role is complicated by the fact that more than one isoform may be expressed in a single cell type. Functional properties of glucose transporters (GLUT1-GLUT9) have been characterized by expression studies in *Xenopus* oocytes, bacteria, low eukaryotes and cultured cells (Gould and Lienhard, 1989; Keller et al., 1989; Birnbaum, 1989; Vera and Rosen, 1989; Kayano et al., 1990; Gould et al., 1991;Waddell et al., 1992; Cohen et al., 1996; Doege et al., 2000ab; Ibberson et al., 2000; Carayannopoulos et al., 2000). The use of *Xenopus* oocytes for the functional expression of different members of the transporter family has considerable value in defining the kinetic properties and sugar specificities. The *Xenopus* oocytes are well suited for studying the functional properties of heterologously expressed glucose transporters due to the low level of endogenous glucose transport activity in uninjected oocytes and the ease of microinjections of mRNA into oocytes. The proteins synthesized from injected mRNA in oocytes are generally correctly modified and targeted to the correct cellular location (Gould and Lienhard, 1989; Keller et al., 1989; Vera and Rosen, 1989; Colville et al., 1993).

Table 3 and 4 summarise the main kinetic parameters and the substrate selectivity of glucose transporters. There are quite major differences between the kinetic values reported in different studies since uptake conditions such as substrate concentration, pH, temperature and steady-state approaches (zero-trans / equilibrium exchange) can have a significant effect on the kinetic values. GLUTs do not transport disaccharides, but those like maltose can bind to the extracellular surface of transporter and inhibit glucose transport. Cytochalasin B and phloretin are the most commonly used inhibitors of facilitative glucose transport, cyanide (KCN) is the inhibitor of proton-dependent transport and phloridzin is used as an inhibitor of sodium/glucose co-transport.

Table 3. Kinetic parameters of the glucose transporters expressed in *Xenopus* oocytes

Isoform	Km (~mM) 3-OMG	Km (~mM) 2-DOG	Km (~mM) D-glucose	Other transported sugars (Km ~mM)
GLUT1	18 [a] 21 [i] 26 [h]	6.9 [d]	17 [b] 2.3-2.6 [k]	D-galactose 17 [d]
GLUT2	42 [a] 32 [c]	16 [d] 11 [e]	66 [b]	D-galactose 36 [d] 86 [e] D-fructose 67 [e]
GLUT3	11 [a]	1.8 [d] 1,4 [e]		D-galactose 6 [d] 8.5 [e]
GLUT4	7.2 [c] 4.3 [h] 1.8 [i]	4.6 [d]	4-7 [b]	
GLUT5				D-fructose 6 [f] 12 [j]
GLUT8		2.4 [g]		

K_m values were determined by *zero*-trans uptake* or equilibrium-exchange° experiments. § tested in cell line.
a) °Gould et al., 1991, b) § reviewed in Gould et al., 1991 c) °Buchs et al., 1995, d) *Burant and Bell, 1992, e) °Colville et al., 1993, f) *Burant et al., 1992, g) *Ibberson et al., 2000, h) °Nishimura et al., 1993, i) °Keller et al., 1989, j) § Inukai et al., 1995, k) *Woodrow et al., 2000.

Table 4. Substrate selectivity of different glucose transporters expressed in *Xenopus* oocytes

Isoform/ Substrate	Competitors: D-glucose	L-glucose	D-mannose	L-mannose	D-xylose	D-fructose	D-galactose
GLUT1							
D-glucose [d]			↓		↔		↔
2-DOG [a]	↓	↔	↓	↔	↔	↔	
GLUT2							
2-DOG [a]	↓	↔	↓	↔	↔	↓	
3-OMG [a]	↔	↔	↓	↔	↔	↓	
D-fructose [e]	↓					↓	↓
GLUT3							
2-DOG [a,c]	↓	↔	↓	↔	↓	↔	↓
3-OMG [a]	↔	↔	↓	↔	↓	↔	
GLUT5							
D-fructose [e, f]	↔ [e] ↓ [f]		↔		↔	↓	↔ [e] ↓ [f]
2-DOG [f]	↓		↔		↔	↓	↓
GLUT8							
2-DOG [b]	↓	↔				↓	↓

↔ no effect, ↓ inhibited. a) Gould et al., 1991, b) Ibberson et al., 2000, c) Colville et al., 1993, d) Woodrow et al., 2000, e) Burant et al., 1992, f) Miyamoto et al., 1994.

GLUT1

GLUT1 cDNAs have been functionally expressed in *Xenopus* oocytes, CHO cells, 3T3L1 cells, COS cells, Sf9 insect cells and transgenic mice (reviewed in Mueckler, 1994a). GLUT1 has very broad substrate specificity, transporting a wide range of aldoses but very low affinity for fructose (K_m approx. 1.5M) indicating that this transporter is not the normal route to transport this sugar into cells. The C-1 chair conformation of the sugar appears to be the structure that is most efficiently transported since the K_m value for D-glucose (in *zero*-trans conditions) is around 2 mM but the K_m for L-glucose is greater than 3 M (reviewed in Burant et al., 1991; Baldwin, 1993). Transport of 2-DOG (2-deoxy-D-glucose) in *Xenopus* oocytes by hGLUT1 was inhibited by D-glucose and D-mannose but L-glucose, L-mannose, D-xylose, L-xylose and D-fructose did not significantly decrease the transport rate (Gould et al., 1991). GLUT1 transport activity is sensitive to the cytochalasin B, the K_i values for D-glucose, 2-DOG and 3-OMG transport being around 1 μM (Burant and Bell, 1992; Due et al., 1995; Woodrow et al., 2000).

GLUT2

GLUT2 is a low-affinity, high-turnover transport system, which is unique among GLUTs in transporting both D-glucose (K_m around 66 mM) and D-fructose (K_m around 67 mM). GLUT2 is also capable of handling galactose (Gould et al., 1991). The transport activity of fructose is

lower than that of 2-DOG, 3-OMG or D-galactose (Burant and Bell, 1992). Transport of 2-DOG in *Xenopus* oocytes was inhibited by D-glucose, D-mannose and D-fructose but L-glucose, L-mannose, D-xylose and L-xylose had no inhibitory effect (Gould et al., 1991). The transport of 3-OMG in *Xenopus* oocytes was decreased by 45% in the presence of D-fructose and 80% in presence of D-mannose but other sugars had no effect (see Table 4). Transport of D-fructose was inhibited by D-glucose, D-galactose and cytochalasin B (K_i 10 μM) (Burant et al., 1992). Binding of cytochalasin B to GLUT2 is weak, having 10-fold lower affinity (K_i for 2-DOG 7.5 μM) than GLUT1 (Burant and Bell, 1992). Colville et al. (1993) reported that the transport of 2-DOG, D-fructose and D-galactose are non-competitively inhibited by cytochalasin B, whereas D-maltose, D-glucose and D-fructose are competitive inhibitors of 2-DOG transport. To test whether D-glucose is a competitive inhibitor of D-fructose transport, they examined ethylideneglucose (a glucose analogue known to interact only with the outward-facing binding site) and 2,5-anhydro-D-mannitol (a reduced fructose analogue). These analogues clearly inhibited both 2-DOG and D-fructose transport, suggesting that these sugars use the same outward-facing substrate binding site in GLUT2.

GLUT3

The most distinctive characteristic of this isoform is the low K_m (about 10 mM) for 3-OMG, which together with its ubiquitous tissue distribution, may reflect its constitutive role for glucose transport. GLUT3 has higher affinity for sugars than GLUT1, which ensures efficient uptake of glucose by neuronal cells even at low extracellular glucose concentrations. Transport of 2-DOG in *Xenopus* oocytes by hGLUT3 was inhibited by D-glucose, D-mannose, D-xylose, D-maltose, D-galactose but not by L-glucose, L-mannose, L-xylose and D-fructose. On the other hand, the transport of 3-OMG was inhibited by D-mannose and D-xylose whereas other sugars had no inhibitory effect (see Table 4). GLUT3 also has quite high affinity for D-galactose and it is capable of transporting D-xylose (Gould et al., 1991; Colville et al., 1993). GLUT3 transport activity is inhibited by cytochalasin B and the K_i value for 2-DOG transport in *Xenopus* oocytes is reported to be around 0.1 μM (Burant and Bell, 1992).

GLUT4

The GLUT4 has K_m values 1.8-7.2 mM for 3-OMG and around 5mM for 2-DOG transport (Keller et al., 1989; Burant and Bell, 1992; Nishimura et al., 1993; Buchs et al., 1995). The reported K_m values are quite low and in many studies transport activity has been insufficient for substrate selectivity studies. When GLUT4 is expressed in *Xenopus* oocyte, it is predominantly intracellularly localized. This can partly explain the lower level of transport activity in injected oocytes as compared with other GLUTs (Gould et al., 1991; Thomas et al.,

1993). The reported K_i value for cytochalasin B during 3-OMG transport is around 1 μM (Due et al., 1995).

GLUT5 – GLUT9

The expression of hGLUT5 in *Xenopus* oocytes has confirmed that it is a fructose (Km ~6 mM) rather than a glucose transporter and fructose transport is not inhibited by cytochalasin B, D-glucose, D-galactose or sucrose (Burant et al, 1992). The K_m value for fructose in human erythrocytes is about 10 mM and transport is not inhibited by 2-DOG (transported by GLUT1 and GLUT2 in erythrocytes) and cytochalasin B (Concha et al., 1997). Miyamoto et al. (1994) measured transport activity of D-fructose by five different GLUT5 (rat, mouse, rabbit, hamster and guinea-pig) in *Xenopus* oocytes and the transport activity was increased only with rabbit GLUT5 (K_m 11 mM). They also reported differences in the inhibition of D-fructose transport by D-glucose between oocytes (inhibition) and epithelial brush-border membrane of rabbit intestine (no inhibition). Rand et al. (1993) reported that D-fructose uptake by rat GLUT5 was inhibited by D-glucose, which was not the case with hGLUT5.

GLUT7 encodes a functional endoplasmic-reticulum glucose transporter protein when expressed in COS-7 cells. Binding of cytochalasin B to GLUT7 is weak (Waddell et al., 1991, 1992). No other functionality studies have been reported.

The mouse GLUT8 was expressed in COS-7 cells. Over-expression caused an 8-fold increase in D-glucose transport activity and a marked increase in the specific binding of cytochalasin B. The transport activity was similar to that of GLUT4 (Doege et al., 2000b). Rat GLUTX1 was expressed in *Xenopus* oocytes but it had no significant effect on 2-DOG transport. Researchers assumed that this was due to the Leu_{12}-Leu_{13} motif present at the amino-terminal end of the protein. After mutation of these leucine residues to alanines, transport activity in oocytes was increased and transport of 2-DOG (K_m 2.4 mM) was inhibited by cytochalasin B, D-glucose, D-fructose and D-galactose (Ibberson et al., 2000). Uptake studies with mouse GLUT8 (cloned by Carayannopoulos et al., 2000) in *Xenopus* oocytes showed that this isoform increased 2-DOG transport by 3-fold, and functional studies in mouse blastocyst confirmed increased glucose transport and movement of GLUT8 to the plasma membrane after insulin treatment.

Human GLUT9 has been expressed in COS-7 cells. Over expression of GLUT9 increased the D-glucose transport activity only at certain (5 mM) D-glucose concentrations. Over-expression clearly increased the binding of cytochalasin B; no kinetic parameters were determined (Doege et al., 2000a).

2.3 Glucose metabolism in fish

2.3.1 Carbohydrate nutrition in teleost fish

Fish have limited ability to utilize carbohydrates in comparison to mammals. No dietary requirement for carbohydrate has been demonstrated in fish though the total absence of dietary carbohydrate can depress fish growth and increase the muscular-protein degradation (reviewed in Wilson, 1994; Peragón et al., 1999). The recommended dietary level of digestible carbohydrate varies among species, being greater for farmed omni- or herbivorous fish such as common carp (Shimeno et al., 1977) and tilapia (*Oreochromis niloticus* L.) (Anderson et al., 1984). In general, not more than 20% content of digestible carbohydrate is recommended for carnivorous fish (reviewed in Wilson, 1994), though Bergot (1979a) has reported that under certain experimental conditions, rainbow trout can tolerate up to 30% glucose in their diet and can use glucose for their energy needs. A concentration of less than 25% dextrin or gelatinised starch can be used as an energy source by rainbow trout (Lee and Putnam, 1973). It has been demonstrated that gelatinised starch can improve protein and energy utilization, and that it can be as effective as lipids as an energy source for rainbow trout (Pieper and Pfeffer, 1980; Kaushik and Oliva Teles, 1985; Kim and Kaushik, 1992). Utilization of carbohydrates depends on environmental factors. Studies with Atlantic salmon (*Salmo salar*) showed that at summer temperatures (12.5 °C) fish could utilize dietary starch better than in cold water (2 °C) and that incorporation of glucose in liver glycogen was also higher at summer temperatures (Hemre et al., 1995). The effect of salinity on carbohydrate metabolism in liver, kidney and gill has been reported (Soengas et al., 1994; 1995ab) and Wilson (1994) suggested that in general marine and coldwater species are more intolerant to glucose than warm and freshwater species.

Within certain limits, lipids and protein energy in fish feeds can be substituted for carbohydrates without any apparent adverse effect on their growth and physiologic condition. However, feeding of salmonid fish like rainbow trout with a high level of digestible carbohydrate has been reported to result in markedly increased hepatosomatic index and glycogen content, increased mortality and depressed growth (Lee and Putnam, 1973; Bergot, 1979a; Hilton and Atkinson, 1982; Kim and Kaushik, 1992; Brauge et al., 1994).

2.3.2 Digestion and absorption of carbohydrates

The dietary value of carbohydrates for fish appears to be associated with their complexity and molecular weight (Bergot, 1979b). Carbohydrates are converted mainly to glucose by digestion. Glucose, maltose, and sucrose have resulted in the best growth rates, followed in descending order by dextrin, fructose, galactose, potato starch and glucosamine when various

carbohydrate sources were fed to young chinook salmon (*Oncorhynchus tshawytscha*) at a concentration of 10 percent of the diet (Buhler and Halver, 1961). The digestibility of carbohydrates in rainbow trout seems to be higher in freshwater than in saltwater (Storebakken et al., 1998), and in general, omnivorous and herbivorous fish appear to digest carbohydrates more effectively than carnivorous fish. The difference may be related to amylase activity in their digestive system. For example, the amylase activity in omnivorous carp is 10-30 times greater than the corresponding activity in carnivorous rainbow trout (Hofer and Sturmbauer, 1985; Wilson, 1994). Nutrient absorption studies in rainbow trout and Atlantic salmon have shown that absorption of carbohydrates takes place mainly in the pyloric region, anterior and caecal part of the intestine depending on the dietary carbohydrate levels, source of carbohydrate, differences in heat treatments, fish species and in methods of analysis (Austreng, 1978; Krogdahl et al., 1999). In rainbow trout the low absorption in the pyloric region seems to be compensated by higher absorption in both the mid-intestine distal to the pyloric caeca and the distal intestine (Austreng, 1978). In general, fish species that consume carbohydrate-rich diets (herbivores and omnivores) have greater intestinal glucose absorption rates than carnivorous species (reviewed in Soengas and Moon, 1998). Carrier-mediated absorption of glucose by the intestinal brush-border is a Na^+- and energy-dependent process, which is inhibited by phloridzin and has similarities to transport mediated by the mammalian glucose transporter SGLT1 (Ferraris and Ahearn, 1984; Reshkin and Ahearn, 1987ab; Ahearn et al., 1992). Transport activity is related to the water temperatures (Houpe et al., 1997) and it is reported that glucose up-regulates these transporters in species that consume carbohydrate-rich diets (carp) but not in strictly carnivorous species (reviewed in Soengas and Moon, 1998). It appears that carnivorous fish are unable to adapt their digestive function to high carbohydrate diets (Buddington and Hilton, 1987).

2.3.3 Intermediate glucose metabolism in fish

Though digestibility of polysaccharides and absorption of glucose in fish is restricted, it is probably not the main obstacle for using carbohydrate-rich diets, because absorbed sugars are poorly utilized in fish. The studies of carbohydrate metabolism have shown that fish tissues are not capable to rapidly utilize ingested sugars. Different kinds of oral and intravenous glucose tolerant tests have been performed with some species (for review see Moon, 2001) such as rainbow tout (Palmer and Ryman, 1972), common carp (Shimeno et al., 1977; Furuichi and Yone, 1981) and brook trout (*Salvelinus fontinalis*) (Phillips et al., 1948). In each case, the administration of glucose resulted in a persistent hyperglycaemia. There is a tendency that fish respond to a glucose challenge in a species-dependent manner, probably related to food habits (Legate et al., 2001). In general, the more carnivorous the species is, the longer time is needed to clear a glucose load from blood.

In omnivorous mammals, insulin plays a key role in regulation of carbohydrate metabolism. Insulin, as well as glucagon, are known to influence nutrition and metabolism of fish (reviewed in Christiansen and KlungsØyr, 1987; Plisetskaya, 1989). Glucagon stimulates mobilization of hepatic glycogen and lipids as well as hepatic gluconeogenesis (Plisetskaya, 1989). Somatostatins, whose levels are affected by glucose, inhibit insulin and glucagon secretion and stimulate glycogenolysis and lipolysis in salmonid fish. The pancreatic cells, which secrete somatostatins are more sensitive to glucose than insulin-secreting cells and that may partly account for the glucose intolerance in rainbow trout (Harmon et al., 1991). The inability of fish to utilize high levels of dietary carbohydrates has been assumed to be the result of low levels of endogenous insulin (Palmer and Ryman, 1972). However this might not be the case, since radioimmunoassay methods have shown that insulin levels in fish are similar or even higher than those observed in mammals. On the other hand, radioimmunoassay of insulin detects also pro-insulin, and it is unclear whether this precursor has any specific physiological role (Plisetskaya, 1990; Mommsen and Plisetskaya, 1991; Plisetskaya, 1998). Considering the nutritional background, it is not surprising that insulin seems to have a different function in carnivorous fish from that in mammals (Christiansen and KlungsØyr, 1987). Although glucose administration to fish increases insulin secretion and the plasma insulin level (Hilton et al., 1987), there is a view that fish need insulin for regulation of their protein rather than carbohydrate metabolism since certain amino acids appear to be most potent secretagogues of insulin in fish (Mommsen and Plisetskaya, 1991). In rainbow trout hepatocytes, insulin plays an important role in the regulation of glycogen content (Pereira et al., 1995) and additionally there are results, which indicate that insulin is an important regulator of lipid synthesis in the liver of rainbow trout (Cowley and Sheridan, 1993) and that adipose tissue may be a target for the action of insulin in fish (Planas et al., 2000a).

The relative intolerance of fish to large doses of exogenous glucose despite the high level of circulating insulin has been suggested to resemble more the condition known as non-insulin-dependent diabetes mellitus rather than insulin-dependent diabetes (Hilton et al., 1987; Hertz et al., 1989). One possible explanation would be that in fish the interaction of insulin with its receptors is different from the situation in the mammalian system (Ablett et al., 1983; Mommsen and Plisetskaya, 1991). The amount of insulin receptors in rainbow trout muscle tissue is only less than 10% of that in mammals (Gutiérrez et al., 1991) but insulin is able to down-regulate its own receptor number in cardiac muscle cells of brown trout (Moon et al., 1996). Parrizas et al. (1994ab) found that the total insulin binding and tyrosine kinase activity of receptors was highest in common carp and lowest in brown trout. Their results revealed that fish can respond to both acute and maintained insulinemia by increasing the number of

insulin receptors but tyrosine kinase activity is modified only after long-term adaptation. They concluded that the properties of muscle insulin receptors might be related to fish nutritional preferences. All these studies imply that glucose intolerance, typical for carnivorous fish, does not seem to be just a consequence of either insulin deficiency or impaired hormone-receptor binding.

The enzymes of the major carbohydrate metabolic pathways such as glycolysis, tricarboxylic acid cycle, pentose phosphate pathway, gluconeogenesis, and glycogen synthesis are present in fish (Shimeno, 1974; Coway and Walton, 1989). The pentose phosphate pathway may play an important part in the breakdown of glucose in fish. Fideu et al. (1983) found high levels of glucose-6-phosphate dehydrogenase in the liver of rainbow trout and several studies have shown that this activity can be enhanced in fish by a carbohydrate-rich diet (Nagayama et al., 1973; Hilton and Atkinson, 1982). The three key enzymes in gluconeogenesis, D-fructose-diphosphatase, pyruvate carboxylase and phosphoenol pyruvate carboxykinase have been found in several tissues from rainbow trout (Cowey et al., 1977a). Since the natural diet of fish is poor in carbohydrate, gluconeogenesis seems to be important to supply the small, but vital, need of glucose of nervous tissue (reviewed in Christiansen and KlungsØyr, 1987).

The most important carbohydrate metabolic pathway is glycolysis, which leads to the tricarboxylic acid cycle (Coway and Walton, 1989). In rainbow trout, skeletal and heart muscles have the greatest and liver the least glycolytic capacity if this is based on the activities of individual enzymes (Knox et al, 1980). In mammals, glycolysis is regulated by energy charge, nutritional status and hormonal modulation. Knowledge of these regulatory mechanisms in fish is quite limited but some information about the influence of different diets on the activity of the glycolytic enzymes is available (reviewed in Christiansen and KlungsØyr, 1987).

Hexokinases catalyse the first reactions of glycolysis by phosphorylating glucose to glucose-6-phosphate. Phosphorylation deficiency of the liver has been assumed to be one of the rate-limiting steps in utilization of glucose in fish (Nagayama et al., 1973; Nagayama and Ohshima 1974; Cowey et al. 1977b; Sundby et al., 1991; Tranulis et al. 1991). In most mammals, an increase in the blood glucose will lead to the induction of liver hexokinase (glucokinase). Cowey et al. (1977b) were unable to find an enzyme with the properties of hexokinase in rainbow trout fed a high carbohydrate diet, but later Tranulis et al. (1996) detected activity of this enzyme in Atlantic salmon liver. Blin et al. (1999) were first to identify hexokinase-family members at the molecular level in rainbow trout, gilthead seabream (*Sparus aurata*) and common carp. In mammals, hexokinase activity is regulated mainly by alteration of gene

expression by dietary carbohydrates through an indirect action of insulin (reviewed in Panserat et al., 2000b). Panserat et al. (1999, 2000a) reported evidence of glucokinase-like gene expression and its regulation by dietary carbohydrates in the liver of rainbow trout, gilthead seabream and common carp. They found the lowest level of induction of glucokinase expression in the most glucose-tolerant fish, i.e, common carp, and the highest glucokinase expression from less glucose-tolerant fish, i.e, rainbow trout (Panserat et al., 2000b). They also reported that even a single meal (containing 24% of glucose) is sufficient to induce glucokinase expression (Panserat et al., 2001a), thus hepatic glucokinase is probably not the limiting step accounting for the low dietary glucose utilization. Additionally, Krasnov et al. (1999) expressed recombinant human GLUT1 and rat hexokinase II and obtained evidence that glucose transport but not hexokinase II activity was rate-limiting in fish embryos.

2.3.4 Existence of facilitative glucose transporters

Insufficient peripheral glucose uptake may contribute to poor utilization of carbohydrates and poor glucose tolerance in fish. Peripheral glucose uptake has been studied in fish enterocytes, erythrocytes, heart, brain and muscle cells.

In addition of Na^+-dependent transport of glucose across the brush-border of intestine, Reshkin and Ahearn (1987ab) reported the presence of facilitative Na^+-independent, phloretin and cytochalasin B-sensitive transport in basolateral membrane of fish intestine. This result was consistent with results reported by Soengas and Moon (1998) indicating the possible presence of facilitative glucose transporters in fish intestine.

The regulation of glucose transport in mammalian cardiac muscle is accomplished by reversible translocation of GLUT4 to the cell surface (Slot et al., 1991). Fish heart utilizes glucose as a metabolic fuel under aerobic conditions. Rodnick et al. (1997) studied the glucose uptake in American eel (*Anguilla rostrata*) cardiac muscle and transport activity was inhibited by cytochalasin B pointing to the involvement of a facilitative glucose transporter. It was concluded in that study that increased utilization of glucose may involve the recruitment of facilitative glucose transport proteins to the cell surface of cardiac myocytes or an increase in the activity of glucose transporters already residing at the cell surface. There was also cytochalasin B -insensitive transport activity present in cardiac muscle and it might reflect either non-transporter-mediated process or the presence of glucose transporter isoform, which was not sensitive to cytochalasin B. It was proposed that glucose transport could be the rate-controlling step for glucose utilization in eel cardiac muscle.

In mammals, insulin-sensitive skeletal muscle and adipose tissue are principally responsible for eliminating a glucose load from blood by increasing activity of facilitative glucose transporters. In fish, muscle represents approximately 50% of total body weight and must be considered to be the key to the disposal of glucose from blood (Moon, 2001). In brown trout (*Salmo trutta*) it was shown that skeletal muscle increases glucose uptake after a glucose load, but not enough to prevent hyperglycaemia (Blasco et al., 1996). Wright et al. (1998) reported that the peripheral resistance to glucose in omnivorous tilapia could be due to lack of GLUT4 and the very limited tissue distribution of GLUT1. They were unable to detect GLUT4 protein from different tissue samples (skeletal muscle, cardiac muscle, adipose tissue, islets and brain) with Western blots and GLUT1 was found only in cardiac muscle and brain. The amount of GLUT1 in cardiac muscle of tilapia was about 10-fold higher than that of rat and these results were consistent with those reported for the American eel cardiac muscle (Rodnick et al.,1997). Legate et al. (2001) reported stereospecific and saturable glucose transport in white muscle membrane of rainbow trout, American eel and black bullhead catfish (*Ameiurus melas*), but cytochalasin B and phloretin did not significantly inhibit the transport activity. They also analysed the presence of GLUTs by using Northern and Western blot analysis and did not detect any mammalian-type GLUT1 or GLUT4 transporters.

A wide variety of substrates can be used for energy production in fish erythrocytes (for review see Nikinmaa and Tiihonen, 1994). The glucose permeability of most nucleated erythrocytes, like fish erythrocytes, is low and has been proposed that glucose transport is the rate-limiting step for glucose utilization in these cells. In most teleost species investigated, carrier-mediated glucose transport by erythrocytes is reported to be either quite low or undetectable. Tse and Young, (1990) studied the glucose transport in erythrocytes of Japanese eel (*Anguilla japonica*), paddyfield eel (*Monopterus albus*) and rainbow trout. The presence of facilitative transporters was indicated only in several individuals of Japanese eel. In erythrocytes of these fish, transport of 3-OMG was saturable (K_m 1.2-1.5 mM) and sensitive to cytochalasin B. Transport was also inhibited by phloretin, D-glucose, 2-DOG and D-galactose but not by D-fructose and L-glucose. They did not find cytochalasin B-sensitive 3-OMG uptake in erythrocytes of paddyfield eel or rainbow trout. All of these three species had also cytochalasin B -insensitive uptake of 3-OMG, D-glucose and L-glucose, and it was speculated that there was some cytochalasin B-insensitive sugar transport system in these cells as is the case in rabbit erythrocytes (Albert, 1984). Tiihonen and Nikinmaa (1995) reported cytochalasin B and phloretin-sensitive 3-OMG uptake in carp erythrocytes but because the transport activity was not saturable, they concluded that either there were no glucose transporters in these cells or their affinity was very low. Soengas and Moon, (1995c) reported studies with American eel erythrocytes where transport of 3-OMG was saturable,

stereospecific (no transport of L-glucose) and sodium independent. The transport activity was clearly inhibited by cytochalasin B and phloretin. The K_m for 3-OMG (~10,4 mM) was 8.5 times higher than that reported for Japanese eel (Tse and Young, 1990) and they concluded that it might be related to genetic differences between these two species.

In contrast to teleost fish, the presence of facilitative glucose transporters has been easily detected in red blood cells of agnathas. Erythrocytes from Pacific hagfish (*Eptatretus stouti*) have a high level cytochalasin B-sensitive 3-OMG transport activity (Ingermann et al., 1984). Young et al. (1994) carried out kinetic and molecular studies with Pacific hagfish erythrocytes. Transport was rapid, saturable (K_m 0.88 mM in intact erythrocytes), stereospecific and cytochalasin B-sensitive and unlike Japanese eel erythrocytes (Tse and Young, 1990) there was no extreme individual variation in permeability. Photoaffinity labelling and immunoblotting experiments clearly indicated that the hagfish transporter had similarities with the human erythrocyte transporter GLUT1. Tiihonen and Nikinmaa (1991) detected moderate saturable and phloretin-sensitive 3-OMG transport in erythrocytes of river lamprey (*Lampetra fluviatilis*) and they concluded that there was both carrier-mediated facilitative diffusion and simple diffusion through the plasma membrane of erythrocytes.

Aldegunde et al. (2000) proposed the existence of facilitative glucose transporters in brain of rainbow trout. They noticed a clear 3-OMG transport activity in blood-brain barrier whose kinetic properties were similar to 3-OMG transport in fish erythrocytes (Soengas and Moon, 1995c). They concluded that since glucose together with lactate is the main metabolic fuel of fish brain and because the glucose levels in brain are normally very low in both fish and mammals, brain relies on a continuous glucose influx from the blood and this explains the possible existence of a phylogenetically conserved transport system.

To conclude, utilization of carbohydrates in fish is poor in relation to higher vertebrates and that can be due either to lack or to low expression of facilitative glucose transporters. When this study was initiated, results of uptake analyses indicated the existence of facilitative glucose transporters in fish but no genes had been cloned. The molecular identification of fish GLUT1 and GLUT2 is reported in this thesis. At the same time, a fish GLUT4-like cDNA was isolated (Planas et al., 2000b).

3 AIMS OF THE PRESENT STUDY

Knowledge of glucose transport system of lower vertebrates is limited and occurrence of GLUT-like genes and proteins in fish has been criticized. Results of glucose uptake studies in fish cells and tissues have indicated the presence of facilitative glucose transporters in fish but no genes have been cloned. Cloning of fish glucose transporters may help to elucidate the molecular basis of inefficient sugar metabolism in fish. The study of fish GLUTs is also needed for a better understanding of the molecular evolution of this multi-gene family and structure to function relationships of glucose transporters.

The aims of the present study were:

1. To identify and clone fish glucose transporters (**I-III**)

2. To analyse phylogeny and structure of fish glucose transporters with respect to other glucose transporters (**I-III**)

3. To determine the expression patterns of fish glucose transporters (**I, III, IV**)

4. To determine the transport activity and functional properties of fish glucose transporters (**I-IV**)

4 MATERIALS AND METHODS

4.1 Fish and cell cultures

Rainbow trout and Artic char (*Salvelinus alpinus*) eggs, and newly hatched rainbow trout alevins were delivered from the Hatsina hatchery (Saaristomerenkala OY) or the Tervo Fisheries Research and Aquaculture (**I**; **III**; **IV**). Fin samples from different salmonid species were kindly provided by Dr. J. Piironen (Saimaa Fisheries Research and Aquaculture) (**I**) and common carp epithelioma papulosum cyprini (EPC) cell line by Dr. M. Bearzotti (INRA, Jouy-en-Josas, France) (**II**). Adult rainbow trouts were from Kuopio University Aquaculture Research Unit (**I**; **III**).

4.2 PCR-cloning

RNA was isolated from tissues and cells by using TRIzol reagent (GibcoBRL), treated with RQ1 RNase-free DNase (Promega) and cDNA was synthesized (**I**; **II**; **III**). Rainbow trout (**I**;**III**) and EPC cell line (**II**) glucose transporters were cloned by using a combination of reverse transcription-polymerase chain reaction (RT-PCR) and rapid amplification of cDNA ends (RACE). Amplification was performed by using self-designed primers and GibcoBRL kits (**I**; **II**; **III**). PCR products were separated with electrophoresis and purified with gel-extraction kit (Qiagen). PCR products were sequenced directly or cloned into TOPO-vectors (Invitrogen) and sequencing was carried out with Thermo Sequenase fluorescent labelled primer cycle sequencing kit with 7-deaza-dGTP and A.L.Fexpress DNA sequencer (Amersham Pharmacia Biotech) (**I**; **II**; **III**).

4.3 Sequence analysis

Sequence similarity searches in the GenBank and dbEst databases were carried out using the BLAST 2.0 program (Altschul et al., 1997) (**I-III**). Sequence comparison analyses were performed with ClustalW program (Thompson et al., 1994) and protein secondary structure was predicted with SOSUI (Hirokawa et al., 1998) (**I-III**). PHYLIP (Felsenstein, 1989) was used for phylogenetic analysis of related proteins. The distance matrix was computed with PROTDIST and the unrooted tree constructed with the NEIGHBOR was viewed with the DRAWTREE (**I**; **III**). Transmembrane domains (TMD) were predicted with the TMHMM program (http://www.cbs.dtu.dk/services/) (**III**).

4.4 DNA dot blot and PCR analysis (I)

To estimate the number of GLUT genes in rainbow trout genome, genomic DNA was isolated using proteinase K digestion and phenol extraction. DNA was blotted with serial dilutions of plasmid containing OnmyGLUT1 (*Onchorhychus mykiss* glucose transporter type 1) insert

and hybridized with DIG-labelled OnmyGLUT1 probe. Chemiluminescent detection with CSPD was carried out according to the manufacturer's protocol (Boehringer-Mannheim). The optical density of spots was measured with Quantity one 4.0 (BioRad) and the linear range of data was used to estimate the copy number.

To study existence of GLUT genes in genomes of phylogenetically related fish species; Arctic char, landlocked salmon (*Salmo salar*), brown trout, whitefish (*Coregonus lavaretus*) and smelt (*Osmerus eperlanus*) DNA from fin samples was extracted with proteinase K and amplified with PCR using primers designed to conserved areas of GLUT. PCR products were gel extracted (Qiagen), blotted and hybridized with the probe using method described above.

4.5 Expression studies

Expression of OnmyGLUT1 and 2 in tissues of adult rainbow trout (**I**), embryos and tissues of 1- year old fish (**III**) was analysed with RT-PCR. Expression of OnmyGLUT1 was also studied in course of embryonic development. The developmental stage of embryo was determined using one somite pair unit (τ_s) according to Gorodilov (1996). Samples were collected at blastula (25 τ_s), early gastrula (36 τ_s), mid and late gastrula (42 and 50 τ_s), 5-7 somites (60-62 τ_s), 14-15 somites (69-70 τ_s), 33-35 somites (88-90 τ_s), 60-62 somites (115-117 τ_s), early vitelline plexus (130 τ_s) and middle vitelline plexus (150 τ_s). At every stages expression was determined with RT-PCR. Cloned OnmyGLUT1 was used to synthesize antisense RNA probe using *in vitro* transcription with T7 RNA polymerase (MBI Fermentas) in presence of DIG-oxigenin-11-UTP (Boehringer Mannheim). Probe was used for whole mount *in situ* hybridization in fixed embryos and detection was carried out with DIG Nucleic Acid Detection kit (Boehringer Mannheim). Stained embryos were mounted in glycerol and photographed using Olympus SZX9 stereomicroscope (**IV**).

4.6 Transport assays in embryos and cells

Hexose transport activity was measured in rainbow trout and Artic char embryos and dissociated embryonic cells (**I**; **III**; **IV**), in EPC cell line (**II**) and in isolated rainbow trout hepatocytes (**III**) using labelled glucose analogs (3-OMG and 2-DOG).

Kinetics of labelled sugars was determined without pre-incubation. When the effect of commonly used inhibitors such as cytochalasin B and phloretin (inhibit facilitative glucose transport), cyanide (KCN, inhibit proton-dependent transport, **II**) and phloridzin (inhibit sodium/glucose co-transport) was analysed, cells were pre-incubated with inhibitor before adding label. Sugars tested for competitive inhibition were added directly into the incubation medium without pre-incubation. To account for passive diffusion and non-specific binding of

label, phloretin (50 μM) or cytochalasin B (50 μM) was added in parallel incubation. Radioactivity was quantified with scintillation counter. Transport activity was normalised for protein measured with Protein assay kit (BioRad) and kinetic parameters were determined from data transformed with Lineweaver-Burk and Eadie-Hofstee plots (**II**).

To account for contribution of transport in hexose uptake by embryonic cells, 2-DOG which is phosphorylated by hexokinase was used. Total and non-phosphorylated 2-DOG were measured. Phosphorylated sugar was removed using the method described by Colville et al., (1993). Hexokinase activity was measured in this experiment using a spectrophotometric method (**IV**).

4.7 Functional assessment of recombinant GLUTs

For functionality studies in *Xenopus laevis* oocytes, rainbow trout OnmyGLUT1 was amplified with RT-PCR from newly hatched rainbow trout alevin RNA with special primers designed to introduce a *Bcl* I enzyme restriction site and a strong eukaryotic Kozak consensus. OnmyGLUT1 was cloned into *Bgl*II sites of pSPGT1 vector which contains 5′-and 3′-untranslated sequences of *Xenopus* ß-globin mRNA. Construct was sequenced and transcribed *in vitro*. Oocytes were prepared as described in Penny et al. (1998) and injected with either mRNA (15ng) or Rnase-free water. Functionality of OnmyGLUT1 transport was determined in mRNA- and water-injected *Xenopus* oocytes. Uptake measurements were made 24-72 hours after injection without pre-incubation of inhibitors or competitors (*zero*-trans conditions). Uptake was corrected for the water-injected controls. Kinetic parameters were estimated by nonlinear regression analysis using a Michaelis-Menten model and K_i was calculated with a one-site competition model (PRISM Ver.2, GraphPad) (**IV**).

The functionality of OnmyGLUT2 was analysed by cloning into vector containing a strong CMV promoter (Invitrogen) and microinjection into rainbow trout eggs as described in Krasnov et al., (1999). Four days after microinjections, expression of CMVOnmyGLUT2 and endogenous OnmyGLUTs were analysed with RT-PCR. Hexose transport activity and the effect of inhibitors were compared to the two different group of control embryos: embryos microinjected with CMVLacZ (Invitrogen) to account for an effect of transgene on the development of embryos and intact controls (no microinjection) (**III**).

4.8 Statistics

The statistical differences from controls in uptake measurements were analysed using Student's t-test (**II**; **III**; **IV**).

5 RESULTS

5.1 Cloning of fish glucose transporters (I-III)

First a full length GLUT cDNA was cloned from rainbow trout alevins (**I**). A 463 bp fragment with a high similarity to mammalian GLUT1 was amplified and next 3′- and 5′-ends were PCR cloned using gene-specific primers designed upon this sequence. Two 3′-end products of different size were amplified (1500 and 750 bp) and were shown to share similarity to the C-termini of mammalian and avian GLUT1. They were named as OnmyGLUT1A and OnmyGLUT1B and their deduced amino acid sequences were 83% identical. No sequence similar to the OnmyGLUT1A 3′- untranslated region (UTR) was found whereas the OnmyGLUT1B 3′-UTR shared similarity with Tc1-like transposon (Figure 2 in **I**). After cloning of 5′-UTR of OnmyGLUT1A (330 bp) the full length cDNA of OnmyGLUT1A (2766 bp) was cloned and verified with sequencing. OnmyGLUT1A contained one open reading frame encoding a predicted protein of 492 amino acids, which shared 77-79% identity with chick, mouse and human GLUT1 proteins (Figure 3 in **I** and Figure 2).

A full length carp glucose transporter cDNA (3060bp) named as CyiGLUT1, was cloned from EPC cells using the same approach. First, a PCR product of 484 bp sharing similarity with mammalian and avian glucose transporters was obtained followed by cloning of 5′- and 3′-ends. The length of 3′- and 5′-UTRs was 1281 bp and 341 bp, respectively. The CyiGLUT1 contained one open reading frame encoding a predicted protein of 478 amino acids, which shared 78-83% identity with mouse, chick and human GLUT1 proteins (Figure 5 in **II** and Figure 2).

A partial sequence of OnmyGLUT2 (293 bp) provided by S. Panserat (INRA, France) was used for PCR cloning of full length OnmyGLUT2 cDNA (1927 bp) from rainbow trout liver. The OnmyGLUT2 contained one open reading frame encoding a predicted protein of 482 amino acids, which had 52-58% identity with mouse, human and chicken GLUT2 proteins (Figure 2 in **III** and Figure 2).

DNA dot blot analysis implied that rainbow trout genome may contain three to six copies of GLUT genes indicating that new isoforms may be found (Figure 5 in **I**). PCR amplification and blotting of fin DNA samples showed that GLUTs can be also found from phylogenetically related species. A 1100bp products from Artic char, landlocked salmon and brown trout, and 900bp products from white fish and smelt were detected (Figure 6 in **I**).

5.2 Structure and phylogeny of fish glucose transporters (I-III)

The length of OnmyGLUT1 amino acid sequence was the same as human GLUT1 sharing 93% identity to CyiGLUT1. The N-terminus of carp glucose transporter was shorter than those of mouse and chick protein by 12 and 11 amino acids, respectively (Figure 5 in **II**). A specific feature of GLUT2, an elongated extra-cellular loop between the first and second transmembrane segments was found in OnmyGLUT2 (Figure 2 in **III**). In Figure 2, the amino acid sequences of OnmyGLUT1A, CyiGLUT1 and OnmyGLUT2 are aligned with human GLUT1, GLUT2 and brown trout GLUT4. Many of the key amino acids involved in substrate selection and transport activity are conserved in fish glucose transporters (see Figure 2).

The phylogenetic relationship between three fish (OnmyGLUT1A, OnmyGLUT2 and CyiGLUT1), higher vertebrate and invertebrate GLUTs was analysed. The distance matrix analysis suggested that fish GLUT1 proteins share a common ancestor with avian and mammalian GLUT1 and that fish GLUT1 proteins diverged from the tetrapod GLUT1 lineage after the segration of GLUT1 and GLUT3[1] (Figure 4 in **I** and Figure 1 in **III**). The matrix analyses showed that OnmyGLUT2 shared a common ancestor with other GLUT2 isoforms including the brown trout glucose transporter type 4 (StGT4) cloned by Planas et al., (2000b), which shared a common ancestor with other GLUT4 isoforms (Figure 1 in **III**).

[1] In **I** there is an error at this point; OnmyGLUT1 and CyiGLUT1 diverged from the tetrapod GLUT1 lineage after (not prior to) segregation of GLUT1 and GLUT3 paralogs .

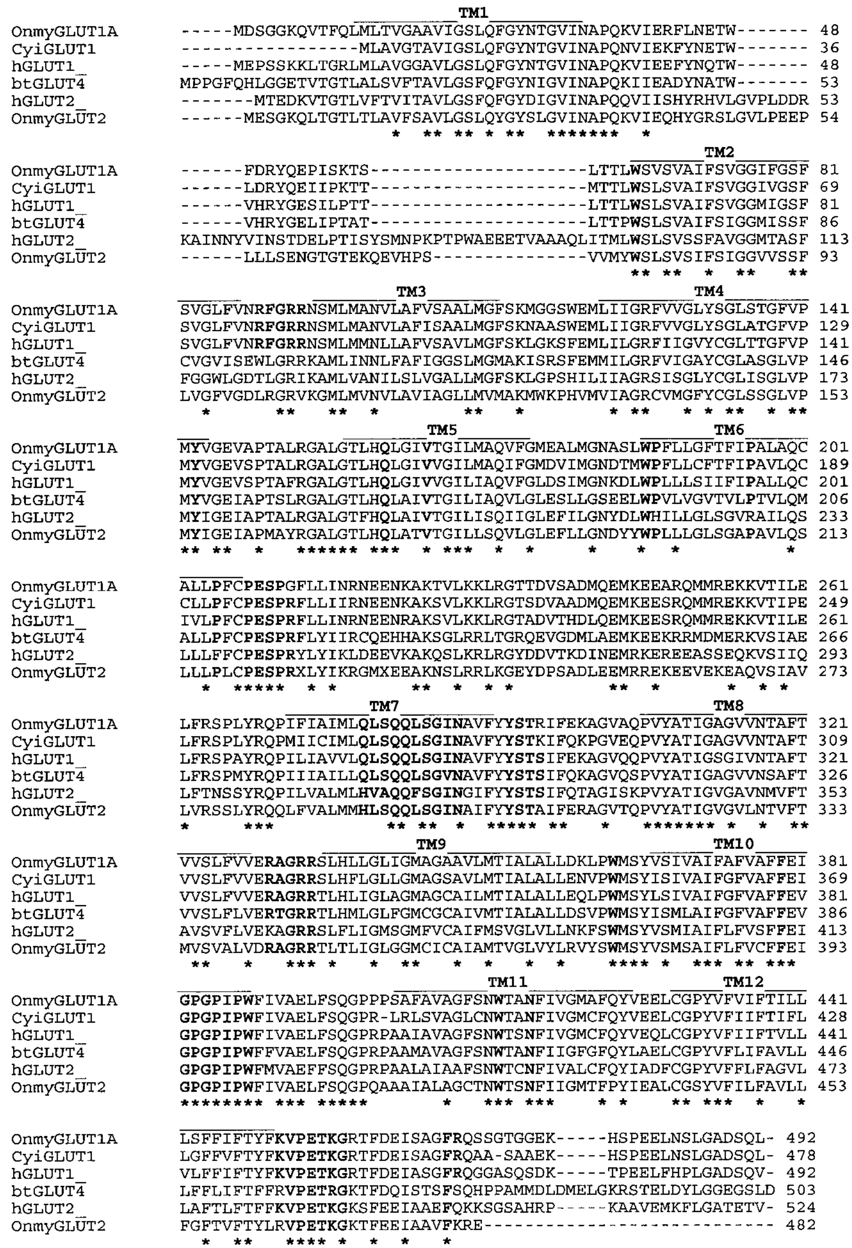

Figure 2. The amino acid sequences of OnmyGLUT1A, CyiGLUT1 and OnmyGLUT2 were aligned with human GLUT1 (Swiss-Prot accession number P11166), human GLUT2 (Swiss-Prot accession number P11168) and brown trout GLUT4 (btGLUT4) (GenBank accession number AF247395) using ClustalW. Transmembrane domains are predicted according to human GLUT1 using TMHMM program (http://www.cbs.dtu.dk/services/). *amino acid residues shared by all proteins. The key amino acids for substrate selectivity and transport activity are in bold.

5.3 Expression of fish glucose transporters (I, III, IV)

Expression studies with RT-PCR in adult rainbow trout tissues found an abundance of OnmyGLUT1 in heart and low-level expression in skeletal muscle, liver, spleen, blood and brain. The cloned and sequenced cDNA from cardiac muscle appeared identical to OnmyGLUT1A (**I**).

The spatial patterns of OnmyGLUT1 expression during rainbow trout embryonic development were analysed using whole-mount *in situ* hybridization. The distribution of transcripts was ubiquitous at early developmental stages. In early gastrula, OnmyGLUT1 was expressed throughout the embryo, especially on the edges of the blastodisc, in the region of the germ ring and terminal node (Figure 3A in **IV**). At early somitogenesis and in the following developmental stages expression was observed throughout the embryo and blastoderm (Figure 3B in **IV**). The spatial pattern of expression gradually became increasingly restricted in the course of development and the uneven distribution of the probe was first seen at the 14-15 somites stage. Transcripts were detected in forebrain, especially in the region of the eye vesicles (Figure 3C in **IV**). In addition, expression was observed in neural crest during the whole study period (Figure 3C-F in **IV**) and expression was most apparent in the anterior part of the embryo after completion of somitogenesis (Figure 3E,F in **IV**). OnmyGLUT1 expression was also seen in the gill arches, pectoral fins, upper jaw, primordia of mouth lips and olfactory organs. Over the course of development, somitic expression decreased along the rosto-caudal axis. No hybridization was observed on the dorsal surface of the brain, in the cranial dorsal ectoderm, notochords, heart, liver, kidney and gut.

Expression of OnmyGLUT2 was analysed with RT-PCR in embryos at different developmental stages; blastula, gastrula, during somitogenesis and at early vitelline plexus. OnmyGLUT2 expression was first detected at the mid stages of somitogenesis (34 somite pairs). Expression of OnmyGLUT2 was detected in the liver, kidney and intestine of 1-year old fish. The results were verified with cloning and sequencing of PCR products (**III**).

5.4 Transport activity and functional properties of fish GLUTs (I-IV)

OnmyGLUT1 is the predominant isoform in rainbow trout embryos. Uptake measurements in rainbow trout and Arctic char embryos were designed to determine the 3-OMG transport activity and its sensitivity to inhibitors and to study the stereospecificity of the transport activity. Cytochalasin B inhibited the uptake of 3-OMG in a dose-dependent manner (Figure 1 in **I**) and there was no difference in sensitivity to cytochalasin B between rainbow trout and Arctic char embryos. Phloretin inhibited transport only at a high concentration (200 μM). Hexose transport in embryos was found to be stereospecific since D-glucose inhibited the 3-

OMG uptake to 47% whereas the effect of L-glucose was not significant (Figure 1 in **I**). Uptake of 3-OMG was measured in rainbow trout embryos at different developmental stages and uptake was clearly inhibited by cytochalasin B. Additionally, hexose transport activity was measured in rainbow trout dissociated embryonic cells using 2-DOG, which is phosphorylated by hexokinase. Cytochalasin B decreased the content of 2-DOG phosphate in cells to a greater extent than that of non-phosphorylated 2-DOG, indicating that transport can be the rate-limiting step for the delivery of glucose to embryonic fish cells (Table 2 in **IV**). The hexokinase activity in embryos was 2 orders of magnitude greater than that of hexose transport and therefore it was not rate-limiting in our experiment. (**IV**).

To study the functional properties of OnmyGLUT1 in detail, it was expressed in *Xenopus* oocytes. The rate of D-glucose uptake by oocytes was found to be 2.3-fold and 13.9-fold greater than that of 3-OMG and D-fructose respectively. Thus D-glucose was used in subsequent studies. Transport of D-glucose was sodium independent, saturable and K_m values ranged from 8.3 to 14.9 mM. Accumulation of labelled D-glucose was decreased in the presence of 3-OMG, 1-deoxy-D-glucose (1-DOG), 2-DOG, 3-DOG, 6-DOG and D-mannose, whereas L-glucose, D-fructose, D-galactose, D-xylose, D-ribose, D-allose, L-sorbitol and mannitol had no inhibitory effect (Table 1 in **IV**). Transport of D-glucose was inhibited by cytochalasin B and phloretin but the inhibitory effect of phloridzin was detected only at high concentrations (over 500 μM) (Figure 2 in **IV**). The K_i values determined for cytochalasin B ranged from 1.4 to 2.3 μM.

CyiGLUT1 is expressed at a high level in EPC cells. Uptake experiments in this cell line were designed to determine the kinetics and sensitivity of 3-OMG transport to inhibitors and to study the stereospecificity of transport activity. Transport of 3-OMG in EPC cells was saturable with a K_m value of 8.5 mM (Figure 1, 2 in **II**). Transport was totally blocked by 10 μM of cytochalasin B and 50 μM of phloretin. KCN had no inhibitory effect on transport and phloridzin decreased 3-OMG uptake only at high concentrations, indicating that transport was carried out through facilitative glucose transporters (Figure 3 in **II**). Transport was found to be stereospecific; uptake of 3-OMG was markedly reduced in the presence of 3-OMG, 2-DOG, D-glucose, D-mannose, and D-xylose, but not in presence of mannitol, L-glucose, D-galactose, D-fructose, D-ribose and sucrose (Figure 4 in **II**).

OnmyGLUT2 is expressed in the rainbow trout liver. The uptake of 3-OMG by isolated rainbow trout hepatocytes was inhibited by both cytochalasin B and phloretin. Uptake was also inhibited by 2-DOG but not by D-glucose, D-fructose, D-galactose and mannitol (Figure 3 in **III**). To verify functionality of OnmyGLUT2, it was cloned into CMV vector and

microinjected into embryos. Expression of GLUTs was analysed with RT-PCR and expression of CMVOnmyGLUT2 but not of endogenous OnmyGLUT1 was detected in microinjected embryos. In contrast, in control embryos, OnmyGLUT1 expression was detected but there was no endogenous OnmyGLUT2 expression. The development of transformed embryos due the to over-expression of the transgene was slower than that of intact embryos. For this reason CMVLacZ controls were used in the uptake measurements. Uptake of 3-OMG in the CMVOnmyGLUT2 group was 1.29-fold greater than in the control group ($P<0.01$). The inhibition of cytochalasin B was found in CMVOnmyGLUT2 ($P<0.005$) but not in CMVLacZ microinjected embryos (Figure 4A in **III**). Comparison between OnmyGLUT2 microinjected embryos and intact controls was found to be difficult due the expression of endogenous GLUT1. During earlier studies, we determined that transport in embryos was relatively insensitive to phloretin unlike transport in hepatocytes therefore phloretin was used to discriminate between endogenous and recombinant CMVOnmyGLUT2 transport activity. The inhibition of 2-DOG transport with phloretin (100 mM) was significantly greater ($P<0.05$) in CMVOnmyGLUT2 microinjected embryos than in non-injected controls indicating that recombinant OnmyGLUT2 was capable of transporting sugar.

6 DISCUSSION

6.1 Identification and structure of fish glucose transporter

When this work was started, no facilitative glucose transporters from fish had been cloned and their existence had remained questionable, although there were hints that fish cells might transport glucose through a facilitative transporter system.

Cloning of facilitative glucose transporters was started from newly hatched rainbow trout alevins since relatively high levels of glucose transport activity had been observed at this stage of the life cycle. Two different 3′-end products of glucose transporters, OnmyGLUT1A and OnmyGLUT1B, were cloned sharing 83% identity at the amino acid level. OnmyGLUT1B 3′-UTR shared similarity with Tc1-like transposon pseudogenes identified in a number fish species (Izsvák et al., 1995; Ivics et al., 1996). A commonly accepted view is that insertion of transposon elements near the coding region of genes impairs their expression (Labrador and Corces, 1997) but surprisingly, a Tc1-like transposon was incorporated in the 3′-UTR region of the rainbow trout GLUT gene without affecting the expression of the gene. Cloning of OnmyGLUT1A was continued and resulted in isolation of a full length (2766 bp) OnmyGLUT1A cDNA encoding a predicted protein of 492 amino acids, which shared its highest similarity with avian and mammalian GLUT1 isoforms (**I**). At the same time, a full length (3060 bp) CyiGLUT1 cDNA was cloned from carp EPC cell line encoding a predicted protein of 478 amino acids and having 78-83% identity to mouse, chick and human GLUT1 proteins. The central part of the CyiGLUT1 protein sequence was similar to other GLUTs whereas terminal domains were more divergent. The N-terminus of the carp glucose transporter was shorter than that of the other GLUT1 proteins and also shorter than that of other mammalian GLUT isoforms. Its predicted transmembrane structure contained only 11 TM segments and it is possible that the lack of several N-terminal amino acids may affect the secondary and tertiary structure of CyiGLUT1 (**II**). Results have shown that both N-terminal and C-terminal domains of the GLUT1 are needed to form a stable protein (Cope et al., 1994).

The highest similarity of different glucose transporters can be seen in the transmembrane areas (Kayano et al., 1990; Burant et al., 1991) and this is true for fish glucose transporters. Both OnmyGLUT1A and CyiGLUT1 contain the GADSQL sequence at the C-end, which is found in all GLUT1 isoforms. The pentametric (RXGRR) motifs located in loop 2 and 8 are important in determining the proper orientation of these cytoplasmic loops as well as their flanking TM segments (Gould and Holman, 1993; Sato and Mueckler, 1999). The residues important for conformation and proper orientation of the protein such as Lys_{451}, Phe_{467} and Arg_{468} are conserved as well. A recognition motif for D-glucose (QLS) in TM7 is present as

well as conserved tryptophan residues (Trp_{65}, Trp_{186}, Trp_{363}, Trp_{388} and Trp_{412}), which have important role in structure and catalytic activity of the transporters. Trp_{388} is also known as the binding site of cytochalasin B. Conserved areas important for transport activity such as proline residues in TM6 and 10, PES(/T)PR in loop 6, GW/PGPIPW in TM10, VPETKG in C-terminal tail, Val_{165} in TM5, Tyr_{143} in TM4, Tyr_{293} in TM7, Phe_{379} in TM10 and Asn_{415} in TM11 can be found. Both these proteins contain $Q_{282}QXSGXN_{288}XXXYYY$ motif, which is known as a part of the excofacial binding site. The conserved STS motif in loop 7, which is involved in the conformational alterations during the transport process is present in OnmyGLUT1A and CyiGLUT1 but in both of these proteins the last serine residue in this motif is replaced by arginine and lysine, respectively (see Figure 2). Doege et al. (1998) reported that mutation of this serine residue in rat GLUT4 had no effect on the functional properties and this may be true for these proteins as well.

The full length (1927 bp) cDNA of OnmyGLUT2 cloned from rainbow trout liver, encodes a predicted protein of 482 amino acids having its highest similarity to avian and mammalian GLUT2 (**III**). It has a long extracellular loop between the first and second TM segments, which is also found in other GLUT2 proteins. The divergence of GLUT2 proteins is greatest in this region (Figure 2). The length of this loop is 30 amino acids in hGLUT1, but in human, chicken and rainbow trout GLUT2 it contains 64, 74 and 42 amino acids, respectively. The length of TM segments and the loops vary depending on which programs are used for predictions. The C-termini of GLUT2 proteins are diverged and this is also true for OnmyGLUT2. TM7-8 in mammalian GLUT2 have been reported to be crucial for the ability to transport D-fructose and TM8-12 motifs have an overall role in substrate selectivity (Buchs et al., 1995; Arbuckle et al., 1996; Wu et al., 1998). The QLS motif in TM7, which is responsible for D-glucose transport in GLUT1 protein is replaced by HVA in human GLUT2, HMA in mouse GLUT2, QIS in chicken GLUT2 and by HLS in OnmyGLUT2. In human GLUT2, replacement of HVA with QLS reduced the ability of the protein to transport D-fructose (Wu et al., 1998). The conserved Val_{197} residue, which is important for GLUT2 transport activity is found in OnmyGLUT2 as well as the conserved areas important for transport activity and proper conformation of GLUTs (see Figure 2).

The phylogenetic relationship between three fish glucose transporters and glucose transporters from higher vertebrates and invertebrates suggested that fish GLUT1 proteins share a common ancestor with avian and mammalian GLUT1 and that GLUT1 proteins diverged from tetrapod GLUT1 lineage after the segregation of GLUT1 and GLUT3 isoforms. In the phylogenetic tree, OnmyGLUT2 was also clustered with other GLUT2 proteins. Similar results were reported by Planas et al. (2000b) who cloned GLUT4-like cDNA from brown

trout. It is likely that diversification of this multigene family occurred at an early stage of vertebrate evolution since fish have different isoforms of glucose transporters and all fish GLUTs share a common ancestor with the same type of GLUTs from other species **(I-III)**.

At present, four glucose transporters of three different types have been cloned from fish species and most likely new isoforms await to be found. Dot blot analysis suggested that there might be at least three to six copies of GLUT genes in rainbow trout genome. PCR and blotting analyses detected GLUT genes in genomes of phylogenetically related species. The primers used in these PCR analyse in theory could amplify different isoforms of glucose transporters since they were designed to detect conserved sequences (VPMY and SGINAVF) found in several mammalian GLUT1, GLUT3 and GLUT4 isoforms **(I)**.

6.2 Tissue expression of fish glucose transporter

In mammals, GLUT1 is the most ubiquitously distributed isoform with the highest level of expression being found in fetal tissues, erythrocytes, brain, kidney and colon (reviewed in Gould and Bell, 1990; Burant et al., 1991). In adult rainbow trout, OnmyGLUT1 was expressed mainly in heart but low-level expression could be found in skeletal muscle, liver, spleen, blood and brain tissues. These results are in accordance with the results reported by Wright et al. (1998) and Aldegunde et al. (2000). Western blot analyses in tilapia found four times greater GLUT1 protein levels in cardiac muscle than in brain, and facilitative glucose transport activity was detected in brain of rainbow trout. In most of the teleost fish species investigated, carrier-mediated glucose transport by erythrocytes is reported to be either quite low or undetectable and in concordance with this, we detected only low level of OnmyGLUT1 expression in erythrocytes of rainbow trout **(I)**.

In mammals, the expression of GLUT1 varies during embryogenesis. Both mRNA and protein are accumulated during pre-implantation in parallel with an increase in glucose transport activity (Hogan et al., 1991; Morita et al., 1992; Dan-Goor et al., 1997) and mammalian GLUT1 is expressed in all embryonic and foetal tissues, especially in brown adipose tissue, heart, liver and skeletal muscle (Werner et al., 1989; Aghayan et al., 1992; Santalucia et al., 1992; Postic et al., 1994). OnmyGLUT1 seems to be the predominant isoform in fish embryos and we analysed the expression patterns of OnmyGLUT1 in rainbow trout embryos from blastula to the end of somitogenesis and during early/middle vitelline plexus. The distribution of transcripts was ubiquitous at early developmental stages (blastula), and during early, middle and late gastrula, OnmyGLUT1 was expressed throughout the embryo, especially on the edges of the blastodisc in the region of the germ ring and terminal node, where the density of cells and active morphogenetic processes is highest (Figure 3A in **IV**). The gastrulation in

salmonid fish is completed when the dense core including the anlagen of the axial organ complex develops in the middle of the embryonic shield (Gorodilolov 1989, 1996). This consists of the notochord, two bands of unsegmented prospective somite mesoderm and neural plate. Gastrulation is followed by somitogenesis when 70-72 somite pairs and primordia of almost all organs and functional systems are formed. At early somitogenesis and in the following developmental stages, expression of OnmyGLUT1 was observed throughout the embryo and blastoderm. The spatial pattern of expression gradually became increasingly restricted over the course of development, and the uneven distribution of hybridization was first seen at the 14-15 somites while abundant expression was observed in the forebrain, especially in the region of the eye vesicles and in neural crest (Figure 3B, C in **IV**). After somitogenesis and during vascularization of the yolksac (vitelline plexus) hybridization of the probe was most apparent in the head part of embryo and OnmyGLUT1 expression was also seen in the gill arches, pectoral fins, upper jaw, primordia of mouth lips and olfactory organs (Figure 3D-F in **IV**). No expression of OnmyGLUT1 was observed on the dorsal surface of the brain, in the cranial dorsal ectoderm, notochords, heart, liver, kidney and gut. Unexpectedly, no expression was detected in heart, though in adult rainbow trout OnmyGLUT1 is expressed mainly in cardiac muscle and there are many results showing that mammals embryonic heart depends on glucose during early organogenesis and GLUT1 is important during this period (Smoak and Branch, 2000).

The neural crest is a population of cells located at the borders between the neural plate and lateral ectoderm along the entire axis of the embryo. The neural crest gives rise to the connective, skeletal and muscle tissues of the head and it produces skeleton, pigmented cells and nervous roots in the trunk/tail and this important structure is found exclusively in vertebrates (Gans and Northcutt, 1983; Langille and Hall, 1989; Couly et al., 1993; Köntges and Lumsden, 1996; Artinger et al., 1999; García-Castro and Bronner-Fraser, 1999; Gorodilov, 2000). Expression of OnmyGLUT1 was abundant in neural crest region during somitogenesis and early/middle vitelline plexus (Figure 3 C-F in **IV**). Our results clearly indicated the importance of glucose transport activity and GLUT1 in these areas during embryonic development of rainbow trout embryos.

In the course of development, somitic expression in rainbow trout embryos decreased along the rosto-caudal axis and was undetectable in the anterior trunk myotomes (derivatives of the somites) in contrast to hybridization in the posterior trunk and tail somites. Expression of mammalian GLUT1 is also reduced during the differentiation of skeletal muscle (Santalucia et al., 1992). Myogenic differentiation includes the fusion of myoblasts into multinuclear myotubes, with subsequent upregulation of muscle-specific protein expression. Guillet-

Deniau et al. (1994) found expression of three GLUT isoforms during foetal myoblast differentiation in rat; GLUT1 mRNA and protein were abundant only in myoblasts, GLUT3 mRNA and protein increased markedly during cell fusion but decreased in contracting myotubes and GLUT4 mRNA and protein were found only from myotubes. These results clearly showed that in mammals there is a switch in glucose transporter isoform expression during myogenic differentiation, but this remains to be studied in fish.

OnmyGLUT2 was expressed in liver, kidney and intestine of 1-year old rainbow trout. Panserat et al. (2001b) reported similar results of RT-PCR experiments with juvenile rainbow trout. Reshkine and Ahearn (1987ab) claimed the possible presence of facilitative glucose transport activity in fish intestine and this was later confirmed by Soengas and Moon (1998) who carried out studies in isolated enterocytes of the black bullhead. In mammals, GLUT2 has a similar expression pattern; it mediates the uptake and release of glucose in liver and participates in transepithelial transport of absorbed and reabsorbed glucose in the small intestine and kidney. In mammals, expression of GLUT2 is seen during embryogenesis from the 8-cell stage onward and expression increases throughout development (Hogan et al., 1991; Postic et al., 1994). During embryonic development of rainbow trout, expression of OnmyGLUT2 was first detected at the middle of somitogenesis (34 somite pairs) but not in blastula and gastrula. The onset of expression coincides with the development of primordial kidney ducts, which begins at the 23 somite pairs stage and the appearance of liver rudiments at the stage of 56 somite pairs (Gorodilov, 1996) (**III**).

6.3 Activity and functional properties of fish glucose transporters

Analysis of the derived amino acids sequences suggested that the residues involved in glucose transport are well conserved in OnmyGLUT1, CyiGLUT1 and OnmyGLUT2 proteins. Glucose uptake studies in embryos and cells were carried out to confirm their transport activity.

Uptake of 3-OMG in rainbow trout and Arctic char embryos at the end of somitogenesis was stereospecific and inhibited by cytochalasin B in a dose-dependent manner. Phloretin inhibited the transport activity only at high concentrations, indicating that the transport system present in these embryos was not as sensitive to this inhibitor as the mammalian glucose transporters (reported K_i values of phloretin 2.4-40 μM) (Jung and Rampal, 1977; Ciaraldi et al., 1986) (**I**). Additionally, uptake of 3-OMG was studied during embryonic development of rainbow trout and transport activity was inhibited by cytochalasin B during gastrula and later developmental stages (**IV**). Experiments with 2-DOG suggested that transport, not hexokinase activity, was rate-limiting step for the delivery of glucose to embryonic fish cells (**IV**). A

similar conclusion was drawn in experiments examining the transfer of hGLUT1 and rat HKII in rainbow trout embryos (Krasnov et al., 1999).

To obtain direct evidence of transport activity and to study functional properties of OnmyGLUT1, this protein was expressed in *Xenopus* oocytes. Expression of OnmyGLUT1 in oocytes increased significantly the sodium-independent, saturable D-glucose uptake. The K_m values of D-glucose ranged from 8.3 to 14.9 mM being similar to the K_m (10.4 mM) reported in red blood cells of American eel (Soengas and Moon, 1995c). The reported K_m of mammalian GLUT1 for D-glucose is around 2.5 mM and for 2-DOG 6.9 mM (Burant and Bell, 1992; Woodrow et al., 2000) being slightly lower than the value for OnmyGLUT1. It is known that 3-OMG appears to have a lower affinity for the glucose transporter than 2-DOG and D-glucose (Gould et al., 1991; Burant and Bell, 1992). This was also true in our studies where OnmyGLUT1 had higher transport activity for D-glucose than for 3-OMG. There are potential pitfalls in using D-glucose or 2-DOG in uptake studies since it is possible that the measured kinetics are influence by hexokinase activity (Jacobs et al., 1990). However, many studies have shown that hexokinase activity in *Xenopus* oocytes is not a limiting factor for transport activity (Gould et al., 1991; Burant and Bell, 1992; Colville et al., 1993). Transport of D-glucose was inhibited by cytochalasin B (K_i 1.4-2.3 μM) and phloretin, but the inhibitory effect of phloridzin was detected only at high concentrations. The K_i for cytochalasin B was similar to that of mammalian GLUT1 during D-glucose, 2-DOG and 3-OMG uptake (K_i around 1 μM) in *Xenopus* oocytes (Burant and Bell, 1992; Due et al., 1995; Woodrow et al., 2000). Competitive inhibition analyses were designed to address the substrate selectivity of OnmyGLUT1. The transport activity of D-glucose decreased in the presence of 1-DOG, 2-DOG, 3-DOG and 6-DOG, suggesting that the removal of hydroxyl group from these positions did not eliminate the ability to compete with D-glucose. D-mannose, the C2 epimer of D-glucose decreased D-glucose transport activity, indicative of an insignificant role of equatorial hydroxyl in C2. OnmyGLUT1 showed higher transport activity for D-glucose than 3-OMG, suggesting that substitution of the C3 hydroxyl group reduced the transport activity. L-glucose, D-fructose, D-galactose, D-xylose, D-ribose, D-allose, L-sorbitol and mannitol did not compete with D-glucose, thus OnmyGLUT1 has a similar substrate preference as mammalian GLUT1 (Gould et al., 1991; Woodrow et al., 2000) and the transporter present in Japanese eel erythrocytes (Tse and Young, 1990). The functional properties of OnmyGLUT1 confirmed it to be similar to the mammalian type 1 glucose transporter.

To characterize the glucose transporter in the EPC cell line, 3-OMG uptake was studied. Saturation of 3-OMG uptake in EPC cells was observed in relation to both incubation time and concentration of substrate. Since phloridzin inhibited the uptake only at high

concentrations and the reported K_i of phloridzin for sodium-dependent cotransport is 5 μM (Yokota et al., 1983), the existence of sodium-dependent glucose transporter in EPC cells was unlikely. In terms of sensitivity to cytochalasin B and phloretin, the transport system in EPC cells was similar to human GLUT1 (Jung and Rampal, 1977). The transporter expressed in the EPC cell line, recognized monosaccharides in a ring form and its substrate preference was similar to mammalian GLUT1 and GLUT3. Interestingly, 3-OMG transport was inhibited by D-xylose, whose competitive effect on 3-OMG transport has been reported for mammalian GLUT3 (Gould et al., 1991). K_m for 3-OMG (8.5 mM) and substrate specificity were similar to mammalian cells (K_m for 3-OMG 13.2 mM) (Takakura et al., 1991) and OnmyGLUT1 expressed in *Xenopus* oocytes. There was no direct evidence that hexose uptake in EPC cells was mediated by CyiGLUT1. However no other glucose transporter was found in these cells though the primers used for PCR cloning could amplify the different GLUT isoforms. It is known that expression of GLUT1 isoform is increased dramatically in transformed cells and most established mammalian cell lines exclusively harbour GLUT1 (reviewed in McGowan et al., 1995).

To characterize the glucose transporter present in rainbow trout hepatocytes, 3-OMG uptake was studied. Transport was inhibited by cytochalasin B and also with phloretin, which did not inhibit the transport activity in rainbow trout embryos. Transport activity of mammalian GLUT2 is less sensitive to cytochalasin B than other isoforms (except GLUT5) and because GLUT2 has also low affinity for D-glucose, it is possible that cytochalasin B is recognized by the glucose binding site (see Burant and Bell, 1992). Transport activity in hepatocytes was not as sensitive to cytochalasin B as transport activity in rainbow trout embryos and in *Xenopus* oocytes, which expressed OnmyGLUT. In contrast to mammalian GLUT2, transport of 3-OMG in rainbow trout hepatocytes was not inhibited by D-fructose. This may indicate that unlike other GLUT2s, OnmyGLUT2 is not capable of transporting D-fructose. This may be due to changes in the HV/MA motif in TM7 needed for D-fructose transport. In our experiments 2-DOG clearly inhibited 3-OMG uptake but the lack of competition with D-glucose was surprising, since D-glucose inhibited 2-DOG uptake both in rat hepatocytes (Elliott et al., 1984) and in *Xenopus* oocytes expressing mammalian GLUT2 (Gould et al., 1991).

To verify OnmyGLUT2 transport activity, it was expressed under CMV promoter in embryos. Comparison between CMVOnmyGLUT2 microinjected embryos and intact controls was difficult due to expression of endogenous GLUT1 and therefore phloretin was used to discriminate between CMVOnmyGLUT2 and endogenous OnmyGLUT1. Our results showed that CMVOnmyGLUT2 increased transport activity in embryos. The moderate effect of the

CMVOnmyGLUT2 on transport activity was probably due to the relatively low transformation rate since usually expression of transgene can be seen only in 2-5% of rainbow trout blastula cells (Krasnov et al., 1999). Studies of functional properties and substrate selectivity of OnmyGLUT2 will need expression of the recombinant protein.

7 SUMMARY AND CONCLUSIONS

At the onset of this study, no glucose transporter had been cloned from lower vertebrates, their very existence in fish was a mater of speculation. The results presented in this thesis prove, without doubt, their existence. Three GLUTs of two different types were cloned in this study and at the same time one more type of fish GLUT was identified by another research group. Results of dot blot hybridization experiments suggested that new GLUT isoforms are likely to be found in fish. A phylogenetic study showed that diversification of this multi-gene family of glucose transporters occurred at an early stage of vertebrate evolution.

Direct evidence for the importance of glucose transporters was obtained with embryonic cells. OnmyGLUT1 was the predominant embryonic isoform, and was especially abundant in neural tissues. In adult fish, OnmyGLUT1 was expressed mainly in the heart, which is in accordance with the high level of glucose metabolism in this tissue. As well as mammalian GLUT2, OnmyGLUT2 was expressed in the liver, kidney and intestine and it is likely to be involved in similar physiological processes as its mammalian counterpart. High level of CyiGLUT1 expression in the EPC cell line suggested that as in mammals, glucose metabolism in fish cells maybe enhanced by malignant transformation.

The functional properties of fish GLUT1 were studied in detail. Recombinant OnmyGLUT1 was expressed in *Xenopus* oocytes and glucose transport in EPC cells, which is probably mediated by CyiGLUT1, was analysed. The results revealed that these proteins are fully functional and are similar to mammalian GLUT1 in terms of kinetics and substrate selectivity. The ability of OnmyGLUT2 to transport glucose was confirmed by gene transfer into rainbow trout embryos and its activity was found in isolated hepatocytes. The functional properties of this transporter remain to be studied. In contrast to GLUT1, the amino acid sequence of GLUT2 was much less conserved. In particular, variation was found in the domain, which is important for transport of fructose. Further studies into the fish GLUT2 will add to our understanding of the structure to function relationship in this transporter.

Transfer of human GLUT1 cDNA cloned with all-tissue fish and viral promoters preceded this study. Expression of these transgenes augmented glucose metabolism in rainbow trout embryos (Krasnov et al., 1999) and a slight tendency to improved carbohydrate utilization was also observed in transformed fish (Pitkänen et al., 1999). Next, fish glucose transporters will be transferred and expressed in fish under the regulation of muscle-specific promoters that have been cloned recently by our group. This will hopefully elucidate the influence of fish glucose transporters on glucose metabolism in fish muscle.

The results of this study are evidence of the functional significance of glucose transporters for fish. Obviously poor utilisation of carbohydrates in carnivorous fish is not attributable to lack of glucose transporters or their abnormal properties. As in mammals, fish GLUTs may be under the regulation of insulin and other hormones and biologically active peptides but because of the pecularities of carnivorous fish carbohydrate metabolism it is no wonder if regulation differs from mammals. With respect to further studies, cloning of fish glucose transporters as well as modern tools of transgenesis, proteomics and microarrays represent a powerful approach to study gene expression, cellular location and trafficking of GLUTs with respect to endocrine regulation and various physiological conditions. These methods provide tools to study the influence of one or several genes on glucose metabolism of the whole fish and they may help to elucidate the physiological role of facilitative glucose transporters in fish.

8 REFERENCES

Ablett R F, Taylor M J and Selivonchick D P: The effect of high-protein and high-carbohydrate diets on (I^{125})iodoinsulin binding in skeletal muscle plasma membranes and isolated hepatocytes of rainbow rout (*Salmo gairdneri*). Br J Nutr 50: 129-139, 1983

Aghayan M, Rao L V, Smith R M, Jarett L, Charron M J, Thorens B and Heyner S: Developmental expression and cellular localization of glucose transporter molecules during mouse preimplantation development. Development 115: 305-312, 1992

Ahearn G A, Behnke R D, Zonno V and Storelli C: Kinetic heterogeneity of Na-D-glucose cotransport in teleost gastrointestinal tract. Am J Physiol 263: 1018-1023, 1992

Albert S G: Cytochalasin B does not serve as a marker of glucose transporter in rabbit erythrocytes. Biochem Int 9: 93-103, 1984

Aldegunde M, Andrés M D and Soengas J L: Uptake of 3-O-methyl-D-[U-^{14}C]glucose into brain of rainbow trout: possible effects of melatonin. J Comp Physiol 170B: 237-243, 2000

Altschul S F, Madden T L, Schaffer A A, Zhang J, Zhang Z, Miller W and Lipman D J: Gapped BLAST and PSI- BLAST: a new generation of protein database search programs. Nucleic Acids Res 25: 3389-3402, 1997

Allard W J and Lienhard G E: Monoclonal antibodies to the glucose transporter from human erythrocytes. Identification of the transporter as a Mr = 55,000 protein. J Biol Chem 260: 8668-8675, 1985

Anderson J, Jackson A J, Matty A J and Capper B S: Effects of dietary carbohydrate and fiber on the tilapia *Oreochromis niloticus* (Linn). Aquaculture 37: 303-314, 1984

Araki S, Yang J, Hashiramoto M, Tamori Y, Kasuga M and Holman G D: Subcellular trafficking kinetics of GLUT4 mutated at the N-and C-termini. Biochem J 315: 153-159, 1996

Arbucle M I, Kane S, Porter L M, Seatter M J and Gould G W: Structure-function analysis of the liver-type (GLUT2) and brain-type (GLUT3) glucose transporters: expression of chimeric transporters in *Xenopus* oocytes suggests an important role for putative transmembrane helix 7 in determining substrate selectivity. Biochemistry 35: 16519-16527, 1996

Artinger K B, Chitnis A B, Mercola M and Driever W: Zeprafish *narrowminded* suggests a genetic link between formation of neural crest and primary sensory neurons. Development 126: 3969-3979, 1999

Asano T, Shibasaki Y, Kasuga M, Kanazawa Y, Takaku F, Akanuma Y and Oka Y: Cloning of a rabbit brain glucose transporter cDNA and alteration of glucose transporter mRNA during tissue development. Biochem Biophys Res Commun 154: 1204-1211, 1988

Austreng E: Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. Aquaculture 13: 265-272, 1978

Baldwin J M, Lienhard G E and Baldwin S A: The monosaccharide transport system of the human erythrocyte. Orientation upon reconstitution. Biochim Biophys Acta 599: 699-714, 1980

Baldwin S A: Mammalian passive glucose transporters: members of an ubiquitous family of active and passive transport proteins. Biochim Biophys Acta 1154: 17-49, 1993

Barrett M P, Walmsley A R and Gould G W: Structure and function of facilitative sugar transporters. Curr Opin Cell Biol 11: 496-502, 1999

Bergot F: Carbohydrate in rainbow trout diets: effect of the level and source of carbohydrate and the number of meals on growth and body composition. Aquaculture 18: 157-167, 1979a

Bergot F: Specific problems posed by carbohydrate utilization in the rainbow trout. Ann Nutr Aliment 33: 247-257, 1979b

Birnbaum M J, Haspel H C and Rosen O M: Cloning and characterization of a cDNA encoding the rat brain glucose-transporter protein. Proc Natl Acad Sci USA 83: 5784-5788, 1986

Birnbaum M J: Identification of a novel gene encoding an insulin-responsive glucose transporter protein. Cell 57: 305-315, 1989

Blasco J, Fernandez-Borras J, Marimon I and Requena A: Plasma glucose kinetics and tissue uptake in brown trout in vivo: Effect of an intravascular glucose load. J Comp Physiol B 165: 534-541, 1996

Blin C, Panserat S, Médale F, Gomes E, Brêque J, Kaushik S and Krishnamoorthy R: Teleost liver hexokinase- and glucokinase-like enzymes: partial cDNA cloning and phylogenetic studies in rainbow trout *(Oncorhychus mykiss)*, common carp *(Cyprinus carpio)* and gilthead seabream *(Sparus aurata)*. Fish Physiol Biochem 21: 93-102, 1999

Brauge C, Medale F and Corraze G: Effect of dietary carbohydrate levels on growth, body composition and glycaemia in rainbow trout, *Oncorhychus mykiss*, reared in seawater. Aquaculture 123: 109-120, 1994

Buchs A, Wu L, Morita H, Whitesell R R and Powers A C: Two regions of GLUT2 glucose transporter protein are responsible for its distinctive affinity for glucose. Endocrinology 136: 4224-4230, 1995

Buchs A E, Sasson S, Joost H G and Cerasi E: Characterization of GLUT5 domains responsible for fructose transport. Endocrinology 139: 827-831, 1998

Buddington R K and Hilton J W: Intestinal adaptations of rainbow trout to changes in dietary carbohydrate. Am J Physiol 253: G489-G496, 1987

Buhler D R and Halver J E: Nutrition of salmonid fishes. 9. Carbohydrate requirements of chinook salmon. J. Nutr 74: 307-318, 1961

Burant C F, Sivitz W I, Fukumoto H, Kayano T, Nagamatsu S, Seino S, Pessin J E and Bell G I: Mammalian glucose transporters: structure and molecular regulation. Rec Progr Hormone Res 47: 349-388, 1991

Burant C F and Bell G I: Mammalian facilitative glucose transporters: evidence for similar substrate recognition site in functionally monomeric proteins. Biochem 31: 10414-10420, 1992

Burant C F, Takeda J, Brot-Laroche E, Bell G I and Davidson N O: Fructose transporter in human spermatozoa and small intestine is GLUT5. J Biol Chem 267: 14523-14526, 1992

Cairns M T, Alvarez J, Panico M, Gibbs A F, Morris H R, Chapman D and Baldwin S A: Investigation of the structure and function of the human erythrocyte glucose transporter by proteolytic dissection. Biochim Biophys Acta 905: 295-310, 1987

Calamia J and Manoil C: lac permease of Escherichia coli: topology and sequence elements promoting membrane insertion. Proc Natl Acad Sci USA 87: 4937-4941, 1990

Calderhead D M, Kitagawa K, Tanner L I, Holman G D and Lienhard G E: Insulin regulation of the two glucose transports in 3T3-L1 adipocytes. J Biol Chem 265: 13801-13808, 1990

Camps M, Castello A, Munoz P, Monfar M, Testar X, Palacin M and Zorzano A: Effect of diabetes and fasting on GLUT-4 (muscle/fat) glucose-transporter expression in insulin-sensitive tissues. Heterogeneous response in heart, red and white muscle. Biochem J 282: 765-772, 1992

Carayannopoulos M O, Chi M M-Y, Pingsterhaus J M, McKnight R A, Mueckler M, Devaskar S U and Moley K H: GLUT8 is a glucose transporter responsible for insulin-stimulated glucose uptake in the blastocyst. Proc Natl Acad Sci USA 97: 7313-7318, 2000

Charron M J, Brosius F C 3rd, Alper S L and Lodish H F: A glucose transport protein expressed predominately in insulin-responsive tissues. Proc Natl Acad Sci USA 86: 2535-2539, 1989

Charron M J, Katz E B and Olson A L: GLUT4 gene regulation and manipulation. J Biol Chem 274: 3253-3256, 1999

Christiansen D C and KlungsØyr L: Metabolic utilization of nutrients and the effects of insulin in fish. Comp Biochem Physiol 88B:701-711, 1987

Ciaraldi T P, Horuk R and Matthaei S: Biochemical and functional characterization of the rat liver glucose-transport system. Comparison with the adipocyte glucose-transport system. Biochem J 240: 115-123, 1986

Cohen N R, Knecht D A and Lodish H F: Functional expression of rat GLUT1 transporter in *Dictyostelium discoideum*. Biochem J 315: 971-975, 1996

Colville C A, Seatter M J, Jess T J, Gould G W and Thomas H M: Kinetic analysis of the liver-type (GLUT2) and brain-type (GLUT3) glucose transporters in *Xenopus* oocytes: substrate specificities and effects of transport inhibitors. Biochem J 290: 701-706, 1993

Concha I I, Velásquez F V, Martínez J M, Angulo C, Droppelmann A, Reyes A M, Slebe J C, Vera J C and Golde D W: Human erythrocytes express GLUT5 and transport fructose. Blood 89: 4190-4195, 1997

Cope D L, Homan G D, Baldwin S A and Wolstenholme A J: Domain assembly of the GLUT1 glucose transporter. Biochem J 300: 291-294, 1994

Cope D L, Lee S-H, Melvin D R and Gould G W: Identification of further important residues within the Glut4 carboxy-terminal tail which regulate subcellular trafficking. FEBS Letters 481: 261-265, 2000

Corpe C P, Bovelander F I, Hoekstra J H and Burant C F: The small intestinal fructose transporters: site of dietary perception and evidence for diurnal and fructose sensitive control elements. Biochim Biophys Acta 1402: 229-238, 1998

Corvera S, Chawla A, Chakrabarti R, Joly M, Buxton J and Czech M P: A double leucine within the GLUT4 glucose transporter COOH-terminal domain functions as an endocytosis signal. J Cel Biol 126: 979-989, 1994

Couly G F, Coltey P M and Le Douarin N M: The triple origin of skull in higher vertebrates: a study in quail-chick chimeras. Develompent 117: 409-429, 1993

Coway C B and Walton M J: Intermediary metabolism. In: J.E. Halver (Editor), Fish Nutrition, 2nd edn., Academic Press, New York, pp.259-329, 1989

Cowey C B, de la Higuera M and Adron J W: The effect of dietary composition and of insulin on gluconeogenesis in rainbow trout (*Salmo gairdneri*). Br J Nutr 38: 385-395, 1977a

Cowey C B, Knox D, Walton M J and Adron J W: The regulation of gluconeogenesis by diet and insulin in rainbow trout (*Salmo gairdneri*). Br J Nutr 38: 463-470, 1977b

Cowley D J and Sheridan M A: Insulin stimulates hepatic lipogenesis in rainbow trout, *Oncorhychus mykiss*. Fish Physiol Biochem 11: 421-428, 1993

Cushman S W and Wardzala L J: Potential mechanism of insulin action on glucose transport in the isolated rat adipose cell. J Biol Chem 25: 4758-4762, 1980

Czech M P and Corvera S: Signalling mechanisms that regulate glucose transport. J Biol Chem 274: 1865-1868, 1999

Dan-Goor M, Sasson S, Davarashvili A and Almagor M: Expression of glucose transporter and glucose uptake in human oocytes and preimplantation embryos. Hum Reprod 12: 2508-2510, 1997

Davidson N O, Hausman A M, Ifkovits C A, Buse J B, Gould G W, Burant C F and Bell G I: Human intestinal glucose transporter expression and localization of GLUT5. Am J Physiol 262: C795-C800, 1992

Davies A, Meeran K, Cairns M T and Baldwin S A: Peptide-specific antibodies as probes of the orientation of the glucose transporter in the human erythrocyte membrane. J Biol Chem 262: 9347-9352, 1987

Davies A, Ciardelli T L, Lienhard G E, Boyle J M, Whetton A D and Baldwin S A: Site-specific antibodies as probes of topology and function of the human erythrocyte glucose transporter. Biochem J 266: 799-808, 1990

Derave, W, Lund S, Holman G D, Wojtaszewski J, Pedersen O and Richter E A: Contraction-stimulated muscle glucose transport and GLUT-4 surface content are dependent on glycogen content. Am J Physiol 277: E1103-E1110, 1999

Doege H, Schürmann A, Ohnimus H, Monser V, Holman G D and Joost H-G: Serine-294 and threonine -295 in the exofacial loop domain between helices 7 and 8 of glucose transporters (GLUT) are involved in the conformational alterations during transport process. Biochem J 329: 289-293, 1998

Doege H, Bocianski A, Joost H-G and Schürmann A: Activity and genomic organization of human glucose transporter 9 (GLUT9), a novel member of the family of sugar-transporters predominantly expressed in brain and leucocytes. Biochem J 350: 771-776, 2000a

Doege H, Schürmann A, Bahrenberg G, Brauers A and Joost H -G: GLUT8, a novel member of the sugar transport facilitator family with glucose transport activity. J Biol Chem 275: 16275-16280, 2000b

Due A D, Zhi-chao Q, Thomas J M, Buchs A, Powers A C and May J M: Role of the C-terminal tail of the GLUT1 glucose transporter in its expression and function in *Xenopuss laevis* oocytes. Biochem 34: 5462-5471, 1995

Dwyer D S: Model of the 3-D structure of the GLUT3 glucose transporter and molecular dynamics simulation of glucose transport. Proteins 42: 531-541, 2001

Ebert B L, Firth J D and Ratcliffe P J: Hypoxia and mitochondrial inhibitors regulate expression of glucose transporter-1 via distinct cis-acting sequences. J Biol Chem 270: 29083-29089, 1995

Elliott K R, Bate A J and Craik J D: Specificity of the rat hepatocyte monosaccharide transporter. Int J Biochem 16: 1251-1253, 1984

Escher S A and Rasmuson-Lestander Å: The *Drosophila* glucose transporter gene: cDNA sequence, phylogenetic comparison, analysis of functional site and secondary structure. Hereditas 130: 95-103, 1999

Felsenstein, J: PHYLIP – Phylogeny Inference Package (Version 3.2). Cladistics 5: 164-166 1989

Ferraris R P and Ahearn G A: Sugar and amino acid transport in fish intestine. Comp Biochem Physiol 77A: 397-413, 1984

Fideu M D, Solèr G and Ruiz-Amil M: Nutritional regulation of glycolysis in rainbow trout (*Salmo gairdnerii R.*). Comp Biochem Physiol 74B: 795-799, 1983

Fukumoto H, Seino S, Imura H, Seino Y, Eddy R L, Fukushima Y, Byers M G, Shows T B and Bell G I: Sequence, tissue distribution, and chromosomal localization of mRNA encoding a human glucose transporter-like protein. Proc Natl Acad Sci USA 85: 5434-5438, 1988

Fukumoto H, Kayano T, Buse J B, Edwards Y, Pilch P F, Bell G I and Seino S: Cloning and characterization of the major insulin-responsive glucose transporter expressed in human skeletal muscle and other insulin-responsive tissues. J Biol Chem 264: 7776-7779, 1989

Furuichi M, and Yone Y: Changes of blood sugar and plasma insulin levels of fishes in glucose tolerance tests. Bull Jpn Soc Sci Fish 47: 761-764, 1981

Gans C and Northcutt R G: Neural crest and origin of vertebrates: a new head. Science 220: 268-274, 1983

Garcia J C, Strube M, Leingang K, Keller K and Mueckler M M: Amino acid substitutions at tryptophan 388 and tryptophan 412 of the HepG2 (Glut1) glucose transporter inhibits transport activity and targeting to the plasma membrane in *Xenopus* oocytes. J Biol Chem 267: 7770-7776, 1992

García-Castro M and Bronner-Fraser M: Induction and differentiation of the neural crest. Curr Opin Cell Biol 11: 695-698, 1999

Garippa R J, Judge T W, James D E and McGraw T E: The amino terminus of GLUT4 functions as an internalization motif but not an intracellular retention signal when substituted for the transferrin receptor cytoplasmic domain. J Cell Biol 124: 705-715, 1994

Garippa R J, Johnson A, Parker J, Petrush R L and McGraw T E: The carboxyl terminus of GLUT4 contains a Serine-Leucine-Leucine sequence that functions as a potent internalization motif in Chinese hamster ovary cells. J Bio Chem 271: 20660-20668, 1996

Gorodilov Y N: Comparative analysis of the dynamics of early ontogeny of species of genus *Salmo*. J Ichtyol 29: 19-31, 1989

Gorodilov Y N: Description of the early ontogeny of the Atlantic salmon, *Salmo salar*, with a novel system of interval (state) identification. Env Biol Fish 47: 109-127, 1996

Gorodilov Y N: The fate of Spemann's organizer. Zool Sci 17: 1197-1220, 2000

Gould G W and Lienhard G E: Expression of a functional glucose transporters in *Xenopus* oocytes. Biochemistry 28: 9447-9452, 1989

Gould G W and Bell G I: Facilitative glucose transporters: an expanding family. Trends Biochem Sci 15: 18-23, 1990

Gould G W, Thomas H M, Jess T J and Bell G I: Expression of human glucose transporters in *Xenopus* oocytes: kinetic characterization and substrate specificities of the erythrocytes, liver, and brain isoform. Biochemistry 30: 5139-5145, 1991

Gould G W and Holman G D: The glucose transporter family: structure, function and tissue-specific expression. Biochem J 295: 329-341, 1993

Grover-McKay M, Walsh S A and Thompson S A: Glucose transporter 3 (GLUT3) protein is present in human myocardium. Biochim Biophys Acta 1416: 145-154, 1999

Guillam M-T, Burcelin R and Thorens B: Normal hepatic glucose production in the absence of GLUT2 reveals an alternative pathway for glucose release from hepatocytes. Proc Natl Acad Sci USA 95: 12317-12321, 1998

Guillet-Deniau I, Leturque A and Girard J: Expression and cellular localization of glucose transporters (GLUT1, GLUT3, GLUT4) during differentiation of myogenic cells isolated from rat foetuses. J Cell Sci 107: 487-496, 1994

Gutiérrez J, Åsgård T, Fabbri E and Plisetskaya E M: Insulin-receptor binding in skeletal muscle of trout. Fish Physiol Biochem 9: 351-360, 1991

Haney P M, Levy M A, Strube M S and Mueckler M: Insulin-sensitive targeting of the GLUT4 glucose transporter in L6 myoblasts is conferred by its COOH-terminal cytoplasmic tail. J Cell Biol 129: 641-658, 1995

Hansen P, Gulve E, Gao J, Schluter J, Mueckler M and Holloszy J: Kinetics of 2-deoxyglucose transport in skeletal muscle: effects of insulin and contractions. Am J Physiol 268: C30-C35, 1995

Hansen P A, Han D H, Marshall B A, Nolte L A, Chen M M, Mueckler M and Holloszy J O: A high fat diet impairs stimulation of glucose transport in muscle. Functional evaluation of potential mechanisms. J Biol Chem 273: 26157-26163, 1998

Harmon J S, Eilertson C D, Sheridan M A and Plisetskaya E M: Insulin suppression is associated with hypersomatostatinemia and hyperglucagonemia in glucose- injected rainbow trout. Am J Physiol 261: R609-R613, 1991

Hashiramoto M, Kadowaki T, Clark A E, Muraoka A, Momomura K, Sakura H, Tobe K, Akanuma Y, Yazaki Y, Holman G D and Kasuga M: Site-directed mutagenesis of GLUT1 in helix 7 residue 282 results in perturbation of exofacial ligand binding. J Biol Chem 267: 17502-17507, 1992

Hediger M A, Coady M J, Ikeda T S and Wright E M: Expression cloning and cDNA sequencing of the Na^{+}/glucose co-transporter. Nature 330: 379-381, 1987

Hediger M A, Kanai Y, You G and Nussberger S: Mammalian ion-coupled solute transporters. J Physiol 482: 7S-17S, 1995

Hemre G I, Torrissen O, Krogdahl A A and Lie O: Glucose tolerance in Atlantic Salmon, *Salmo salar* L., dependence on adaptation to dietary starch and water temperature. Aquacult Nutr 1: 69-75, 1995

Hertz Y, Epstein N Abraham M, Madar Z, Hepher B and Gertler A: Effects of metformin on plasma insulin, glucose metabolism and protein synthesis in the common carp (*Cyprinus carpio* L.). Aquaculture 80: 175-187, 1989

Hilton J W and Atkinson J L: Response of rainbow trout (*Salmo gairdneri*) to increased levels of available carbohydrate in practical trout diets. Br. J. Nutr. 47: 597-607, 1982

Hilton J W, Plisetskaya E M and Leatherland J F: Does oral 3,5,3'-triiodo-L-thyronine affect dietary glucose utilization on plasma insulin levels in rainbow trout (*Salmo gairdneri*)? Fish Physiol Biochem 4: 113-120, 1987

Hirokawa T, Boon-Chieng S and Mitaku S: SOSUI: classification and secondary structure prediction system for membrane proteins. Bioinformatics 14: 378-379, 1998

Hofer R and Sturmbauer C: Inhibition of trout and carp alpha-amylase by wheat. Aquaculture 48: 277-283, 1985

Hogan A, Heyner S, Charron M J, Copeland N G, Gilbert D J, Jenkins N A, Thorens B and Schultz G A: Glucose transporter gene expression in early mouse embryos. Development 113: 363-372, 1991

Houpe K L, Malo C and Buddington R K: Dietary lipid and intestinal brush border membrane phospholipid fatty acid composition and glucose transport of channel catfish. Physiol Zool 70: 230-236, 1997

Hresko R C, Kruse M, Strube M and Mueckler M: Topology of the Glut1 glucose transporter deduced from glycosylation scanning mutagenesis. J Biol Chem 269: 20482-20488, 1994

Ibberson M, Uldry M and Thorens B: GLUTX1, a novel mammalian glucose transporter expressed in the central nervous system and insulin-sensitive tissues. J Biol Chem 275: 4607-4612, 2000

Ikeda T S, Hwang E S, Coady M J, Hirayama B A, Hediger M A and Wright E M: Characterization of a Na^{+}/glucose cotransporter cloned from rabbit small intestine. J Membr Biol 110: 87-95, 1989

Ingermann R L, Hall R E, Bissonnette J M and Terwille R C: Monosaccharide transport in erythrocytes of the pacific hagfish *Eptatretus stouti.* Molec Physiol 6: 311-320,1984

Inukai K, Asano T, Katagiri H, Anai M, Funaki M, Ishihara H, Tsukuda K, Kikuchi M, Yazaki Y and Oka Y: Replacement of both tryptophan residues at 388 and 412 completely abolished cytochalasin B photolabelling of the GLUT1 glucose transporter. Biochem J 302: 355-361, 1994

Inukai K, Katagiri H, Takata K, Asano T, Anai M, Ishihara H, Nakuzaki M, Kikuchi M, Yazaki Y and Oka Y:Characterization of rat GLUT5 and functional analysis of chimeric proteins of GLUT1 glucose transporter and GLUT5 fructose transporter. Endocrinology 136: 4850-4857, 1995

Ishihara H, Asano T, Katagiri H, Lin J L, Tsukuda K, Shibasaki Y, Yazaki Y and Oka Y: The glucose transport activity of GLUT1 is markedly decreased by substitution of a single amino acids with a different charge at residue 415. Biochem Biophys Res Commun 176: 922-930, 1991

Ivics Z, Izsvák Z, Minter A and Hackett P B: Identification of functional domains and evolution of Tc*1*-like transposable elements. Proc Natl Acad Sci USA 93: 5008-5013, 1996

Izsvák Z, Ivics Z and Hackett P B: Characterization of a *Tc1*-like transposable element in zebrafish (*Danio rerio*). Mol Gen Genet 247: 312-322, 1995

Jacobs A E M, Oosterhof A and Veerkamp J H: 2-Deoxy-D-glucose uptake in cultured human muscle cells. Biochim Biophys Acta 1051: 230-236, 1990

James D E, Brown R, Navarro J and Pilch P F: Insulin-regulatable tissue express a unique insulin -sensitive glucose transport protein. Nature 333: 183-185, 1988

James D E, Strube M and Mueckler M: Molecular cloning and characterization of an insulin-regulatable glucose transporter. Nature 338: 83-87, 1989

Jessen-Marshall A, Paul N J and Brooker R J: The conserved motif, GXXX(D/E)(R/K)(R/K), in hydrophilic loop 2/3 of the lactose permease. J Biol Chem 270: 16251-16257, 1995

Jones J P, Tapscott E B, Olson A L, Pessin J E and Dohm G L: Regulation of glucose transporters GLUT-4 and GLUT-1 gene transcription in denervated skeletal muscle. J Appl Physiol 84: 1661-1666, 1998

Jung C Y and Rampal A L: Cytochalasin B binding sites and glucose transport carrier in human erythrocyte ghosts. J Biol Chem 252: 5456-5463, 1977

Jung C Y: Proteins that interact with facilitative glucose transporters: implication for function. Exp Physiol 83: 267-273, 1998

Kaestner K H, Christy R J, McLenithan J C, Braiterman L T, Cornelius P, Pekala P H and Lane M D: Sequence, tissue distribution, and differential expression of mRNA for a putative insulin-responsive glucose transporter in mouse 3T3-L1 adipocytes. Proc Natl Acad Sci USA 86: 3150-3154, 1989

Kasahara M and Hinkle P C: Reconstitution and purification of the D-glucose transporter from human erythrocytes. J Biol Chem 252:7384-7390, 1977

Kasahara T and Kasahara M: Tryptophan 388 in putative transmembrane segment 10 of the rat glucose transporter GLUT1 is essential for glucose transport. J Biol Chem 273: 29113-29117, 1998

Katagiri H, Asano T, Ishihara H, Tsukuda K, Lin J-L, Inukai K, Kikuchi M, Yazaki Y and Oka Y:Replacement of intracellular C-terminal domain of GLUT1 glucose transporter with that of GLUT2 increases V_{max} and K_m of transport activity. J Biol Chem 267: 22550-22555, 1992

Kaushik S J and de Oliva Teles A: Effects of digestible energy on nitrogen and energy balance in rainbow trout. Aquaculture 50: 89-101, 1985

Kayano T, Fukumoto H, Eddy R L, Fan Y-S, Byers M G, Shows T B and Bell G I: Evidence for a family of human glucose transporter-like proteins. Sequence and gene localization of a protein expressed in fetal skeletal muscle and other tissues. J Biol Chem 263: 15245-15248, 1988

Kayano T, Burant C F, Fukumoto H, Gould G W, Fan Y-S, Eddy R L, Byers M G, Shows T B, Seino S and Bell G I: Human facilitative glucose transporters. Isolation, functional characterization and gene localization of cDNAs encoding an isoform (GLUT5) expressed in small intestine, kidney, muscle and adipose tissue and an unusual glucose transporter pseudogene-like sequence (GLUT6). J Biol Chem 265: 13276-13282, 1990

Keller K, Strube M and Mueckler M: Functional expression of the human HepG2 and rat adipocyte glucose transporters in *Xenopus* oocytes. J Biol Chem 264: 18884-18889, 1989

Kilgour E, Baldwin S A and Flint D J: Divergent regulation of rat adipocyte GLUT1 and GLUT4 glucose transporters by GH. J Endocrinol 145: 27-33, 1995

Kim J D and Kaushik S J: Contribution of digestible energy from carbohydrates and estimation of protein/energy requirements for growth of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 106: 161-169, 1992

Knox D, Walton M J and Cowey C B: Distribution of enzymes of glycolysis and gluconeogenesis in fish tissues. Mar Biol 56: 7-10, 1980

Koivisto U M, Martinez-Valdez H, Bilan P J, Burdett E, Ramlal T and Klip A: Differential regulation of the GLUT-1 and GLUT-4 glucose transport systems by glucose and insulin in L6 muscle cells in culture. J Biol Chem 266: 2615-2621, 1991

Krasnov A, Pitkänen T I, Reinisalo M and Mölsä H: Expression of human glucose transporter type 1 and rat hexokinase type II complementary DNAs in rainbow trout embryos: effects on glucose metabolism. Mar Biotechnol 1: 25-32, 1999

Krogdahl Å, Nordrum S, Sørensen M, Brudeseth L and Røsjø C: Effects of diet composition on apparent nutrient absorption along the intestinal tract and of subsequent fasting on mucosal disaccharidase activities and plasma nutrient concentration in Atlantic salmon *Salmo salar* L. Aquaculture Nutr 5: 121-133, 1999.

Köntges G and Lumsden A: Rhombencephalic neural crest segmentation is preserved throughout craniofacial ontogeny. Development 122: 3229-3242, 1996

Labrador M and Corces V G: Transposable element-host interactions: regulation of insertion and excision. Ann Rev Genet 31: 381-404, 1997

Langille R M and Hall B K: Developmental processes, developmental sequences and early vertebrate phylogeny. Biol Rev Canb Philos Soc 64: 73-91, 1989

Lee D J and Putnam G B: The response of rainbow trout to varying protein/energy rations in a test diet. J Nutr 103: 916-922, 1973

Legate N J, Bonen A and Moon T W: Glucose tolerance and peripheral glucose utilization in rainbow trout (*Oncorhynchus mykiss*), American eel (*Anguilla rostrata*), and black bullhead catfish (*Ameiurus melas*). Gen Comp Endocrinol 122: 48-59, 2001

Leppens-Luisier G, Urner F and Sakkas D: Facilitated glucose transporters play a crucial role throughout mouse preimplantation embryo development. Hum Reprod 16: 1229-1236, 2001

Lienhard G E, Slot J W, James D E and Mueckler M M: How cells absorb glucose. Scient American January, pp 34-39, 1992

Lin J-L, Asano T, Katagiri H, Tsukuda K, Ishihara H, Inukai K, Yazaki Y and Oka Y: Deletion of C-terminal 12 amino acids of GLUT1 protein does not abolish the transport activity. Biochem Biophys Res Commun 184: 865-870, 1992

Maiden M C J, Davis E O, Baldwin S A, Moore D C M and Henderson P J F: Mammalian and bacterial sugar transport proteins are homologous. Nature 325: 641-643, 1987

Marger M D and Saier M H Jr: A major superfamily of transmembrane facilitators that catalyse uniport, symport and antiport. Trends Biochem Sci 18: 13-20, 1993

Marsh B J, Martin S, Melvin D R, Martin L B, Alm R A, Gould G W and James D E: Mutation analysis of the carboxy-terminal phosphorylation site of GLUT-4 in 3T3-L1 adipocytes. Am J Physiol 275: E412-E422,1998

Marshall B A, Murata H, Hresko R C and Mueckler M: Domains that confer intracellular sequestration of the Glut4 glucose transporter in *Xenopus* oocytes. J Biol Chem 268: 26193-26199, 1993

Martinez-Arca S, Lalioti V S and Sandoval I V: Intracellular targeting and retention of the glucose transporter GLUT4 by the perinuclear storage compartment involves distinct carboxyl-tail motifs. J Cell Sci 113: 1705-1715, 2000

Mathoo J M, Shi Z Q, Klip A and Vranic M: Opposite effects of acute hypoglycemia and acute hyperglycemia on glucose transport and glucose transporters in perfused rat skeletal muscle. Diabetes 48: 1281-1288, 1999

McGowan K M, Long S D and Pekala P H: Glucose transporter gene expression: regulation of transcription and mRNA stability. Pharmac Ther 66: 465-505, 1995

McVie-Wylie A J, Lamson D R and Chen Y T: Molecular cloning of a novel member of the GLUT family of transporters, SLC2A10 (GLUT10), localized on chromosome 20q13.1: A candidate gene for NIDDM susceptibility. Genomics 72: 113-117, 2001

Miyamoto K, Tatsumi S, Morimoto A, Minami H, Yamamoto H, Sone K, Taketani Y, Nakabou Y, Oka T and Takeda E: Characterization of the rabbit intestinal fructose transporter (GLUT5). Biochem J 303: 877-883, 1994

Mommsen T P and Plisetskaya E M: Insulin in fish and agnathans: History, structure and metabolic regulation. Rev Aquat Sci 4: 225-259, 1991

Moon T W, Castejon C, Banos N, Maestro M A, Plisetskaya E M, Gutiérrez J and Navarro I: Insulin and IGF-I binding in isolated trout cardiomyocytes. Gen Comp Endocrinol 103: 264-272, 1996

Moon T W: Glucose intolerance in teleost fish: fact or fiction? Comp Biochem Physiol 129B: 243-249, 2001

Mori H, Hashiramoto M, Clark A E, Yang J, Muraoka A, Tamori Y, Kasuga M and Holman G D: Substitution of tyrosine 293 of GLUT1 locks the transporter into an outward facing conformation. J Biol Chem 269: 11578-11583, 1994

Morita Y, Tsutsumi O, Hosoya I, Taketani Y, Oka Y and Kato T: Expression and possible function of glucose transporter protein GLUT1 during preimplantation mouse development from oocytes to blastocysts. Biochem Biophys Res Commun 188: 8-15, 1992

Mueckler M, Caruso C, Baldwin S A, Panico M, Blench I, Morris H R, Allard W J, Lienhard G E and Lodish H F: Sequence and structure of a human glucose transporter. Science 229: 941-945, 1985

Mueckler M: Facilitative glucose transporters. Eur J Biochem 219: 713-725, 1994a

Mueckler M, Kruse M, Strube M, Riggs A C, Chiu K C and Permutt, M A: A mutation in the Glut2 glucose transporter gene of a diabetic patient abolishes transport activity J Biol Chem 269: 17765-17767, 1994b

Mueckler M, Weng W and Kruse M: Glutamine 161 of Glut1 glucose transporter is critical for transport activity and exofacial ligand binding. J Biol Chem 269: 20533-20538, 1994c

Mueckler K and Makepeace C: Identification of an amino acid residue that lies between the exofacial vestibule and exofacial substrate binding site of the Glut1 sugar permeation pathway. J Biol Chem 272: 30141-30146, 1997

Mueckler K and Makepeace C: Transmembrane segment 5 of the Glut1 glucose transporter is an amphipathic helix that forms part of the sugar permeation pathway. J Biol Chem 274: 10923-10926, 1999

Murakami T, Nishiyama T, Shirotani T, Shinohara Y, Kan M, Ishii K, Kanai F, Nakazuru S and Ebinal Y: Identification of two enhancer elements in the gene encoding the type 1 glucose transporter from the mouse which are responsive to serum, growth factor and oncogenes. J Biol Chem 267: 9300-9306, 1992

Muraoka A, Hashiramoto M, Clark A E, Edwards L C, Sakura H, Kadowaki T, Holman G D and Kasuga M: Analysis of the structural features of the C-terminus of GLUT1 that are required for transport catalytic activity. Biochem J 311: 699-704, 1995

Nagamatsu S, Kornhauser J M, Burant C F, Seino S, Mayo K E and Bell G I: Glucose transporter expression in brain. cDNA sequence of mouse GLUT3, the brain facilitative glucose transporter isoform, and identification of sites of expression by in situ hybridization. J Biol Chem 267: 467-472, 1992

Nagayama F, Ohshima H and Takeuchi T: Activities of hexokinase and glucose dehydrogenase in fish liver. Bull Jap Soc Scient Fish 39: 1349, 1973

Nagayama F and Ohshima H: Studies on the enzyme system of carbohydrate metabolism in fish. 1. Properties of liver hexokinase. Bull Jap Soc Sci Fish 40: 285-290, 1974

Nikinmaa M and Tiihonen K: Substrate transport and utilization in fish erythrocytes. Acta Physiol Scand 152: 183-189, 1994

Nishimura H, Pallardo F V, Seidner G A, Vannucci S, Simpson I A and Birnbaum M J: Kinetics of GLUT1 and GLUT4 glucose transporters expressed in *Xenopus* oocytes. J Biol Chem 268: 8514-8520, 1993

Oka Y, Asano T, Shibasaki Y, Lin J L, Tsukuda K, Katagiri H, Akanuma Y and Takaku F: C-terminal truncated glucose transporter is located into an inward-facing form without transport activity. Nature 345: 550-553, 1990

Olefsky J M: Insulin-stimulated glucose transport minireview series. J Biol Chem 274: 1863, 1999

Onetti R, Baulida J and Bassols A: Increased glucose transport in *ras*-transformed fibroblasts: a possible role for *N*-glycosylation of GLUT1. FEBS Letters 407: 267-270, 1997

Palmer T N and Ryman B E: Studies on oral glucose intolerance in fish. J Fish Biol 4:311-319, 1972

Panserat S, Médale F, Blin C, Krishnamoorthy R and Kaushik S: Glucose homeostasis and characterization of the glucokinase in teleosteans. Cybium 23: 153-159, 1999

Panserat S, Blin C, Médale F, Plagnes-Juan E, Brèque J, Krishnamoorthy J and Kaushik S: Molecular cloning, tissue distribution and sequence analysis of complete glucokinase cDNAs from gilthead seabream (*Sparus aurata*), rainbow trout (*Oncorhychus mykiss*) and common carp (*Cyprinus carpio*). Biochim Biophys Acta 1474: 61-69, 2000a

Panserat S, Médale F, Blin C, Brèque J, Vachot C, Plagnes-Juan E, Gomes E, Krishnamoorthy R and Kaushik S: Hepatic glucokinase is induced by dietary carbohydrates in rainbow trout, gilthead seabream, and common carp. Am J Physiol Regulatory Integrative Comp Physiol 278: R1164-R1170, 2000b

Panserat S, Capilla E, Gutiérrez J, Frappart PO, Vachot C, Plagnes-Juan E, Aguirre P, Breque J and Kaushik S: Glucokinase is highly induced and glucose-6-phosphatase poorly repressed in liver of rainbow trout (*Oncorhychus mykiss*) by a single meal with glucose. Comp Biochem Physiol 128B: 275-283, 2001a

Panserat S, Plagnes-Juan E and Kaushik S: Nutritional regulation and tissue specificity of gene expression for proteins involved in hepatic glucose metabolism in rainbow trout (*Oncorhynchus mykiss*). J Exp Biol 204: 2351-2360, 2001b

Parrizas M, Planas J, Plisetskaya E M and Gutiérrez J: Insulin binding and receptor tyrosine kinase activity in skeletal muscle of carnivorous and omnivorous fish. Am J Physiol 266: R1944-R1950, 1994a

Parrizas M, Banos N, Baro J, Planas J and Gutiérrez J: Up-regulation of insulin binding in fish skeletal muscle by high insulin levels. Regul Pept 53: 211-222, 1994b

Penny J I, Hall S T, Woodrow C J, Cowan G M, Gero A M and Krishna S: Expression of substrate-specific transporters encoded by *Plasmodium falciparum* in *Xenopus laevis* oocytes. Mol Biochem Parasitol 93: 81-89, 1998

Peragón J, Barroso J B, García-Salguero L, de la Higuera M and Lupiáñes J A: Carbohydrates affect protein-turnover rates, growth, and nucleic acid content in the white muscle of rainbow trout (*Oncorhychus mykiss*). Aquaculture 179: 425-437, 1999

Pereira C, Vijayan MM, Storey K B, Jones R A and Moon T W: Role of glucose and insulin in regulating glycogen synthase and phosphorylase activities in rainbow trout hepatocytes. J Comp Physiol 165B: 62-70, 1995

Phillips S M Jr, Tunison A V and Brockway D R: Utilization of carbohydrates by trout. Fish. Res. Bull. NY 11: 1-44, 1948

Pieper A and Pfeffer E: Studies on the comparative efficiency of utilization of gross energy from some carbohydrates, proteins and fats by rainbow trout (*Salmo gairdneri,* R.). Aquaculture 20: 323-332, 1980

Piper R C, Tai C, Slot J W, Hahn C S, Rice C M, Huang H and James D E: The efficient intracellular sequestration of the insulin-regulatable glucose transporter (GLUT-4) is conferred by the NH2 terminus. J Cell Biol 117: 729-743, 1992

Piper R C, Tai C, Kulesza P, Pang S, Warnock D, Baenziger J, Slot J W, Geuze H J, Puri C and James D E: GLUT-4 NH2 terminus contains a phenylalanine-based targeting motif that regulates intracellular sequestration. J Cell Biol 121: 1221-1232, 1993

Pitkänen, T I, Krasnov A, Reinisalo M and Mölsä H: Transfer and expression of glucose transporter and hexokinase genes in salmonid fish. Aquaculture 173: 319-332, 1999

Planas J V, Mendez E, Banos N, Capilla E, Navarro I and Gutiérrez J: Insulin and IGF-I receptors in trout adipose tissues are physiologically regulated by circulating hormone levels. J Exp Biol 203: 1153-1159, 2000a

Planas J V, Capilla E and Gutiérrez J: Molecular identification of a glucose transporter from fish muscle. FEBS Letters 481: 266-270, 2000b

Plisetskaya E M: Physiology of fish endocrine pancreas. Fish Physiol Biochem 7: 39-48, 1989

Plisetskaya E M: Recent studies on fish pancreatic hormones: Selected Topics. Zool Sci 7: 335-353, 1990

Plisetskaya E M: Some of my not so favorite things about insulin and insulin-like growth factors in fish. Comp Biochem Physiol 121B: 3-11, 1998

Postic C, Leturque A, Printz R L, Maulard P, Loizeau M, Granner D K and Girard J: Development and regulation of glucose transporter and hexokinase expression in rat. Am J Physiol 266: E548-E559, 1994

Qi C and Pekala P H: The influence of mRNA stability on glucose transporter (GLUT1) gene expression. Biochem Biophys Res Commun 263: 265-269, 1999

Rand E B, Depaoli A M, Davidson N O, Bell G I and Burant C F: Sequence, tissue distribution, and functional characterization of the rat fructose transporter GLUT5. Am J Physiol 264: G1169-G1176, 1993

Reed B C, Shade D, Alperovich F and Vang M: 3T3-L1 adipocyte glucose transporter (HepG2 class): sequence and regulation of protein and mRNA expression by insulin, differentiation, and glucose starvation. Arch Biochem Biophys 279: 261-274, 1990

Reshkin S J and Ahearn G A: Intestinal glucose transport and salinity adaptation in a euryhaline teleost. Am J Physiol 252: R567-R578, 1987a

Reshkin S J and Ahearn G A: Basolateral glucose transport by intestine of teleost, *Oreochromis mossambicus*. Am J Physiol 252: R579-R586, 1987b

Rodnick K J, Holloszy J O, Mondon C E and James D E: Effects of exercise training on insulin -regulatable glucose-transporter protein levels in rat skeletal muscle. Diabetes 39: 1425-1429, 1990

Rodnick K J, Bailey J R, West J L, Rideout A and Driedzic W R: Acute regulation of glucose uptake in cardiac muscle of the American eel *Anguilla rostrata*. J Exp Biol 200: 2871-2880, 1997

Santalucia T, Camps M, Castello A, Munoz P, Nuel A, Testar X, Palacin M and Zorzano A: Developmental regulation of GLUT-1 (erythroid/hep G2) and GLUT-4 (muscle/fat) glucose transporter expression in rat heart, skeletal muscle, and brown adipose tissue. Endocrinology 130: 837-846, 1992

Sato M and Mueckler M: A conserved amino acid motif (R-X-G-R-R) in the Glut1 glucose transporter is an important determinant of membrane topology. J Biol Chem 274: 24721-24725, 1999

Schürmann A, Keller K, Monden I, Brown F M, Wandel S, Shanahan M F and Joost H-G: Glucose transport activity and photolabelling with 3-(125I)iodo-4-azidophenethylamido-7-O-succinyldeacetyl (IAPS)-forskolin of two mutants at tryptophan-388 and -412 of the glucose transporter GLUT1: dissociation of the binding domains of forskolin and glucose. Biochem J 290: 497-501, 1993

Schürmann A, Doege H, Ohnimus H, Monser V, Buchs A and Joost H-G: Role of conserved Argine and Glutamate residues on the cytosolic surface of glucose transporters for transport function. Biochemistry 36: 12897-12902, 1997

Seatter M J, De La Rue S A, Porter L M and Gould G W: QLS motif in transmembrane helix VII of the glucose transporter family interacts with the C-1 position of D-glucose and is involved in substrate selection at the exofacial binding site. Biochemistry 37: 1322-1326, 1998

Shepherd P R, Gibbs E M, Wesslau C, Gould G W and Kahn B B: Human small intestinal facilitative fructose/glucose transporter (GLUT5) is also present in insulin-responsive tissues and brain. Investigation of biochemical characteristics and translocation. Diabetes 41: 1360-1365, 1992

Shewan A M, Marsh B J, Melvin D R, Martin S, Gould G W and James D E: The cytosolic C-terminus of the glucose transporter GLUT4 contains an acidic endosomal targeting motif distal to the dileucine signal. Biochem J 350: 99-107, 2000

Shimeno S: Studies on carbohydrate metabolism in fishes. Rep. Fish. Lab. Kochi Univ. (Japan) 2: 1-107, 1974

Shimeno S, Hosakawa H, Hirata H and Takeda M: Comparative studies on carbohydrate metabolism of yellowtail and carp. Bull. Jpn. Soc. Sci. Fish 43: 213-217, 1977

Slot J W, Geuze H J, Gigengack S, James D E and Lienhard G E: Translocation of the glucose transporter GLUT 4 in cardiac myocytes of the rat. Proc Natl Acad Sci USA. 88: 7815-7819, 1991

Smoak I W and Branch S: Glut-1 expression and its response to hypoglycemia in the embryonic mouse heart. Anat Embryol 201: 327-333, 2000

Soengas J L, Fuentes J, Andres M D and Aldegunde M: Direct transfer of rainbow trout to seawater induces several changes in kidney carbohydrate metabolism. Rev Esp Fisiol 50: 219-227, 1994

Soengas J L, Aldegunde M and Andrés M D: Gradual transfer to sea water of rainbow trout: effects on liver carbohydrate metabolism. J Fish Biol 47: 466-478, 1995a

Soengas J L, Barciela P, Aldegunde M and Andrés D: Gill carbohydrate metabolism of rainbow trout is modified during gradual adaptation to sea water. J Fish Biol 46: 845-856, 1995b

Soengas J L and Moon T W: Uptake and metabolism of glucose, alanine and lactate by red blood cells of the American eel *Anguilla rostrata*. J Exp Biol 198: 877-888, 1995c

Soengas J L and Moon T W: Transport and metabolism of glucose in isolated enterocytes of the black bullhead *Ictalurus melas*: effects of diet and hormones. J Exp Biol 201: 3263-3273, 1998

Storebakken T, Shearer K D, Refstie S, Lagocki S and McCool J: Interactions between salinity, dietary carbohydrate source and carbohydrate concentration on the digestibility of macronutrients and energy in rainbow trout (*Oncorhychus mykiss*). Aquaculture 163: 347-359, 1998

Stuart C A, Wen G, Gustafson W C and Thompson E A: Comparison of GLUT1, GLUT3, and GLUT4 mRNA and subcellular distribution of their proteins in normal human muscle. Metabolism 49: 1604-1609, 2000

Sundby A, Hemre G-I, Borrebaek B, Christophersen B and Blom A K: Insulin and glucagon family peptides in relation to activities of hepatic hexokinase and other enzymes in fed and starved Atlantic salmon (*Salmo salar*) and cod (*Gadus morhua*). Comp Biochem Physiol 100B: 467-470, 1991

Takakura Y, Kuentzel S L, Raub T J, Davies A, Baldwin S A and Borchardt R T: Hexose uptake in primary cultures of bovine brain microvessel endothelial cells. I. Basic characteristics and effects of D-glucose and insulin. Biochim Biophys Acta 1070: 1-10, 1991

Tamori Y, Hashiramoto M, Clark A E, Mori H, Muraoka A, Kadowaki T, Holman G D and Kasuga M: Substitution at Pro^{385} of GLUT1 perturbs the glucose transport function by reducing conformational flexibility. J Biol Chem 269: 2982-2986, 1994

Tetaud E, Barrett M P, Bringaud F and Baltz T: Kinetoplastid glucose transporters. Biochem J 325: 569-580, 1997

Thomas H M, Takeda J and Gould G W: Differential targeting of glucose transporter isoforms heterologously expressed in *Xenopus* oocytes. Biochem J 290: 707-715, 1993

Thomas-Delloye V, Marmonier F, Duchamp C, Pichon-Georges B, Lachuer J, Barré H and Crouzoulon G: Biochemical and functional evidences for a GLUT-4 homologous protein in avian skeletal muscle. Am J Physiol 277: R1733-R1740, 1999

Thompson J D, Higgins D G and Gibson T J: CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22: 4673-4680, 1994

Thorens B, Sarkar H K, Kaback H R and Lodish H F: Cloning and functional expression in bacteria of a novel glucose transporter present in liver, intestine, kidney, and beta-pancreatic islet cells. Cell 55: 281-290, 1988

Thorens B, Cheng Z-Q, Brown D and Lodish H F: Liver glucose transporter: a basolateral protein in hepatocytes and intestine and kidney cells. Am J Physiol 259: C279-C285, 1990

Tiihonen K and Nikinmaa M: D-glucose permeability in river lamprey (*Lampetra fluviatilis*) and carp (*Cyprinus carpio*) erythrocytes. Comp Biochem Physiol 100A: 581-584, 1991

Tiihonen K, Nikinmaa M and Lappivaara J: Glucose transport in carp erythrocytes: individual variation and effect of osmotic swelling, extracellular pH and catecholamines. J Exp Biol 198: 577-583, 1995

Tordjman K M, Leingang K A and Mueckler M: Differential regulation of the HepG2 and adipocyte/muscle glucose transporters in 3T3L1 adipocytes. Effect of chronic glucose deprivation. Biochem J 271: 201-207, 1990

Tranulis M A, Christophersen B, Blom A K and Borrebaek B: Glucose dehydrogenase, glucose-6-phosphate dehydrogenase and hexokinase in liver of rainbow trout (*Salmo gairdneri*). Effects of starvation and temperature variations. Comp Biochem Physiol 99B: 687-691, 1991

Tranulis M A, Dregni O, Christophersen B, Krogdahl A and Borrebaek B: A glucokinase-like-enzyme in the liver of Atlantic salmon (*Salmo salar*). Comp Biochem Physiol 114B: 35-39, 1996

Tse C M and Young J D: Glucose transport in fish erythrocytes: variable cytochalasin-B-sensitive transport activity in the common eel (*Anguilla japonica*) and transport deficiency in paddyfield eel (*Monopterus albus*) and rainbow trout (*Salmo gairdneri*). J Exp Biol 148: 367-383, 1990

Valera A, Pujol A, Gregori X, Riu E, Visa J and Bosch F: Evidence from transgenic mice that myc regulates hepatic glycolysis. FASEB J 9: 1067-1078, 1995

Vera J C and Rosen O M: Functional expression of mammalian glucose transporters in *Xenopus laevis* oocytes: evidence for cell-dependent insulin sensitivity. Mol Cell Biol 9: 4187-4195, 1989

Verhey K J and Birnbaum M J: A Leu-Leu sequence is essential for COOH-terminal targeting signal of GLUT4 glucose transporter in fibroblasts. J Biol Chem 269: 2353-2356, 1994

Viñals F, Ferré J, Fandos C, Santalucia T, Testar X, Palacín M and Zorzano A: Cyclic adenosine 3′, 5′-monophosphate regulates GLUT4 and GLUT1 glucose transporter expression and stimulates transcriptional activity of the GLUT1 promoter in muscle cells. Endocrinology 138: 2521-2529, 1997

Waddell I D, Scott H, Grant A and Burchell A: Identification and characterization of a hepatic microsomal glucose transport protein. T3 of the glucose-6-phosphatase system? Biochem J 275: 363-367, 1991

Waddell I D, Zomerschoe A G, Voice M W and Burchell A: Cloning and expression of a hepatic microsomal glucose transport protein. Comparison with liver plasma-membrane glucose-transport protein GLUT2. Biochem J 286: 173-177, 1992

Walker P S, Ramlal T, Sarabia V, Koivisto U M, Bilan P J, Pessin J E and Klip A: Glucose transport activity in L6 muscle cells is regulated by the coordinate control of subcellular glucose transporter distribution, biosynthesis, and mRNA transcription. J Biol Chem 265: 1516-1523, 1990

Walmsley A R, Barrett M P, Bringaud F and Gould G W: Sugar transporters from bacteria, parasites and mammals: structure-activity relationships. Trends Biochem Sci 23: 476-481, 1998

Wandel S, Schürmann A, Becker W, Summers S A, Shanahan M F and Joost H G: Substitution of conserved tyrosine residues in helix 4 (Y143) and 7 (Y293) affects the activity, but not IAPS-forskolin binding, of the glucose transporter GLUT4. FEBS Letters 348: 114-118, 1994

Weiler-Guttler H, Zinke H, Mockel B, Frey A and Gassen H G: cDNA cloning and sequence analysis of the glucose transporter from porcine blood-brain barrier. Biol Chem Hoppe Seyler 370: 467-473, 1989

Wellner M, Monden I, Mueckler M M and Keller K: Functional consequences of proline mutations in the putative transmembrane segments 6 and 10 of the glucose transporter GLUT1. Eur J Biochem 227: 454-458, 1995

Werner H, Adamo M, Lowe W L, Roberts C T and LeRoith D: Developmental regulation of rat brain/Hep G2 glucose transporter gene expression. Mol Endocrinol 3: 273-279, 1989

Wilson R P: Utilizations of dietary carbohydrate by fish. Aquaculture 124: 67-80, 1994

Woodrow C J, Burchmore R J and Krishna S: Hexose permeation pathways in *Plasmodium falciparum*- infected erythrocytes. Proc Natl Acad Sci USA 97: 9931-9936, 2000

Wright J R Jr, O'Hali W, Yang H, Han X-X and Bonen A: GLUT4-deficiency and severe peripheral resistance to insulin in the teleost fish tilapia. Gen Comp Endocrinol 111: 20-27, 1998

Wu L, Fritz J D and Powers A C: Different functional domains of GLUT2 glucose transporter are required for glucose affinity and substrate selectivity. Endocrinology 139: 4205-4212, 1998

Yang J C, Clark A E, Kozka I J, Cushman S W and Holman G D: Development of an intracellular pool of glucose transporters in 3T3-L1 cells. J Biol Chem 267: 10393-10399, 1992

Yokota K, Nishi Y and Takesue Y: Effect of phloretin on Na^+-dependent D-glucose uptake by intestinal brush border membrane vesicles. Biochem Pharmacol 32: 3453-3457, 1983

Young J D, Yao S Y-M, Tse C M, Davies A and Baldwin S A: Functional and molecular characterization of a primitive vertebrate glucose transporter: studies of glucose transport by erythrocytes from the Pacific hagfish (*Eptatretus stouti*). J Exp Biol 186: 23-41, 1994

Zhang J Z, Behrooz A and Ismail-Beigi F: Regulation of glucose transport by hypoxia. Am J Kindey Dis 34: 189-202, 1999